Experimental and theoretical study on high temperature induced changes in chlorophyll *a* fluorescence oscillations in barley leaves upon 2 % CO_2

D. LAZÁR[*,+], R. KAŇA[*], T. KLINKOVSKÝ[**], and J. NAUŠ[*]

Laboratory of Biophysics, Faculty of Science, Palacký University, tř. Svobody 26, 771 46 Olomouc, Czech Republic[*]
TK8-Software, Na Mlynářce 3, 757 01 Valašské Meziříčí, Czech Republic[**]

Abstract

Oscillations in many of photosynthetic quantities with a period of about 1 min can be routinely measured with higher plant leaves after perturbation of the steady state by sudden change in gas phase. Among all hypotheses suggested so far to explain the oscillations, an effect of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activation status to control the oscillations is highly probable, at least upon high temperature (HT) treatment when *in vivo* RuBPCO activity controlled by RuBPCO activase (RuBPCO-A) decreases. Therefore, we measured the oscillations in fluorescence signal coming from barley leaves (*Hordeum vulgare* L. cv. Akcent) after their exposure for various time intervals to different HTs in darkness. We also evaluated steady state fluorescence and CO_2 exchange parameters to have an insight to functions of electron transport chain within thylakoid membrane and Calvin cycle before initiation of the oscillations. The changes in period of the oscillations induced by moderate HT (up to 43 °C) best correlated with changes in non-photochemical fluorescence quenching (q_N) that in turn correlated with changes in gross photosynthetic rate (P_G) and rate of RuBPCO activation (k_{act}). Therefore, we suggest that changes in period of the oscillations caused by moderate HT are mainly controlled by RuBPCO activation status. For more severe HT (45 °C), the oscillations disappeared which was probably caused by an insufficient formation of NADPH by electron transport chain within thylakoid membrane as judged from a decrease in photochemical fluorescence quenching (q_P). Suggestions made on the basis of experimental data were verified by theoretical simulations of the oscillations based on a model of Calvin cycle and by means of a control analysis of the model.

Additional key words: *Hordeum vulgare*; model; NADPH; ribulose-1,5-bisphosphate carboxylase/oxygenase and its activase.

Introduction

Under certain conditions, photosynthesising organism exhibits damped oscillations in measured quantity. The period and damping is different for particular organism but the oscillations in higher plant leaves have the period of about 1 min and are weakly damped enabling them to maintain typically for about 3–10 min. These oscillations are induced by a perturbation of the steady state reached by a change in conditions of irradiation or gas phase. The oscillations were most often measured in chlorophyll (Chl) *a* fluorescence signal, and rate of oxygen (O_2) evolution and carbon dioxide (CO_2) fixation (Walker *et al*. 1983, Walker and Sivak 1985, Sivak and Walker 1985, 1986, 1987, Malkin 1987) but generally every quantity related to photosynthetic function measured so far

Received 29 April 2004, *accepted* 1 November 2004.
[+]Corresponding author; fax: +420 58 5225737; e-mail: lazard@seznam.cz
Abbreviations: ADP – adenosine 5'-diphosphate; ATP – adenosine 5'-triphosphate; Chl – chlorophyll; F_0 (F_0') – minimal fluorescence for dark (light) adapted state; FBPase – fructose 1,6-bisphosphate phosphatase; F_M (F_M') – maximal fluorescence for dark (light) adapted state; F_S – steady state fluorescence for light adapted state; F_V (F_V') – variable fluorescence for dark (light) adapted state; F_V/F_M – maximal quantum yield of photosystem 2 photochemistry; GAPDH – NADP:glyceraldehyde-3-phosphate dehydrogenase; HT – high temperature; k_{act} – apparent rate constant of RuBPCO activation; NADP – nicotinamide adenine dinucleotide phosphate; NADPH – reduced nicotinamide adenine dinucleotide phosphate; P_G – steady state gross photosynthetic rate; P_N – steady state net photosynthetic rate; PAR – photosynthetically active radiation; PGA – 3-phosphoglycerate; PGK – 3-phosphoglycerate kinase; P_i – orthophosphate; PS – photosystem; q_N – steady state non-photochemical fluorescence quenching; q_P – steady state photochemical fluorescence quenching; *R* – dark respiration; RPK – ribulose-5-phosphate kinase; RuBP – ribulose-1,5-bisphosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBPCO-A – ribulose-1,5-bisphosphate carboxylase/oxygenase activase; RuP – ribulose-5-phosphate; TP – triosephosphate.
Acknowledgements: Grant Agency of the Czech Republic financially supported this work by grant number 204/02/P071. This work was also supported by grant of the Ministry of Education of the Czech Republic number MSM 153100010.

oscillates (for reviews see Walker 1992, Giersch 1994). The oscillations in the rate of O_2 evolution and CO_2 fixation are parallel and in phase but anti-parallel to the oscillations in fluorescence signal that anticipates the oscillations in the former two quantities (Delieu and Walker 1983, Walker *et al*. 1983, Quick and Horton 1984, 1986, Walker and Sivak 1985, 1986, Sivak and Walker 1986, Stitt and Schreiber 1988). Further, the oscillations in the rate of O_2 evolution and CO_2 fixation calculated from oscillation in the fluorescence signal agree with experimentally measured oscillations in the rate of O_2 evolution and CO_2 fixation (Keiller and Walker 1990, Seaton and Walker 1990) showing that measurement of oscillations in one quantity (*e.g*. fluorescence) is enough because the oscillations in other quantities are mutually connected.

Several hypotheses were suggested to explain the oscillations. The insufficiency of ATP caused by competition for ATP between 3-phosphoglycerate kinase (PGK) and ribulose-5-phosphate kinase (RPK) during Calvin cycle was suggested to be possible origin of the oscillations (Walker *et al*. 1983). As concentration of orthophosphate (P_i) can affect the amplitude of the oscillation, P_i can also play a significant role in the oscillations (Walker and Sivak 1985, Sivak and Walker 1986). Feedback inhibition of cytosolic fructose 1,6-bisphosphate phosphatase (FBPase) by fructose 2,6-bisphosphate with a delay caused by other reactions in the sucrose synthesis in cytosol was also suggested as a possible source of the oscillations (Stitt *et al*. 1988). It was also suggested that imbalance in the supply of ATP and NADPH to Calvin cycle caused by insufficient function of the photosynthetic electron transport chain can cause the photosynthetic oscillations (Laisk *et al*. 1991). Participation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), the key enzyme in Calvin cycle, was also suggested on the basis of theoretical analyses as a possible source of the oscillations (Ryde-Pettersson 1991, Roussel 1998) which was supported by experimental finding that transgenic tobacco plants with decreased amount of RuBPCO lack the oscillations in fluorescence signal and in the rate of O_2 evolution in contrast to the control plants (Stitt *et al*. 1991). The above mentioned results tend to conclude that several processes control the oscillations as suggested on the basis of analysis of fluorescence oscillations by Fourier transformation (Ferimazova *et al*. 2002).

Although with different success, there was also an effort to mathematically model the hypotheses mentioned above and the photosynthetic oscillations as such (Giersch 1986, Hahn 1986, Laisk and Walker 1986, 1989, Horton and Nicholson 1987, Laisk *et al*. 1989, Giersch and Sivak 1991, Karavaev and Kukushkin 1993, Kocks and Ross 1995, Rovers and Giersch 1995, Buschmann and Gradmann 1997, Kukushkin 1997, Laisk and Eichelmann 1998, Roussel 1998, Khuznetsova and Kukushkin 1999, Gradmann 2001).

The photosynthetic oscillations were almost always measured at room temperature. The oscillations in fluorescence signal and rate of O_2 evolution and CO_2 fixation measured with leaves of both C_3 and C_4 plants are suppressed upon high temperature (HT) treatment (Bilger *et al*. 1987, Raghavendra *et al*. 1995, Bukhov *et al*. 1998). However, no detailed insight into changes in the photosynthetic oscillations caused by HT stress has been done so far. Therefore, measurements of changes in the photosynthetic oscillations caused by HT could bring information as for the origin and control of the oscillations.

In this work we measured the Chl *a* fluorescence oscillations induced by 2 % CO_2 with HT-stressed barley leaves. From evaluation of other fluorescence parameters (F_V/F_M, q_P, and q_N) and measurement of the rate of CO_2 fixation, we were able to quantify changes in reactions occurring in thylakoid membrane and in Calvin cycle, respectively, caused by HT. To verify our suggestions based on the experiments, we developed a simple theoretical model for the photosynthetic oscillations that includes (almost) all suggested origins of the oscillations. By changing the parameters of the model with respect to the measured and in-literature reported results, we simulated oscillations in fluorescence signal and compared them with the experimentally measured oscillations. Further, we used an analogy with metabolic control analysis and calculated to what extent the parameters of the model control the parameters of simulated oscillations. We discussed our results with respect to action of HT on photosynthetic apparatus.

Materials and methods

Plants and HT incubation: Barley (*Hordeum vulgare* L. cv. Akcent) seedlings were cultivated at 25 °C in perlit substrate supplied with Knop solution. They were exposed to light–dark cycles (16 h "white light"/8 h dark) with the irradiance of about 90 μmol m^{-2} s^{-1} of PAR at the top of the seedlings. The experiments were performed with the first leaves of 14-d-old plants (growth phase 1.2 according to Zadoks *et al*. 1974) during dark phase of the growth cycle. Whole plants in a flowerpot were put into a temperature-controlled box where they were incubated in dark at given temperature and time interval.

Measurement of fluorescence signal, evaluation of fluorescence parameters, and initiation of the oscillations in fluorescence signal: After the incubation, a leaf was detached and fluorescence signal was immediately measured from a central part of the leaf in dark at room temperature and in ambient air using a *PAM 2000* fluorometer (*Walz*, Effeltrich, Germany). Weak modulated red radiation ($\lambda = 655$ nm) was used for the measurement of fluorescence signal. First, minimal fluorescence F_0 (dark-adapted state) was measured followed by the determination of maximal fluorescence F_M (dark-adapted

state) caused by application of saturating "white light" pulse of 10 000 μmol m^{-2} s^{-1} of PAR and duration of 0.8 s. 100 s after the application of the saturating pulse, when fluorescence signal dropped to the original F_0 level, continuous actinic red radiation (λ = 655 nm) of about 290 μmol(photon) m^{-2} s^{-1} was switched on. After seven min, when a steady state of fluorescence was reached, the saturation pulse was applied to measure the maximal fluorescence F_M' for the light-adapted state. Five seconds after the end of the saturating pulse, the "actinic light" was switched off and far-red radiation (λ = 735 nm) of 55 μmol(photon) m^{-2} s^{-1} was switched on for 5 s to determine minimal fluorescence F_0' for the light-adapted state. The value of fluorescence signal just before application of the saturating pulse to determine F_M' was considered as F_S in further calculations. The following fluorescence parameters were evaluated from the measured values: maximal quantum yield of photosystem 2 (PS2) photochemistry expressed as F_V/F_M (Kitajama and Butler 1975), steady state photochemical fluorescence quenching expressed as $q_P = (F_M' - F_S)/F_V'$ (Bilger and Schreiber 1986), and steady state non-photochemical fluorescence quenching expressed as $q_N = (F_V - F_V')/F_V$ (Bilger and Schreiber 1986), where variable fluorescence is expressed as $F_V = F_M - F_0$ and $F_V' = F_M' - F_0'$. For the latest review on the fluorescence quenching parameters see Roháček (2002). The irradiance was in all cases measured with the quantum radiometer *LI-189* (*LI-COR*, Lincoln, USA).

40 s after the application of the saturating pulse to determine F_M', the central part of the leaf was flushed by a gas containing 2 % of CO_2 (the rest was air) to initiate oscillations in fluorescence signal. The gas flow was provided by a tube (diameter of 3 mm) mounted directly on the light guide of the fluorometer ensuring the flushing to and fluorescence detection from the same part of the leaf. End of the light guide and of the tube was 3 mm above the leaf surface. For an example of typical measured curve see Fig. 1.

Measurement of CO_2 exchange rate: Immediately after the incubation of leaves for 39 min at different temperatures (room; 32, 35, 40, and 43 °C), gas exchange of leaves was measured after equilibration with ambient CO_2 concentration (250 μmol mol^{-1}) maintained by soda lime (*ADC BioScientific*, Hoddesdon, Hearts, UK) at room temperature (24.0±0.3 °C) using open gas exchange system *LCA-4* (*Analytical Development Co.*, Hoddesdon, UK). The water pressure deficit of the air being supplied to the chamber was lowered to 1.0±0.2 kPa by anhydrous calcium sulphate (*W.A. Hammond Drierite Co.*, Xenia, OH, USA). Central parts of seven leaves, which occupied whole area of the gasometric leaf chamber (6.25 cm^2) were used for the measurements. First, the increase in CO_2 concentration due to dark respiration (R) was measured for five min. Then, saturated continual actinic "white light" (276 μmol m^{-2} s^{-1} of PAR) very similar to that used for the fluorescence measurements was switched on and gas exchange was recorded at 15-s intervals for at least 10 min to determine RuBPCO activation. The steady state net photosynthetic rate (P_N) was detected after 25-min exposure to the "actinic light".

P_N was calculated using the equations of Caemmerer and Farquhar (1981) and gross photosynthetic rate (P_G) was calculated as a sum of R and P_N. The apparent rate constant of the RuBPCO activation (k_{act}) was determined from the RuBPCO phase (between the 1st and the 9th min of the increase in P_N) reflecting the activation of RuBPCO (Woodrow and Mott 1989, Mott and Woodrow 1993). The RuBPCO phase was separated from stomatal phase by normalising photosynthesis to intercellular concentration C_i of 250 μmol mol^{-1} by assuming a linear relationship between P_N and C_i (Woodrow and Mott 1989). The rate constant k_{act} was determined by plotting natural logarithm of difference between normalised photosynthesis and maximal normalised photosynthesis *versus* time (Woodrow and Mott 1989) where the slope of the plot gave k_{act}.

Theoretical simulations of the oscillations: Time course of reactants in the model was simulated using *Gepasi* software version *3.21* (P. Mendes, University of Wales, Aberystwyth, UK) that was designed for the simulation of chemical and biochemical kinetics (Mendes 1993). *Gepasi* uses a routine *LSODA* (Livermore Solver of Ordinary Differential Equations) for numerical solution of a set of differential equations. The initial amounts of particular reactants in the model and values of the rate constants of particular reactions were the entrance parameters for each simulation. See the Appendix for details on the used theoretical model.

Control analysis of the period and initial amplitude of the oscillations: We have used analogy with the metabolic control analysis (MCA) to find to what extent the period and initial amplitude of the oscillations are controlled by changes in the values of the rate constants and initial amount of reactants in the model. In MCA (for reviews see Fell 1992, Visser and Heijnen 2002), in addition to other parameters, the control coefficient is defined as a relative change in steady-state model variable X_j (usually concentration or flux) upon a relative change in steady-state of selected parameter of the model (usually concentration or activity or rate constant) p_i as:

$$C_{p_i}^{X_j} = \frac{\delta X_j / X_j}{\delta p_i / p_i} = \frac{\delta \ln X_j}{\delta \ln p_i} .$$

As oscillations do not represent steady state, we have used only analogy with MCA where X_j was the period or the initial amplitude of the oscillations and p_i was the value of the rate constant or the initial amount of reactants in the model. To calculate the control coefficient, the rate constant or initial amount of reactant was changed by both $+\delta p_i$ and $-\delta p_i$ around its reference value (as

listed in Table 2) and the oscillations were simulated from which the period or initial amplitude of the oscillations was determined (see below). The magnitude of δp_i change was ±2.5 and 5.0 % for all explored processes. Practically, the control coefficient represents a slope of a line fitted to points of the $\ln(X_j)$ *versus* $\ln(p_i)$ graph.

Recently, a new procedure for determining the control coefficients for theoretical oscillations based on analysis of the oscillations by Fourier transformation was introduced (Reijenga *et al*. 2002). Use of Fourier transformation for determination of particular periods contributing to the overall oscillations is powerful when many periods of the oscillations occur. This is, however, not the case of our oscillations because they are highly damped. Therefore, we determined the period of simulated oscillations using autocorrelation function. In this procedure, the values of an oscillating function for given time t are shifted to time $t+\tau$, where τ represents a time shift, and a correlation coefficient between the original and shifted oscillation functions is calculated. The shift is realised for many different τ values. Auto-correlation function is then a course of the correlation coefficient in dependence on the time shift. Therefore, the auto-correlation function calculates self-similarity of the original and shifted function and is maximal (*i.e*. equals 1) for zero time-shift. A non-zero time shift corresponding to the first maximum in the auto-correlation function represents the period of the oscillations.

The initial amplitude of simulated oscillations was determined as the size of the first maximum in the oscillations.

Results and discussion

Experiments: To avoid effects of the electron transport steps within thylakoid membrane and of enzyme activations (the so called photosynthetic induction) on the photosynthetic oscillations, the leaf was first irradiated in ambient atmosphere for 7 min to reach a steady state fluorescence signal. After such irradiation, activities of most of Calvin cycle enzymes [chloroplastic FBPase, NADP: glyceraldehyde-3-phosphate dehydrogenase (GAPDH), PGK, RPK, and RuBPCO] was already maximal (Leegood and Walker 1980). After reaching the steady state of fluorescence signal, the leaf was blown by an atmosphere with increased amount of CO_2 (2 %) that induced the oscillations in fluorescence signal. Such approach to initiate the oscillations was also used before (Ogawa 1982, Walker *et al*. 1983, Sivak and Walker 1986, Peterson *et al*. 1988, Laisk *et al*. 1991). An example of measurement of the oscillations in fluorescence signal using this measuring technique is presented for control leaf in Fig. 1, and the insert of Fig. 1 shows examples of the fluorescence oscillations measured after different HT treatments.

Fig. 2*A* shows in detail the dependence of period of oscillations on the time of incubation at different temperatures. The changes in period of the oscillation for given

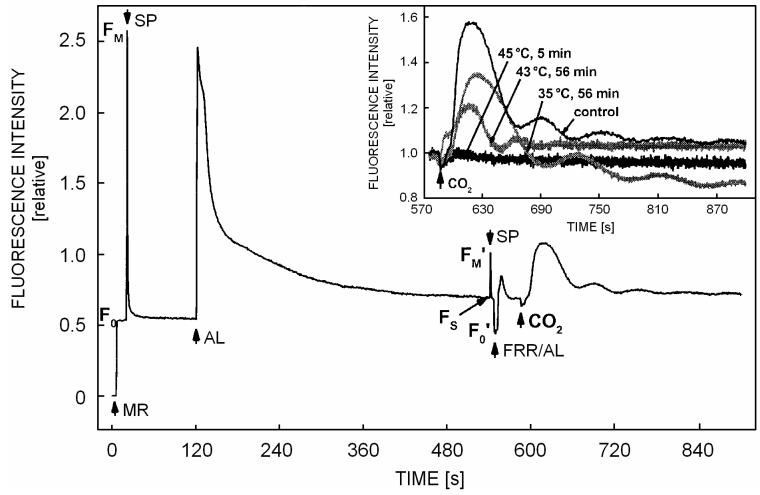

Fig. 1. Example of the routine for measuring basic fluorescence parameters and oscillations in fluorescence signal. Typical curve measured with control (*i.e*. no high-temperature treatment) barley leaf is shown. *The arrows* indicate application of measuring radiation (MR), "actinic light" (AL), far-red radiation (FRR), and saturating pulse (SP). AL was switched off during application of FRR. Each measurement started in ambient air concentration of CO_2 and when steady state fluorescence was reached, air with 2 % of CO_2 was blown to the leaf (at 580 s). F_0 and F_M indicate minimal and maximal, respectively, fluorescence for dark-adapted state, F_S indicates steady state fluorescence for light-adapted state, and F_0' and F_M' indicate minimal and maximal, respectively, fluorescence corresponding to the light-adapted state. *Insert* shows typical examples of oscillations obtained with control barley leaf (the same as in the main figure) and with barley leaves after various high temperature treatments (see denotation in the insert). The curves in the insert are normalised to F_S. See the Materials and methods for further details.

HT treatment were probably not significant because they occurred within ±SD range. Interestingly, periods of the oscillations for the leaves incubated at 35 °C were in general higher when compared to the control leaves. Moreover, further increase in the temperature of incubation resulted in a decrease of periods of the oscillations (Fig. 2*A*). Finally, at 45 °C no oscillations were detected (the insert in Fig. 1). The initial amplitude of the oscillations (*i.e*. the amplitude of the first maximum in the oscillations) decreased with increased temperature (see the insert to Fig. 1) and the incubation of the leaf for longer times in all given HTs also caused an increase of the damping of the oscillations (data not shown).

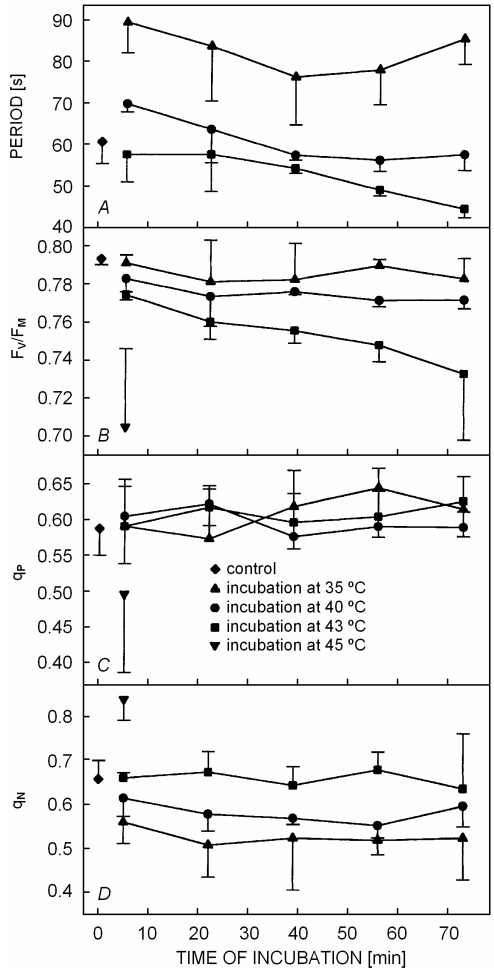

Fig. 2. Experimentally measured period of the oscillations in fluorescence signal (*A*), maximal quantum yield of PS2 photochemistry expressed by F_V/F_M (*B*), and photochemical and non-photochemical fluorescence quenching expressed by q_P (*C*) and q_N (*D*), respectively, in dependence on temperature and duration of high temperature treatment (see the legend inside *C* which is valid for all panels) of barley leaves. F_V/F_M, q_P, and q_N were measured in ambient air after the high temperature treatment and before the measurement of period of the oscillations (see Fig. 1) that were measured in air with 2 % of CO_2. *Vertical bars* represent SD calculated from 3–4 measurements. For further details see the Materials and methods.

To explore the origin of changes in period of the oscillations induced by HT, the basic fluorescence parameters (F_0, F_M, F_S, F_M', and F_0', see Fig. 1) were measured at room temperature and ambient atmosphere after the HT treatment. These parameters were used for calculations of F_V/F_M and steady state values of q_P and q_N (see the Materials and methods). The goal of evaluation of the F_V/F_M, q_P, and q_N was to obtain a description of photosynthetic function after the HT treatments just before initiation of the oscillations. For a given HT treatment, no significant changes of fluorescence parameters with increasing duration of incubation were found (Fig. 2*B,C,D*). Only the F_V/F_M slightly decreased with prolonged incubation at 43 °C (Fig. 2*B*, *squares*) but this decrease was less than 10 % and the final value of the F_V/F_M in this case was still high (about 0.73). Hence this HT treatment caused only a small alteration of PS2 function. HT treatment up to 43 °C had no effect on q_P (Fig. 2*C*) which reflects relative number of open (*i.e*. functional) PS2 centres upon irradiation (for review see Roháček 2002) and hence informs about the extent of NADPH formation. Therefore, because the F_V/F_M and q_P had generally different temperature behaviour than the period of the oscillations, it was unlikely that changes in the period of the oscillations up to 43 °C were caused by changes in the maximal quantum yield of PS2 photochemistry and by insufficiency of NADPH.

However, even 5-min treatment at 45 °C caused a decrease in q_P (Fig. 2*C*, *down triangle*). This decrease was also accompanied by a decrease of the F_V/F_M (Fig. 2*B*, *down triangle*), even if in this case F_V/F_M was still high. However, a decrease in q_P for leaves incubated for 5 min at 45 °C could be a reason for disappearance of the oscillation in this case (see the insert in Fig. 1) because a decrease in q_P reflects insufficiency of open (*i.e*. functional) PS2 centres upon irradiation, which can lead to insufficient formation of NADPH. Pastens and Horton (1996b) suppose a suppression of NADPH formation upon HT treatment *in vivo*. Therefore, we suggest that disappearance of the oscillations caused by HT treatment (45 °C) is mainly due to insufficiency of NADPH.

The changes in periods of oscillations caused by HT well correlated with changes in the steady state non-photochemical quenching of fluorescence expressed by q_N. The first increase (for 35 °C treatment) followed by a decrease (for 40 and 43 °C treatments) in period of the oscillations were exactly anti-parallel to changes in q_N caused by HT (compare Fig. 2*A* and 2*D*). Further, after 5-min incubation at 45 °C when the oscillations disappeared, q_N was about 0.84 (see Fig. 2*D*, *down triangles*). The correlation between changes in the period of oscillations and changes in q_N is presented for fixed duration of the HT treatment (39 min) in detail in Fig. 3*A* (relative values of the parameters with respect to the control are presented). No obvious relationship between changes in the period and in F_V/F_M or q_P was found (Fig. 3*B*) in this case. Qualitatively the same behaviour of the

fluorescence parameters and period of the oscillations were found for all measured durations of HT treatment (data not shown).

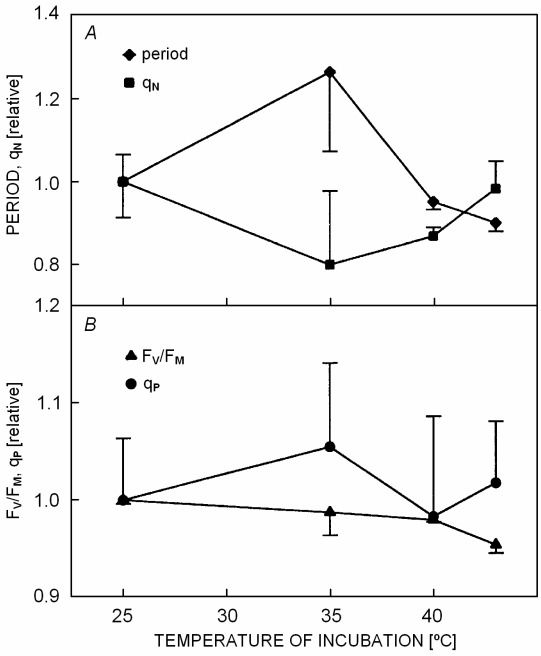

Fig. 3. Experimentally measured dependence of period of the oscillations and q_N (*A*) and F_V/F_M and q_P (*B*) on high temperature treatment that lasted 39 min. Normalised values from Fig. 2 were evaluated with respect to values at room temperature (25 °C).

The initial HT-induced decrease in q_N followed by its increase as shown in Fig. 3*A* was already reported (Weis and Berry 1988, Pastens and Horton 1996a). It is accompanied by anti-parallel changes in the rate of CO_2 fixation (Harley *et al*. 1985, Weis and Berry 1988, Lieth and Pasian 1990, Bunce 2000) leading to a correlation between HT-induced changes in q_N and the rate of CO_2 fixation (Law and Crafts-Brandner 1999). Therefore, we explored in another experiment if the correlation also existed in our case. We measured the steady state value of q_N and P_G with the leaves after their incubation for 39 min at different HTs and found (Fig. 4*A*) a correlation (R = –0.984) between q_N and P_G.

The HT-induced inhibition in the rate of CO_2 fixation correlates with RuBPCO activation (Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000). Therefore we evaluated an overall rate constant of RuBPCO activation (k_{act}) from an increase in the rate of CO_2 fixation in time (Woodrow and Mott 1992, Hammond *et al*. 1998a,b) measured at room temperature and ambient atmosphere immediately after incubation of the leaves for 39 min at different HT. We found a correlation (R = 0.953) between k_{act} and P_G (Fig. 4*B*). All these results confirmed a consequent correlation between period of the oscillations and q_N (Fig. 3*A*), between q_N and P_G (Fig. 4*A*), and between P_G and k_{act} (Fig. 4*B*). Therefore we suggest that the period of the oscillations upon HT stress up to 43 °C is mainly controlled by RuBPCO activation status.

The above measured changes in P_G and k_{act} induced by HT were not caused by stomata closure. The calculated values of C_i, stomatal conductance (g_S), and transpiration rate (E) all increased with HT treatment indicating stomata opening (data not shown).

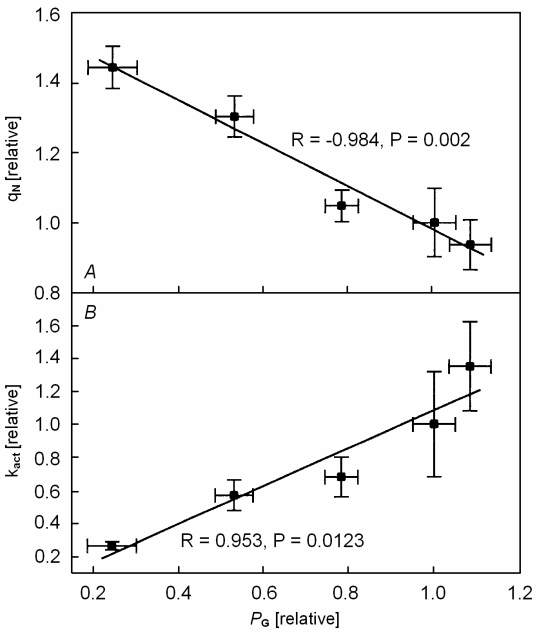

Fig. 4. Correlation between q_N and P_G (*A*) and between k_{act} and P_G (*B*). The data were obtained after incubation of barley leaves for 39 min at different high temperatures and represent normalised values with respect to values at room temperature (25 °C) whose co-ordinate is [1, 1]. *The bars* represent SD calculated from 3–4 measurements. R and P are Pearson correlation coefficient and the P-value of the correlation, respectively.

Our suggestion about the control of the period of the oscillations mainly by RuBPCO activation is a logical consequence and complementation of results presented in literature about action of HT on RuBPCO (for review see Salvucci and Crafts-Brandner 2004). Moderate HT stress causes *in vivo* a decrease in carboxylation activity of RuBPCO (Weis 1981a,b, Kobza and Edwards 1987). This inhibition of RuBPCO carboxylation activity by HT is not caused by increased affinity of RuBPCO to oxygen at HT because HT-induced decrease in carboxylation activity of RuBPCO was observed upon both aerobic and anaerobic conditions (Kobza and Edwards 1987). As the activity of RuBPCO *in vitro* increases up to about 60 °C (Crafts-Brandner and Salvucci 2000, Salvucci *et al*. 2001), the decreased RuBPCO activity *in vivo* reflects a decrease in numbers of active (catalytically competent) RuBPCO sites. An enzyme called RuBPCO activase

(RuBPCO-A) controls the decrease (for reviews see Portis 1992, Salvucci and Ogren 1996). Even if the activity of RuBPCO-A *in vitro* slightly increases up to about 42–45 °C (Crafts-Brandner *et al*. 1997, Crafts-Brandner and Salvucci 2000, Salvucci *et al*. 2001), it is inhibited by HT *in vivo* (Feller *et al*. 1998, Law and Crafts-Brandner 1999, Crafts-Brandner and Salvucci 2000, Salvucci *et al*. 2001). This is probably caused by perturbation of RuBPCO-A subunits' interactions (Crafts-Brandner and Law 2000) or by unfavourable ATP/ADP ratio (Crafts-Brandner and Salvucci 2000). All these effects on RuBPCO-A activity together with possible perturbation of interaction between RuBPCO and RuBPCO-A (Crafts-Brandner and Law 2000) can lead to inhibition of RuBPCO activity at HT *in vivo*. Also a HT-induced denaturation of RuBPCO-A (Salvucci *et al*. 2001) and/or association of RuBPCO-A with thylakoid membrane at HT (Rokka *et al*. 2001), both leading to a loss of RuBPCO-A, can result in inhibition of RuBPCO activity. Reduced thioredoxin (Trx, for reviews see Jacquot *et al*. 2000, Schürmann and Jacquot 2000) is also necessary for activation of RuBPCO-A (Zhang and Portis 1999, Motohashi *et al*. 2001, Ruuska *et al*. 2002, Zhang *et al*. 2002) and small subunit of RuBPCO (Motohashi *et al*. 2001). Therefore, a HT-induced inhibition of Trx reduction and/or a change in interaction between Trx and RuBPCO and/or RuBPCO-A could be also a reason for HT-induced decrease in RuBPCO activity *in vivo*. In summary, mainly under high concentration of CO_2 and HT, that are exactly the conditions used in this work, the activity of RuBPCO is major limitation of CO_2 fixation *in vivo* (Crafts-Brandner and Salvucci 2000, Jensen 2000, Salvucci and Crafts-Brandner 2004).

Theoretical simulations: Further, we used mathematical modelling to find if HT-induced changes in some parameters of a model can lead to changes in period of the oscillation as measured after the HT treatment. A usual tactic in modelling is to investigate a low-dimensional simplified model because complicated and large model increases dimensionality of the model that complicates the mathematical treatment (Kærn and Hunding 1999). The increased dimensionality can even lead to impossibility to reveal desired and expected property of the model as it was discussed when modelling the photosynthetic oscillations (Laisk *et al*. 1989). Therefore, the model used by us in this work is rather simple and contains only some aspects of photosynthesis.

The scheme of the model is shown in Fig. 7 in the Appendix where one can find also detailed description of the model and values of the rate constants and initial conditions used for simulations. Briefly, as experimental results suggest that period of the oscillations is controlled by RuBPCO activation status and because a decrease in RuBPCO activity *in vivo* with increasing temperature is well documented in literature (see above), the model was formulated to describe and stress a role of RuBPCO activation status in the photosynthetic oscillations. Therefore, the core of model is reactions occurring in the Calvin cycle, including also formation of NADPH and ATP. Except for the ´fructose 2,6-bisphosphate´ hypothesis, our model includes all hypotheses suggested so far for the origin of the photosynthetic oscillations (see the Introduction). Rejection of reactions employing the ´fructose 2,6-bisphosphate´ hypotheses from our model is based on the fact that the photosynthetic oscillations were also measured with intact chloroplasts (Veljović-Janović and Cerović 1991). As the model does not contain PS2 from which fluorescence signal is emitted (for reviews see Krause and Weis 1991, Dau 1994, Govindjee 1995, Lazár 1999), fluorescence was assumed to be proportional to the concentration of NADPH (see Rovers and Giersch 1995) for the case of simulations of the fluorescence photosynthetic oscillations (see also the Appendix). The basic simulation of the fluorescence oscillations is presented in Fig. 5*A*. The oscillations have a period of about 58 s, very similar as the oscillations measured at room temperature (about 60 s, see Figs. 1 and 2). Fig. 5*A* also shows the oscillations in the rate of CO_2 fixation (see the Appendix for definition) simulated on the basis of our model. These oscillations are anti-parallel to the oscillations in fluorescence signal and are somewhat out of phase as it was reported in the literature (see the Introduction).

Even if we suggest that the HT-induced changes in the period of the oscillations are mainly controlled by RuBPCO activation, it cannot be completely ruled out that also HT-induced changes in activities of other enzymes of Calvin cycles participate (but only in a minor way) in determination of P_G and consequently in a partial control of the period of the oscillations. For example, it was found that the activity of GAPDH, an enzyme which together with PGK catalyse reduction of 3-phosphoglycerate (PGA) to triosephosphate (TP) upon consumption of ATP and NADPH, increases with increasing temperature *in vivo* (up to 30–35 °C; Weis 1981b, Fridlyand *et al*. 1997). On the other hand, activity of the second enzyme in Calvin cycle that utilises ATP, RPK, is *in vivo* temperature independent (up to 38–45 °C; Weis 1981b, Kobza and Edwards 1987). Further, activity of chloroplastic FBPase only slightly decreases with increasing temperature *in vivo* (up to 38–45 °C; Weis 1981b, Kobza and Edwards 1987).

Therefore, on the basis of these facts and our results, we further explored the photosynthetic oscillations by simulations in which we changed values of some model parameters according to our results and literature. In such a way simulated oscillations are shown in Fig. 5*B–E*. Increase in RuBPCO activity (k_{cat}) as well as increase in RuBPCO-A activity (k_7) observed *in vitro* at HT (Crafts-Brandner and Salvucci 2000, Salvucci *et al*. 2001) both resulted in a decrease in period of the oscillations (Fig. 5*B*). The HT-induced increase in rate of PGA reduction (k_2) observed *in vivo* (Weis 1981b, Fridlyand *et al*.

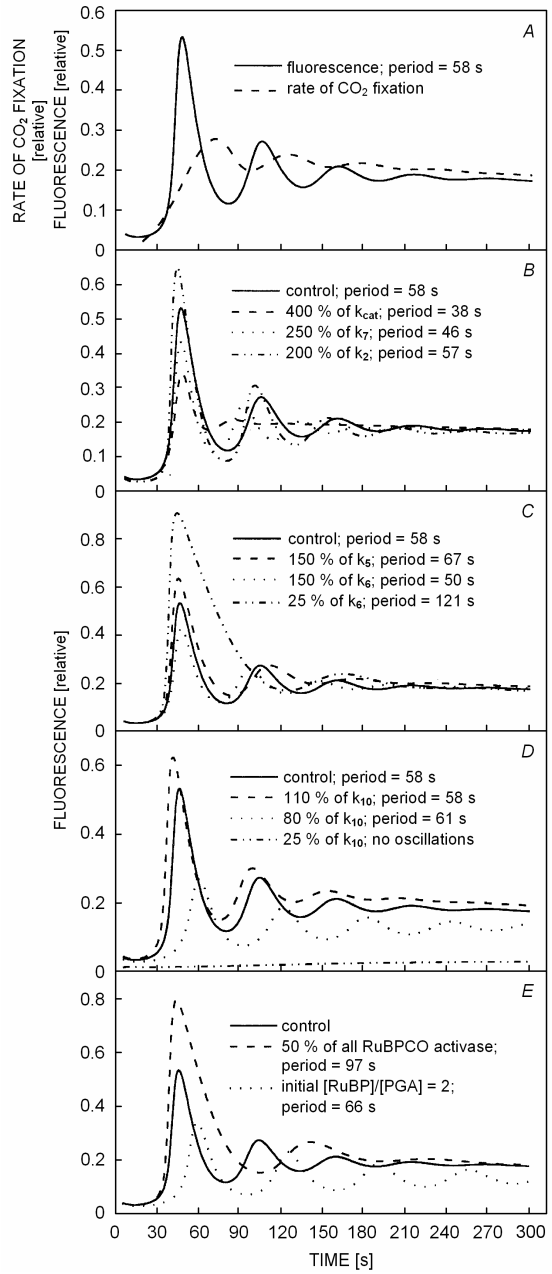

Fig. 5. Theoretically simulated oscillations in fluorescence signal (*A–E*) and in rate of CO_2 fixation (*A*) using our model (Fig. 7). (*A*): The simulations for room temperature (control in panels *B–E*) using values of the rate constants and initial conditions as listed in Table 2. (*B–D*): Oscillations simulated for various % changes in indicated rate constants (related to values listed in Table 2) keeping values of the rest of the rate constant and initial conditions as listed in Table 2. (*E*): The initial conditions of some forms in the model were changed in the simulations keeping the initial conditions of the rest of forms in the model and values of the rate constants as listed in Table 2: 50 % of all RuBPCO-A was simulated using $[\text{RuBPCO-A}_A]_0 = 0.25$ mM whereas initial [RuBP]/[PGA] = 2 used $[\text{RuBP}]_0 = 10$ mM and $[\text{PGA}]_0 = 5$ mM in the simulation. The % changes in the rate constants or changes in the initial conditions were taken according to our results or according to the references mentioned in the text.

1997) almost did not change period of the oscillations (Fig. 5*B*). Suggested faster rate of RuBPCO inactivation (k_5) to occur *in vivo* upon HT stress (Crafts-Brandner and Salvucci 2000) resulted in a small increase in period of the simulated oscillations (Fig. 5*C*). Changes in the rate of RuBPCO activation (k_6) as observed *in vivo* by us (Fig. 4*B*) and suggested to occur *in vivo* upon HT stress (Crafts-Brandner and Law 2000) strongly altered the period of oscillations (Fig. 5*C*). Changes (80–110 %) in the rate of NADPH formation (k_{10}) as deduced from changes in q_P observed *in vivo* only slightly affected period of the oscillations (Fig. 5*D*). However, even a small decrease in k_{10} (to 80 %) resulted in the lowest amplitude of the oscillations from all so far explored simulations (Fig. 5). If a further decrease in k_{10} was considered (to 25 %), simulated oscillations had very small amplitude which means that the oscillations disappeared (Fig. 5*D*) as observed in the experiments (insert to Fig. 1). Decrease in the amount of RuBPCO-A caused by HT *in vivo* (Rokka *et al*. 2001, Salvucci *et al*. 2001) led to a pronounced increase in period of the oscillations (Fig. 5*E*). Only a small increase in period of the oscillations was simulated (Fig. 5*E*) when initial amounts of RuBP and PGA were assumed as observed *in vivo* upon a HT treatment (Kobza and Edwards 1987, Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000).

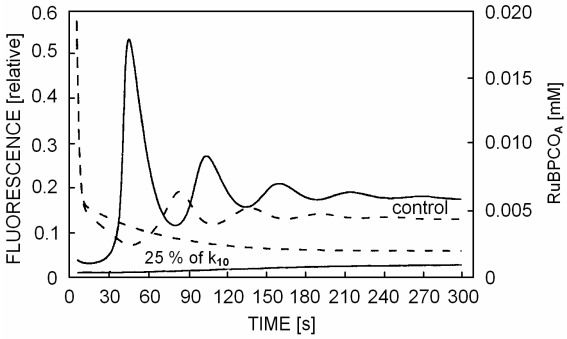

Fig. 6. Theoretical time courses of fluorescence signal (*solid lines*) and concentration of active RuBPCO (*dashed lines*) simulated for room temperature (control) and for 25 % of k_{10}. The fluorescence curves are those presented in Fig. 5.

To show that the RuBPCO activation status is related to the oscillations, we also simulated time course of active RuBPCO. For the case of simulation of the oscillations at room temperature (the control in Fig. 6), the concentration of active RuBPCO oscillated as expected. Moreover, maximal values in fluorescence signal (the control solid line) were accompanied by minimal contents in active RuBPCO (the control dashed line) and *vice versa*. Such behaviour is expected because when RuBPCO is active, Calvin cycle functions and absorbed photon energy is used for photochemistry and not for fluorescence emission, and *vice versa*. At simulation of fluorescence signal for 25 % of k_{10} where no oscillations appeared (the 25 % of k_{10} solid line in Fig. 6) there were,

as expected, no oscillations in content of active RuBPCO (the 25 % of k_{10} dashed line in Fig. 6).

Control analysis: Previous results showed changes in theoretically simulated oscillations caused by changes in the selected model parameters as they were reported in literature or implied from our results. To quantify to what extent all model parameters control the oscillations, we have used analogy with the metabolic control analysis (MCA) for further theoretical exploration of the oscillations. In MCA (for reviews see Fell 1992, Visser and Heijnen 2002), the control coefficient C, describing to what extent the particular model parameter (concentration or activity or rate constant) controls a selected variable of the model (concentration or flux), is defined. Positive C indicates that increase in the model parameter results in increase of the model variable, and on the other hand, negative C means that the increase in the model parameter results in decrease of the model variable. Higher absolute value of C indicates higher control of the model variable by the model parameter. The MCA was originally developed for analysis of the steady states and therefore we used only analogy with MCA to analyse the oscillations (see the Materials and methods for more details).

Table 1. Values of the control coefficients C by which particular initial concentration or rate constant controls the period of the initial amplitude of the oscillations. For more details on the evaluation of the control coefficients see the Materials and methods.

Initial amount	C for the period	for initial amplitude	Rate constant	C for the period	for initial amplitude
TP	0.029	-0.078	k_1	-0.311	-0.462
PGA	0.297	-0.682	k_2	-0.042	0.334
RuBP	0.294	-0.686	k_3	0.043	-0.159
RuP	0.025	-0.082	k_4	0.284	-0.732
Complex	-0.015	-0.090	k_5	0.302	0.480
$RuBPCO_A$	-0.361	-0.460	k_6	-0.424	-0.526
$RuBPCO_I$	-0.071	-0.091	k_7	-0.241	-0.140
$RuBPCO\text{-}A_A$	-0.523	-0.709	k_8	0.043	-0264
$RuBPCO\text{-}A_I$	-0.195	-0.124	k_9	-0.007	0.009
NADP	-0.124	1.920	k_{10}	-0.164	1.953
NADPH	-0.071	0.945	k_{11}	0.007	-0.046
ATP	0.025	-0.355	k_{12}	-0.011	0.043
ADP	-0.007	-0.414	k_{-12}	0.011	-0.051
P_i	0.029	-0.078	k_{cat}	-1.491	-0.374

The values of the control coefficients for both the period and initial amplitude of the oscillations calculated for all rate constants and initial concentrations of the reactants in the model are listed in Table 1. The highest control of the period of the oscillations is by the initial concentration of active RuBPCO-A, active RuBPCO, PGA, and RuBP. As the control coefficients of both active RuBPCO-A and active RuBPCO are negative, decrease in the amount of active RuBPCO-A *in vivo* caused by HT (Rokka *et al*. 2001, Salvucci *et al*. 2001) and consequent decrease in the amount of active RuBPCO would both lead to increase in period of the oscillations as observed for moderate HT treatment (see Figs. 2*A* and 3*A*). The same effect on the period of the oscillations would be caused also by increase (positive control coefficient) in the amount of RuBP found for HT treatment *in vivo* (Kobza and Edwards 1987, Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000). The decrease in the amount of PGA found for HT treatment *in vivo* (Kobza and Edwards 1987, Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000) would lead to a decrease (positive control coefficient) in the period of the oscillations as observed for HT treatment (see Figs. 2*A* and 3*A*).

The highest control of the period of the oscillations by rate constants is exerted by the rate constant of intrinsic carboxylation of RuBP to PGA by RuBPCO (k_{cat}), RuBPCO activation by RuBPCO-A (k_6), binding of RuBP and CO_2 to RuBPCO to form catalytic complex (k_1), RuBPCO inactivation (k_5), restoration of RuBP from RuP (k_4), and RuBPCO-A activation (k_7) (see scheme of the model in the Appendix). As the control coefficients of both k_{cat} and k_7 are negative, increase in k_{cat} and k_7 observed after HT treatment *in vitro* (Crafts-Brandner and Salvucci 2000, Salvucci *et al*. 2001) would both lead to decrease in period of the oscillations as observed for the HT treatment (see Figs. 2*A* and 3*A*). Both the *in vivo* effects occurring upon HT stress, *i.e*. increase in k_5 (positive control coefficient – Crafts-Brandner and Salvucci 2000) and decrease in k_6 (negative control coefficient – Crafts-Brandner and Law 2000, see our Fig. 4*B*) might lead to increase in period of the oscillations as observed for moderate HT treatment (see Fig. 2*A*). As the value of k_4 *in vivo* was temperature independent (up to 38–45 °C; Weis 1981b, Kobza and Edwards 1987) and as there are no data in literature about HT-induced changes of k_1, we could only speculate about their effects on period of the oscillations.

As seen from the insert to Fig. 1, the initial amplitude of the oscillations (*i.e*. the amplitude of the first maximum in the oscillations) decreases with increasing HT stress and after even 5-min incubation at 45 °C the oscillations disappear (*i.e*. they have zero initial amplitude). Therefore, evaluation of the control coefficients of the initial amplitude of the oscillations calculated for all rate constants and initial concentrations of the reactants in the model can be also helpful for better understanding of changes in the oscillations caused by HT. Table 1 shows that the highest control of the initial amplitude of the oscillations is by the initial concentration of NADP and NADPH and by the rate constant of NADPH formation from NADP (k_{10}). As all three above mentioned control coefficients are positive, a suppression of NADPH formation upon HT treatment *in vivo* as deduced from changes in q_P observed *in vivo* by us (Fig. 2*C*) and also suggested elsewhere (Pastens and Horton 1996*b*) would result in disappearance of the oscillations. This is in line with disappearance of the simulated oscillations for higher decrease in k_{10} (Figs. 5*D* and 6). Decrease of the initial amplitude of the oscillations would be also caused by increase (negative control coefficient) in the initial concentration of RuBP found for HT treatment *in vivo* (Kobza and Edwards 1987, Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000).

However, the initial amplitude of the oscillations can also increase with increasing HT treatment. The increase can be caused by a decrease in the initial concentration of PGA and RuBPCO-A (both have negative control coefficients) which was both found for HT treatment *in vivo* (Kobza and Edwards 1987, Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000, Rokka *et al*. 2001, Salvucci *et al*. 2001). Also an increase in k_5 (positive control coefficient) suggested to occur *in vivo* upon HT stress (Crafts-Brandner and Salvucci 2000) as well as decrease in k_6 (negative control coefficient) suggested to occur *in vivo* upon HT stress (Crafts-Brandner and Law 2000) and measured by us (Fig. 4*B*) would both lead to an increase in the initial amplitude of the oscillations. Because of the reasons mentioned above when describing control of period of the oscillations by k_1 and k_4, we could only speculate also here about effect of k_1 and k_4 on the initial amplitude of the oscillations.

Theoretical simulations of the oscillations and calculation of the control coefficients presented in Fig. 5 and Table 1, respectively, showed that period and the initial amplitude of the oscillations are affected not only by one factor (rate constant or initial amount) but all studied factors somehow affect parameters of the oscillations. This is in agreement with Ferimazova *et al*. (2002) who applied Fourier transformation for analysis of the fluorescence oscillations. They found that the measured oscillations consist from several partial oscillations characterised by different frequencies indicating contribution of different processes. Also in the case of often-explored phenomenon of yeast glycolytic oscillations, the control of their parameters was shared by all parameters of the model (Teusink *et al*. 1996, Reijenga *et al*. 2002). Therefore, it is impossible to strictly say which only one event is the origin of the oscillations. However, in the framework of our theoretical model, the most pronounced changes in the period and amplitude of the oscillations were associated with reactions dealing with RuBPCO activation status and NADPH formation, respectively.

The control of period of the oscillations upon HT treatment mainly by RuBPCO activation status is due to a property of RuBPCO that it can undergo reversible inactivation. Enzymes with this property are sometimes called ´hysteretic´ or ´mnemonical´ enzymes and are common in regulatory enzymes (Roussel 1998). A key role of RuBPCO activation upon HT treatment is well known for the steady state rate of CO_2 fixation (see above). Moreover, the activation status of RuBPCO also determines the rate of CO_2 fixation under saturating concentration of CO_2 (Crafts-Brandner and Salvucci 2000) that is in contrast to generally accepted view that the rate of CO_2 fixation under saturating concentration of CO_2 is determined by phosphate turnover (Caemmerer 2000). As the original model by Farquhar *et al*. (1980) and its derivatives that are now used for predictions of the rate of photosynthesis assume fully activated RuBPCO, a significant error can occur in the predictions (Bernacchi *et al*. 2001, Leuning 2002, Medlyn *et al*. 2002). Therefore, when using models to predict the rate of photosynthesis, at least upon HT and/or high CO_2 concentration, one must use corrections for the RuBPCO activation status (for review see Salvucci and Crafts-Brandner 2004).

References

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis, A.R., Jr., Long, S.P.: Improved temperature response functions for models of Rubisco-limited photosynthesis. – Plant Cell Environ. **24**: 253-259, 2001.

Bilger, W., Schreiber, U.: Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. – Photosynth. Res. **10**: 303-308, 1986.

Bilger, W., Schreiber, U., Lange, O.L.: Chlorophyll fluorescence as an indicator of heat induced limitation of photosynthesis in *Arbutus unedo* L. – In: Tenhunen, J.D., Catarino, F.M., Lange, O.L., Oechel, W.C. (ed.): Plant Response to Stress. Pp. 391-399. Springer-Verlag, Berlin – Heidelberg – New York – London – Paris – Tokyo 1987.

Bukhov, N.G., Boucher, N., Carpentier, R.: Loss of the precise control of photosynthesis and increased yield of non-radiative dissipation of excitation energy after mild heat treatment of barley leaves. – Physiol. Plant. **104**: 563-570, 1998.

Bunce, J.A.: Acclimation of photosynthesis to temperature in eight cool and warm climate herbaceous C_3 species: Temperature dependence of parameters of a biochemical photosynthesis model. – Photosynth. Res. **63**: 59-67, 2000.

Buschmann, P., Gradmann, D.: Minimal model for oscillations

of membrane voltage in plant cells. – J. theor. Biol. **188**: 323-332, 1997.

Caemmerer, S. von: Biochemical Models of Leaf Photosynthesis. – CSIRO Publishing, Collingwood 2000.

Caemmerer, S. von, Evans, J.R., Hudson, G.S., Andrews, T.J.: The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. – Planta **195**: 88-97, 1994.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. – Planta **153**: 376-387, 1981.

Crafts-Brandner, S.J., Law, R.D.: Effect of heat stress on the inhibition and recovery of the ribulose-1,5-bisphosphate carboxylase/oxygenase activation state. – Planta **212**: 67-74, 2000.

Crafts-Brandner, S.J., Salvucci, M.E.: Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. – Proc. nat. Acad. Sci. USA **97**: 13430-13435, 2000.

Crafts-Brandner, S.J., van de Loo, F.J., Salvucci, M.E.: The two forms of ribulose-1,5-bisphosphate carboxylase/oxygenase activase differ in sensitivity to elevated temperature. – Plant Physiol. **114**: 439-444, 1997.

Dau, H.: Molecular mechanisms and quantitative models of variable photosystem II fluorescence. – Photochem. Photobiol. **60**: 1-23, 1994.

Delieu, T.J., Walker, D.A.: Simultaneous measurement of oxygen evolution and chlorophyll fluorescence from leaf pieces. – Plant Physiol. **73**: 534-541, 1983.

Farquhar, G.D., Caemmerer, S. von, Berry, J.A.: A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. – Planta **149**: 78-90, 1980.

Fell, D.A.: Metabolic control analysis: a survey of its theoretical and experimental development. – Biochem. J. **286**: 313-330, 1992.

Feller, U., Crafts-Brandner, S.J., Salvucci, M.E.: Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. – Plant Physiol. **116**: 539-546, 1998.

Ferimazova, N., Küpper, H., Nedbal, L., Trtílek, M.: New insights into photosynthetic oscillations revealed by two-dimensional microscopic measurements of chlorophyll fluorescence kinetics in intact leaves and isolated protoplasts. – Photochem. Photobiol. **76**: 501-508, 2002.

Fridlyand, L.E., Backhausen, J.E., Holtgrefe, S., Kitzmann, C., Scheibe, R.: Quantitative evaluation of the rate of 3-phosphoglycerate reduction in chloroplasts. – Plant Cell Physiol. **38**: 1177-1186, 1997.

Giersch, C.: Oscillatory response of photosynthesis in leaves to environmental perturbations: A mathematical model. – Arch. Biochem. Biophys. **245**: 263-270, 1986.

Giersch, C.: Photosynthetic oscillations: Observations and models. – Comments theor. Biol. **3**: 339-364, 1994.

Giersch, C., Sivak, M.N.: A mathematical skeleton model of photosynthetic oscillations. – Proc. roy. Soc. London B **245**: 77-83, 1991.

Govindjee: Sixty-three years since Kautsky: Chlorophyll *a* fluorescence. – Aust. J. Plant Physiol. **22**: 131-160, 1995.

Gradmann, D: Models for oscillations in plants. – Aust. J. Plant Physiol. **28**: 577-590, 2001.

Hahn, B.D.: A mathematical model of the Calvin cycle: analysis of the steady state. – Ann. Bot. **57**: 639-653, 1986.

Hammond, E.T., Andrews, TJ., Mott, K.A., Woodrow, I.E.: Regulation of Rubisco activation in antisense plants of tobacco containing reduced levels of Rubisco activase. – Plant J. **14**: 101-110, 1998a.

Hammond, E.T., Andrews, TJ., Woodrow, I.E.: Regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase by carbamylation and 2-carboxyarabinitol 1-phosphate in tobacco: Insights from studies of antisense plants containing reduced amounts of Rubisco activase. – Plant Physiol. **118**: 1463-1471, 1998b.

Harley, P.S., Weber, J.A., Gates, D.M.: Interactive effects of light, leaf temperature, CO_2 and O_2 on photosynthesis in soybean. – Planta **165**: 249-263, 1985.

Horton, P., Nicholson, H.: Generation of oscillatory behavior in the Laisk model of photosynthetic carbon assimilation. – Photosynth. Res. **12**: 129-143, 1987.

Jacquot, J.-P., Lancelin, J.-M., Meyer, Y.: Thioredoxins: structure and function in plant cells. – New Phytol. **136**: 543-570, 2000.

Jensen, R.G.: Activation of Rubisco regulates photosynthesis at high temperature and CO_2. – Proc. nat. Acad. Sci. USA **97**: 12937-12938, 2000.

Kærn, M., Hunding, A.: The effect of slow allosteric transitions in a coupled biochemical oscillator model. – J. theor. Biol. **198**: 269-281, 1999.

Karavaev, V.A., Kukushkin, A.K.: A theoretical model of light and dark processes of photosynthesis: the problem of regulation. – Biophysics **38**: 958-975, 1993.

Keiller, D.R., Walker, D.A.: The use of chlorophyll fluorescence to predict CO_2 fixation during photosynthetic oscillations. – Proc. roy. Soc. London B **241**: 59-64, 1990.

Khuznetsova, S.A., Kukushkin, A.K.: A new theoretical approach to the study of regulatory links in photosynthesis. – Biophysics **44**: 448-454, 1999.

Kitajama, M., Butler, W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – Biochim. biophys. Acta **376**: 105-115, 1975.

Kobza, J., Edwards, G.E.: Influences of leaf temperature on photosynthetic carbon metabolism in wheat. – Plant Physiol. **83**: 69-74, 1987.

Kocks, P., Ross, J.: Kinetic model for (damped) oscillations of transthylakoid pH in plants. – J. phys. Chem. **99**: 16490-16497, 1995.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Kukushkin, A.K.: The influence of cyclic electron transport around photosystem II on the dampening oscillations in photosynthesis. – Biophysics **42**: 1224-1234, 1997.

Laisk, A., Eichelmann, H.: Towards understanding oscillations: a mathematical model of the biochemistry of photosynthesis. – Phil. Trans. roy. Soc. London B **323**: 369-384, 1989.

Laisk, A., Eichelmann, H., Oja, V., Eatherall, A., Walker, D.A.: A mathematical model of the carbon metabolism in photosynthesis. Difficulties in explaining oscillations by fructose 2,6-bisphosphate regulation. – Proc. roy. Soc. London B **237**: 389-415, 1989.

Laisk, A., Siebke, K., Gerst, U., Eichelmann, H., Oja, V., Heber, U.: Oscillations in photosynthesis are initiated and supported by imbalances in the supply of ATP and NADPH to the Calvin cycle. – Planta **185**: 554-562, 1991.

Laisk, A., Walker, D.A.: Control of phosphate turnover as a rate-limiting factor and possible cause of oscillations in photosynthesis: a mathematical model. – Proc. roy. Soc. London

B **227**: 281-302, 1986.
Laisk, A., Walker, D.A.: A mathematical model of electron transport. Thermodynamic necessity for photosystem II regulation: 'light stomata'. – Proc. roy. Soc. London B **237**: 417-444, 1989.
Law, R.D., Crafts-Brandner, S.J.: Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. – Plant Physiol. **120**: 173-181, 1999.
Lazár, D.: Chlorophyll *a* fluorescence induction. – Biochim. biophys. Acta **1412**: 1-28, 1999.
Leegood, R.C., Walker, D.A.: Autocatalysis and light activation of enzymes in relation to photosynthetic induction in wheat chloroplasts. – Arch. Biochem. Biophys. **200**: 575-582, 1980.
Leuning, R.: Temperature dependence of two parameters in a photosynthesis model. – Plant Cell Environ. **25**: 1205-1210, 2002.
Lieth, J.H., Pasian, C.C.: A model for net photosynthesis of rose leaves as a function of photosynthetically active radiation, leaf temperature, and leaf age. – J. amer. Soc. hort. Sci. **115**: 486-491, 1990.
Malkin, S.: Fast photoacoustic transients from dark-adapted intact leaves: oxygen evolution and uptake pulses during photosynthetic induction – a phenomenology record. – Planta **171**: 65-72, 1987.
Mate, C.J., Caemmerer, S. von, Evans, J.R., Hudson, G.S., Andrews, T.J.: The relationship between CO_2-assimilation rate, Rubisco carbamylation and Rubisco activase content in activase-deficient transgenic tobacco suggests a simple model of activase action. – Planta **198**: 604-613, 1996.
Medlyn, B.E., Dreyer, E., Ellsworth, D., Forstreuter, M., Harley, P.C., Kirschbaum, M.U.F., Le Roux, X., Montpied, P., Strassemeyer, J., Walcroft, A., Wang, K., Loustau, D.: Temperature response of parameters of biochemically based model of photosynthesis. II. A review of experimental data. – Plant Cell Environ. **25**: 1167-1179, 2002.
Mendes, P.: GEPASI: a software package for modelling the dynamics, steady states and control of biochemical and other systems. – Comput. appl. Biosci. **9**: 563-571, 1993.
Motohashi, K., Kondoh, A., Stumpp, M.T., Hisabori, T.: Comprehensive survey of proteins targeted by chloroplast thioredoxin. – Proc. nat. Acad. Sci. USA **98**: 11224-11229, 2001.
Mott, K.A., Woodrow, I.E.: Effects of O_2 and CO_2 on non-steady-state photosynthesis. Further evidence for ribulose-1,5-bisphosphate carboxylase/oxygenase limitation. – Plant Physiol. **102**: 859-866, 1993.
Ogawa, T.: Simple oscillations in photosynthesis of higher plants. – Biochim. biophys. Acta **681**: 103-109, 1982.
Pastens, C., Horton, P.: Effect of high temperature on photosynthesis in beans. II. Oxygen evolution and chlorophyll fluorescence. – Plant Physiol. **112**: 1245-1251, 1996a.
Pastens, C., Horton, P.: Effect of high temperature on photosynthesis in beans. II. CO_2 assimilation and metabolite contents. – Plant Physiol. **112**: 1253-1260, 1996b.
Peterson, R.B., Sivak, M.N., Walker, D.A.: Carbon dioxide-induced oscillations in fluorescence and photosynthesis. Role of thylakoid membrane energization in regulation of photosystem II activity. – Plant Physiol. **88**: 1125-1130, 1988.
Portis, A.R., Jr.: Regulation of ribulose 1,5-bisphosphate carboxylase/oxygenase activity. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 415-437, 1992.
Quick, W.P., Horton, P.: Studies on the induction of chlorophyll fluorescence in barley protoplasts. I. Factor affecting the observation of the oscillations in the yield of chlorophyll fluorescence and the rate of oxygen evolution. – Proc. roy. Soc. London B **220**: 361-370, 1984.
Quick, W.P., Horton, P.: Studies on the induction of chlorophyll fluorescence in barley protoplasts. III. Correlation between changes in the level of glycerate 3-phosphate and the pattern of fluorescence quenching. – Biochim. biophys. Acta **849**: 1-6, 1986.
Raghavendra, A.S., Gerst, U., Heber, U.: Oscillations in photosynthetic carbon assimilation and chlorophyll fluorescence are different in *Amaranthus caudatus*, a C_4 plant, and *Spinacia oleracea*, a C_3 plant. – Planta **195**: 471-477, 1995.
Reijenga, K.A., Westerhoff, H.V., Kholodenko, B.N., Snoep, J.L.: Control analysis for autonomously oscillating biochemical networks. – Biophys. J. **82**: 99-108, 2002.
Roháček, K.: Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. – Photosynthetica **40**: 13-29, 2002.
Rokka, A., Zhang, L., Aro, E.-M.: Rubisco activase: an enzyme with a temperature-dependent dual function? – Plant J. **25**: 463-471, 2001.
Roussel, M.: Slowly reverting enzyme inactivation: a mechanism for generating long-lived damped oscillations. – J. theor. Biol. **19**: 233-244, 1998.
Rovers, W., Giersch, C.: Photosynthetic oscillations and the interdependence of photophosphorylation and electron transport as studied by a mathematical model. – BioSystems **35**: 63-73, 1995.
Ruuska, S.A., Andrews, T.J., Badger, M.R., Price, G.D., Caemmerer, S. von: The role of chloroplast electron transport and metabolites in modulating Rubisco activity in tobacco. Insights from transgenic plants with reduced amounts of cytochrome *b/f* complex or glyceraldehyde 3-phosphate dehydrogenase. – Plant Physiol. **122**: 491-504, 2002.
Ryde-Pettersson, U.: Identification of possible two-reactant sources of oscillations in the Calvin photosynthesis cycle and ancillary pathways. – Eur. J. Biochem. **198**: 613-619, 1991.
Salvucci, M.E., Crafts-Brandner, S.J.: Inhibition of photosynthesis by heat stress: the activation of Rubisco as a limiting factor in photosynthesis. – Physiol. Plant. **120**: 179-186, 2004.
Salvucci, M.E., Ogren, W.L.: The mechanism of Rubisco activase: Insights from studies of the properties and structure of the enzyme. – Photosynth. Res. **47**: 1-11, 1996.
Salvucci, M.E., Osteryoung, K.W., Crafts-Brandner, S.J., Vierling, E.: Exceptional sensitivity of Rubisco activase to thermal denaturation in vitro and in vivo. – Plant Physiol. **127**: 1053-1064, 2001.
Schürmann, P., Jacquot, J.-P.: Plant thioredoxin systems revisited. – Annu. Rev. Plant Physiol. Plant mol. Biol. **51**: 371-400, 2000.
Seaton, G.G.R., Walker, D.A.: Chlorophyll fluorescence as a measure of photosynthetic carbon fixation. – Proc. roy. Soc. London B **242**: 29-35, 1990.
Sivak, M.N., Walker, D.A.: Chlorophyll *a* fluorescence: can it shed light on fundamental questions in photosynthetic carbon dioxide fixation? – Plant Cell Environ. **8**: 439-448, 1985.
Sivak, M.N., Walker, D.A.: Photosynthesis *in vivo* can be limited by phosphate supply. – New Phytol. **102**: 499-512, 1986.
Sivak, M.N., Walker, D.A.: Oscillations and other symptoms of limitation of *in vivo* photosynthesis by inadequate phosphate supply to the chloroplast. – Plant Physiol. Biochem. **25**: 635-648, 1987.
Spreitzer, R.J., Salvucci, M.E.: Rubisco: Structure, regulatory

interactions, and possibilities for a better enzyme. – Annu. Rev. Plant Biol. **53**: 449-475, 2002.

Stitt, M.., Grosse, H., Woo, K.-C.: Interactions between sucrose synthesis and CO_2 fixation. II. Alterations of fructose 2,6-bisphosphate during photosynthetic oscillations. – J. Plant Physiol. **133**: 138-143, 1988.

Stitt, M., Quick, W.P., Schurr, U., Schulze, E.-D., Rodermel, S.R., Bogorad, L.: Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' *rbcS*. II. Flux-control coefficients for photosynthesis in varying light, CO_2, and air humidity. – Planta **183**: 555-566, 1991.

Stitt, M., Schreiber, U.: Interaction between sucrose synthesis and CO_2 fixation. III. Response of biphasic induction kinetics and oscillations to manipulation of the relation between electron transport, Calvin cycle, and sucrose synthesis. – J. Plant Physiol. **133**: 263-271, 1988.

Teusink, B., Bakker, B.M., Westerhoff, H.V.: Control of frequency and amplitudes is shared by all enzymes in three models of yeast glycolytic oscillations. – Biochim. biophys. Acta **1275**: 204-21, 1996.

Veljović-Jovanović, S., Cerović, Z.G.: Induction of oscillations in chlorophyll fluorescence by re-illumination of intact isolated pea chloroplasts. – Planta **185**: 39-400, 1991.

Viil, J.: Carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP): a model on the level of the partial reactions. – In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. V. Pp. 215-218. Kluwer Academic Publ., Dordrecht – Boston – London 1995.

Viil, J., Ivanova, H., Pärnik, T.: Estimation of rate constants of the partial reactions of carboxylation of ribulose-1,5-bisphosphate *in vivo*. – Photosynth. Res. **60**: 247-256, 1999.

Visser, D., Heijnen, J.: The mathematics of metabolic control analysis revisited. – Metab. Eng. **4**: 114-123, 2002.

Walker, D.A.: Concerning oscillations. – Photosynth. Res. **34**: 387-395, 1992.

Walker, D.A., Sivak, M.N.: Can phosphate limit photosynthetic carbon assimilation *in vivo*? – Physiol. vég. **23**: 829-841, 1985.

Walker, D.A., Sivak, M.N.: Photosynthesis and phosphate: a cellular affair? – Trends biochem. Sci. **11**: 176-179, 1986.

Walker, D.A., Sivak, M.N., Prinsley, R.T., Cheesbrough, J.K.: Simultaneous measurement of oscillations in oxygen evolution in chlorophyll *a* fluorescence in leaf pieces. – Plant Physiol. **73**: 542-549, 1983.

Weis, E.: Reversible heat-inactivation of the Calvin cycle: a possible mechanism of the temperature regulation of photosynthesis. – Planta **151**: 33-39, 1981a.

Weis, E.: The temperature sensitivity of dark-inactivation and light-activation of the ribulose-1,5-bisphosphate carboxylase in spinach chloroplasts. – FEBS Lett. **129**: 197-200, 1981b.

Weis, E., Berry, J.A.: Plants and high temperature stress. – In: Long, S.P., Woodward, F.I. (ed.): Plants and Temperature. Pp. 329-346. Company of Biologists, Cambridge 1988.

Woodrow, I.E., Mott, K.A.: Rate limitation of non-steady-state photosynthesis by ribulose-1,5-bisphosphate carboxylase in spinach. – Aust. J. Plant Physiol. **16**: 487-500, 1989.

Woodrow, I.E., Mott, K.A.: Biphasic activation of ribulose bisphosphate carboxylase in spinach leaves as determined from nonsteady-state CO_2 exchange. – Plant Physiol. **99**: 298-303, 1992.

Woodrow, I.E., Mott, K.A.: Modelling C_3 photosynthesis: A sensitivity analysis of the photosynthetic carbon-reduction cycle. – Planta **191**: 421-432, 1993.

Yokota, A., Wadano, A., Murayama, H.: Modelling of continuously and directly analyzed biphasic reaction courses of ribulose-1,5-bisphosphate carboxylase/oxygenase. – J. Biochem. (Tokyo) **119**: 487-499, 1996.

Zadoks, J.C., Chang, T.T., Konzak, C.F.: A decimal code for the growth stages of cereals. – Weed Res. **14**: 415-421, 1974.

Zhang, N., Kallis, R.P., Ewy, R.G., Portis, A.R.: Light modulation of Rubisco in *Arabidopsis* requires a capacity of redox regulation of the larger Rubisco activase isoform. – Proc. nat. Acad. Sci. USA **99**: 3330-3334, 2002.

Zhang, N., Portis, A.R.: Mechanism of light regulation of Rubisco: A specific role for the larger Rubisco activase isoform involving reductive activation by thioredoxin-f. – Proc. nat. Acad. Sci. USA **96**: 9438-9443, 1999.

Appendix

A scheme of the model used for simulations of the photosynthetic oscillations is shown in Fig. 7. Even if the model is rather simple, it includes all necessary aspects of photosynthetic machinery that is, according to current knowledge (see the Introduction), probably involved in the photosynthetic oscillations. The model describes the three parts of Calvin cycle, *i.e.* ribulose-1,5-bisphosphate (RuBP) carboxylation to 3-phosphoglycerate (PGA) catalysed by active ribulose-1,5-bisphosphate carboxylase/oxygenase ($RuBPCO_A$, rate constant k_1 – binding of RuBP and CO_2 to $RuBPCO_A$ to form catalytic complex, and k_{cat} – intrinsic carboxylation catalysed by $RuBPCO_A$), PGA reduction to triosephosphate (TP, k_2), and RuBP regeneration [k_3 – overall formation of ribulose-5-phosphate (RuP) from TPs and k_4 – restoration of RuBP from RuP].

Reactions connected to activation status of RuBPCO are described in detail in the model by means of RuBPCO inactivation from $RuBPCO_A$ to inactive RuBPCO ($RuBPCO_I$, k_5), activation of $RuBPCO_I$ by active RuBPCO activase ($RuBPCO\text{-}A_A$) resulting in inactive $RuBPCO\text{-}A_I$ (k_6), and activation of $RuBPCO\text{-}A_I$ to $RuBPCO\text{-}A_A$ by adenosine 5'-triphosphate (ATP, k_7).

Formation of ATP from adenosine 5'-diphosphate (ADP) and orthophosphate (P_i) is subject of control by reduced nicotinamide adenine dinucleotide phosphate (NADPH), *i.e.* velocity v of ATP formation is expressed as $v = k_8$ [ADP](t) [P_i](t) [NADPH](t), where NADPH is a modifier of velocity of the reaction and concentration of NADPH is not changed by this reaction. Use of NADPH to control the rate of ATP formation by ATP synthase in thylakoid membrane reflects assumption that ATP formation requires proton accumulation in thylakoid lumen due to electron transport steps within the thylakoid membrane. Therefore, the higher number of electrons is transported from PS2 to nicotinamide adenine dinucleotide phosphate (NADP) to form NADPH (see below), the higher amount of protons accumulates in lumen

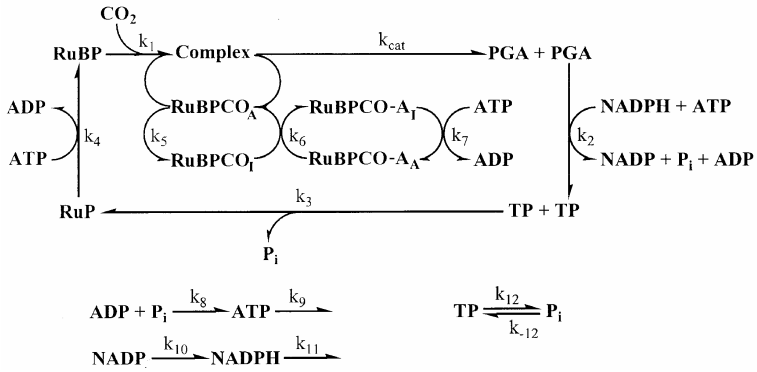

Fig. 7. Scheme of the model used for simulations of the photosynthetic oscillations. For meaning of reactants in the model, particular reactions, and rate constants see the Appendix. For the initial amount of the reactants in the model and values of the rate constants used for simulations of the oscillation at room temperature (denoted as control in Figs. 5 and 6) see Table 2.

Table 2. Values of rate constants and initial amounts of reactants in the model used for simulation of the oscillations at room temperature (denoted as control in Figs. 5 and 6). Dimension of the rate constant: [A]mM^{-2} s^{-1}; [B]s^{-1}; [C]mM^{-1} s^{-1}. The values of rate constants and amounts of reactants were reported in: [D]Caemmerer *et al*. (1994), Mate *et al*. (1996), Viil *et al*. (1999); [E]Yokota *et al*. (1996), Roussel (1998); [F]Mott *et al*. (1993), Woodrow and Mott (1992), Yokota *et al*. (1996), Hammond *et al*. (1998a,b); [G]Woodrow and Mott (1992); [H]Giersch (1986), Woodrow and Mott (1993); [I]Giersch and Sivak (1991), Woodrow and Mott (1993); [J]3–5 mM total RuBPCO (Viil 1995, Roussel 1999); [K]1 RuBPCO-A tetramer per 5–10 RuBPCO hexamers (Mate *et al*. 1996); [L]Rovers and Giersch (1995); [M]Giersch and Sivak (1991); [N]assuming 7 μM CO_2 in ambient (0.035 %) CO_2 level (Viil 1995).

Rate constant	Used value	Reported value	Initial amount	Used value [mM]	Reported value [mM]
k_1 [A]	60	60 [D]	TP	1	–
k_2 [A]	2	–	PGA	10	4–11 [H]
k_3 [B]	0.03	–	RuBP	5	5–6 [I]
k_4 [C]	0.01	–	RuP	1	–
k_5 [B]	1	0.14 – 1 [E]	Complex	0	–
k_6 [C]	0.004	0.001 – 0.008 [F]	$RuBPCO_A$	2.5	– [J]
k_7 [C]	0.004	0.004 [G]	$RuBPCO_I$	0.5	– [J]
k_8 [C]	3	–	RuBPCO-A_A	0.5	– [K]
k_9 [B]	0.001	–	RuBPCO-A_I	0.5	– [K]
k_{10} [B]	0.5	–	NADP	0.8	0.4 [L]
k_{11} [B]	0.005	–	NADPH	0.4	0.8 [L]
k_{12} [B]	0.4	–	ATP	1	1 [L]
k_{-12} [B]	0.4	–	ADP	1.5	1.5 [L]
k_{cat} [B]	3	2.6 – 3.5 [D]	P_i	1	1 [M]
			CO_2	0.5	~0.4 [N]

that leads to faster rate of ATP formation. Further possibilities of control of NADPH and ATP formation used in simulations of the photosynthetic oscillations are discussed in Rovers and Giersch (1995).

Formation of NADPH from NADP (k_{10}) is assumed to reflect function of all electron transport within thylakoid membrane starting from electron separation from water in oxygen evolving complex of PS2 and ending in reduction of NADP to NADPH by electrons delivered by all electron transport chain within thylakoid membrane to ferredoxin (a final electron acceptor from PS1), employing ferredoxin:$NADP^+$ oxidoreductase. Therefore, and because the model does not include description of function of PS2 from which fluorescence signal is emitted (for reviews see Krause and Weis 1991, Dau 1994, Govindjee 1995, Lazár 1999), fluorescence at time t, as presented in Figs. 5 and 6, was assumed to be proportional to the concentration of NADPH at time t as it was suggested and discussed in Rovers and Giersch (1995) for the case of simulations of the photosynthetic oscillations.

Effluxes of ATP and NADPH due to their consumptions in other reactions not included in the model are described by rate constants k_9 and k_{11}, respectively.

Even if cytosolic TP and P_i are not included in the model, an exchange of chloroplastic TP (P_i) with cytosolic P_i (TP) by phosphate translocators is described by the reactions with rate constants k_{12} and k_{-12}, respectively. It is because the amount of TP (P_i) that is leaving chloroplast is assumed to be replaced in chloroplast by the same amount of P_i (TP) from cytosol. Therefore, chloroplastic TP is ´changed´ to chloroplastic P_i and *vice versa* as it is described by the re-

actions with rate constants k_{12} and k_{-12}.

Except of ATP formation as described above, all other reactions followed mass action law. Time courses of reactants in the model were calculated numerically using the software *Gepasi* (see the Materials and methods). In this software, particular reactions are entered as shown in Fig. 7 and knowledge of appropriate set of ordinary differential equations is not necessary. Therefore, the differential equations are not presented here.

The rate of CO_2 fixation presented in Fig. 5*A* was calculated as k_{cat} [Complex](t) (see Fig. 7).

Values of the rate constants and initial amounts of reactants in the model used for simulations of the oscillations at room temperature (called and denoted as control in text and Figs. 5 and 6) are listed in. The amounts of reactants are steady state values that were measured in literature after some time of continuous irradiation as it was done in our experiments. Values of some rate constants not reported in literature were determined by trial-and-error setting to obtain agreement between theoretical and experimental oscillations as it was used by Horton and Nicholson (1987) in the case of simulation of the photosynthetic oscillations. As the values of some rate constants were found in this way, it is not possible to simulate the fluorescence transition from low to high CO_2 level as it was measured in the experiments (Fig. 1) because the values of the rate constants were set to simulate the oscillations upon high CO_2 concentration and can be different for low CO_2 concentration. Also the initial amounts of reactants can be different for low and high CO_2 concentrations (*e.g.* a way of RuBPCO inactivation is different if RuBPCO is carbamylated or not which could result in different amounts of active RuBPCO in different CO_2 concentrations; for reviews see Spreitzer and Salvucci 2002, Salvucci and Crafts-Brandner 2004). Because we want to stress the role of RuBPCO activation status and NADPH insufficiency in control of changes in the period and amplitude, respectively, of the oscillation caused by HT treatment upon high CO_2 concentration, for this purpose it is not important to simulate exactly the fluorescence transition from low to high CO_2 concentration. But note that keeping the values of the rate constants as listed in Table 2 and decreasing the CO_2 concentration to values reported in literature for ambient atmosphere (see the legend to Table 2) resulted in no simulated oscillations (data not shown).