BRIEF COMMUNICATION

Regulation of chloroplast electron transport and photophosphorylation by externally applied protein factor

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Abstract

Pea (*Pisum sativum L.*) chloroplasts contain water-soluble protein factors. They *in vitro* activated the rate of Hill reaction and inhibited the photophosphorylation rate.

Additional key words: Hill reaction; pea; *Pisum sativum*.

Mitochondria contain peptide factors influencing the rate of photochemical reactions (Ivanchenko *et al.* 1987, 1988). The present work shows that also chloroplasts contain water-soluble protein factors regulating the energy-transformation reactions.

Chloroplasts were isolated from the leaves of 7 to 12 d-old pea seedlings in a medium containing 225 mM sucrose and 10 mM NaCl in 50 mM Tris-HCl buffer (pH 7.5) (Gavrilenko *et al.* 1975, Ivanchenko *et al.* 1987, 1988). In order to isolate water-soluble proteins chloroplasts were either treated three times by a 10-fold volume of acetone (temperature 13-15 °C) and then proteins were extracted by water from acetone powder for 30 min, or they were subjected to osmotic shock in distilled water for 15-20 h. In both cases the insoluble residue was removed by centrifugation at 10 000 × *g* during 15 min. Protein concentration was measured by the Lowry method in the modification of Hartree (1972). Chlorophyll (Chl) concentration in suspensions was determined spectrophotometrically according to Arnon (1949). The rate of potassium ferricyanide reduction was measured by the absorbance changes at λ = 420 nm in an *SF-46* spectrophotometer. The rate of photophosphorylation (PP) with phenazine methosulphate was measured from a decrease of P_i content using the same spectrophotometer and its kinetics was indicated by absorbance changes at λ = 650 nm (Nikulina 1965). Irradiance for photochemical activity determination was

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200 W m^{-2}, temperature 20 ± 1 °C. The rate of Hill reaction (HR) in such conditions in control was 0.14-0.22 mol(red. ferricyanide) kg^{-1}(Chl) s^{-1}, whereas the rate of PP was 0.08-0.11 mol(ester. P_i) kg^{-1}(Chl) s^{-1}.

The concentrated water-soluble extract from chloroplasts osmotically shocked by distilled water inhibited PP by 50 % and more and increased the HR rate by a factor of 2 and more (Table 1). The effects of protein factor increased with increasing its concentration. These values characterize the chloroplast protein factor as uncoupler because a similar effect was caused by the typical exogenous uncoupler, NH_4Cl. Thus adding 0.1 kg m^{-3} of chloroplast protein to the sample activated HR by 41 % and 0.5 mM NH_4Cl by 98 %. Simultaneous adding of both protein and NH_4Cl activated the Hill reaction by 136 %. Such additive effect was detected by using protein concentrations within the limits of 0.25-0.5 kg m^{-3}.

Table 1. The influence of 3 or 6 min treatment by combined fractions of chloroplast water-soluble proteins on Hill reaction (HR) and photophosphorylation (PP) rates [%]. Means + S.E.

	Protein [kg m^{-3}]					
	0.25		0.50		1.00	
	Irradiation time [min]					
	3	6	3	6	3	6
Inhibition of PP	41.9± 9.5	55.2±7.9	60.0±6.2	71.9±10.7	74.7±13.0	81.5± 8.6
Activation of HR	100.3±18.8	84.9±5.9	123.5±7.8	152.6±16.9	177.9±15.2	170.7±14.4

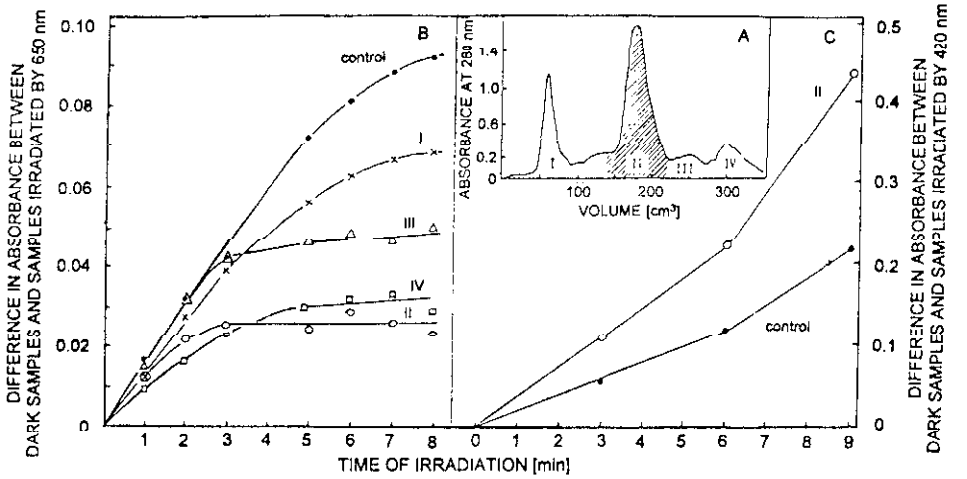

Fig. 1. *A*: Elution profile of bulk extract of water-soluble proteins after gel-filtration on *Acrilex P-30*. *B*: Kinetics of photophosphorylation in absence (control) and presence of the obtained protein fractions (I-IV). *C*: Kinetics of Hill reaction in absence (control) and presence of the most active fraction II. The protein concentrations were 0.25 kg m^{-3} in all variants except control.

Bulk protein extract was then fractionated by gel-filtration method on a column (90.0×1.6 cm) filled with *Acrilex P-30 (Reanal)* (Osterman 1985). The protein content in eluate was determined spectrophotometrically at λ 280 nm. Gel-filtration yielded four fractions: fraction II was the most active (Fig. 1) in inhibiting PP. This fraction increased also more than twice the rate of electron flow to potassium ferricyanide.

Water solution of proteins extracted from previously prepared chloroplast acetone powder gave after separation on the column with *Sephadex G-50 (Pharmacia)* two fractions analogous to the first two ones received after gel-filtration on *Acrilex P-30*. The fraction II was again more active (in concentration of 0.24 kg m^{-3} it completely inhibited PP).

Considering that kinetics of electron transport and photophosphorylation were different, we can think that chloroplast water-soluble protein extract contains more than one active factor with different action on both processes.

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