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Influence of photodynamic processes induced by 2,2'-dipyridyl on the enzymatic system of chlorophyll biosynthesis

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Abstract

The influence of 2,2'-dipyridyl (2,2'-DP) on the activity of one of the enzymes at the initial stages of chlorophyll (Chl) biosynthesis, δ-aminolevulinic acid dehydratase (ALAD; δ-aminolevulinate hydro-lyase, EC 4.2.1.24), as well as on δ-aminolevulinic acid (ALA) accumulation was investigated in green barley (*Hordeum vulgare* L.) leaves. In seven day old green leaves treated with 3 mM 2,2'-DP for 17 h in darkness and subsequently irradiated with "white light" (15 W m^{-2}) for 4, 8, and 24 h the ALAD activity was 51 % as compared to that in untreated leaves. At the same time, the ALA forming system was most sensitive to the photodynamic processes caused by 2,2'-DP. After 8 h of irradiation, ALA synthesis was entirely inhibited. After the treatment the leaves accumulated exceptionally high amounts of Chl precursors such as protoporphyrin IX (Proto), Mg-protoporphyrin IX (Mg-Proto), its monomethyl ester, and protochlorophyllide (Pchlide) that are photosensitizers of photodynamic processes in plants. A comparatively low Chl and carotenoid (Car) destruction was registered during the subsequent 4 and 8 h of irradiation. At the same time, the content of Chl precursors was negligible. The low photodestruction of Chl and Car included in pigment-protein complexes, against the background of fast porphyrin disappearance, and fast decrease of enzymatic activities at the initial stages of Chl production could mean that the photodynamic effect induced by porphyrins accumulated in the presence of 2,2'-DP affected first the Chl enzymatic system and did not change the pool of already synthesized photosynthetic pigments.

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Abbreviations: ALA, δ-aminolevulinic acid; ALAD, δ-aminolevulinic acid dehydratase; Car, carotenoids; Chl, chlorophyll; Chlide, chlorophyllide; DP, 2,2'-dipyridyl; LA, levulinic acid; MgP(E), magnesium-protoporphyrin-IX-monomethyl ester; Mg-Proto, Mg-protoporphyrin IX; PChlide, protochlorophyllide; Proto, protoporphyrin IX.

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Additional key words: δ-aminolevulinic acid; δ-aminolevulinic acid dehydratase; barley; *Hordeum vulgare* L.; protoporphyrin IX; protochlorophyllide; photodestruction.

Introduction

The synthesis of δ-aminolevulinic acid (ALA) is the first step in porphyrin biosynthesis, leading to heme, chlorophyll (Chl), vitamin B_{12}, and other specific plant tetrapyrrole formations (Granick and Sassa 1971, Avissar and Moberg 1995). Higher plants treated in darkness with ALA and ALA+2,2'-DP as a modulator of Chl synthesis (Rebeiz *et al.* 1984) accumulate exceptionally high amounts of protoporphyrin IX (Proto), magnesium-protoporphyrin-IX-monomethyl ester [MgP(E)] and protochlorophyllide (Pchlide). In dark-grown and greening plants, 2,2'-DP acts as a metal chelator, which binds free ions in the tissue and therefore inhibits heme synthesis (Castelfranco and Jones 1975, Rao *et al.* 1981, Hodgins and Van Huystee 1989, Kittsteiner *et al.* 1991). This leads to a lack of feed-back inhibition of the tetrapyrole pathway at ALA formation, as well as at later stages (Duggan and Gassman 1974, Chereshkin and Castelfranco 1982, Kittsteiner *et al.* 1991, Koleva *et al.* 1995). There are no results about the influence of 2,2'-DP on ALA accumulation and ALAD activity in green plants.

The massive accumulation of Chl precursors, especially Proto and MgP(E), which act as photodynamic pigments, results in the generation of singlet oxygen (Becerril and Duke 1989, Neverov *et al.* unpublished), followed by photodestruction of cell membranes and plants (Rebeiz *et al.* 1984). What is more, 2,2'-DP induces inhibition of formation of grana and Chl *a/b* binding proteins (LHCP) and a delay of prolamellar body transformation in irradiated etiolated seedlings (Mostowska and Siedlecka 1995). Taking into account the photodynamic effect of 2,2'-DP (Rebeiz *et al.* 1984, 1988, Mayasich *et al.* 1990, Mostowska 1992), we investigated the ALAD (E.C. 4.2.1.24) activity, the accumulation of ALA and Chl precursors, including Chl, and Car contents under the influence of 2,2'-DP, and the role of the latter in inducing photodynamic damage in green barley leaves.

Materials and methods

Barley seedlings (*Hordeum vulgare* L.) were grown for 7 d under cool-fluorescent tubes (irradiance of 15 W m^{-2}), at a 14/10 light/dark cycle and 23 °C. The leaf segments were placed in Petri dishes with 8 cm^3 of 3 mM 2,2'-DP and incubated in darkness for 17 h. In the experiments where the ALA amount was measured after the 2,2'-DP treatment, the leaf segments were washed and placed into 0.05 M levulinic acid (LA) and incubated in the light or dark for 4, 8, and 24 h.

The ALA amount was estimated, following condensation with acetylacetone, according to Miller *et al.* (1979) with some modifications (Averina *et al.* 1988). The activity of ALA-dehydratase was analysed as described by Mauzerall and Granick (1956) and Shemin (1962). Proto, MgP(E), PChlide and chlorophyllide (Chlide) were extracted with an acetone-NH_4OH mixture (9:1, v/v), and the acetone extract was

washed twice with an equal volume of hexane as described by Rebeiz *et al.* (1984). The precursors were measured fluorimetrically as described by Shlyk *et al.* (1982). The total amount of Chl and Car was measured in 80 % acetone according to Lichtenthaler and Wellburn (1983).

The protein content of the samples was measured after pigment extraction. After centrifugation, the pellet was resuspended in water, and the determination was performed in 0.2 cm^3 of homogenate according to Lowry *et al.* (1951).

Results

Seven-day-old green barley seedlings (*Hordeum vulgare* L.), which had been cut and incubated for 17 h in darkness in a 3 mM 2,2'-dipyridyl solution, showed accumulation of active photosensitizers such as Chl precursors Proto and MgP(E). The sum of Proto and MgP(E) in such leaves was about 73 pmol per seedling. No Proto and MgP(E) were found in the control plants, and the Pchlide amount was twice lower than in the plants treated in the 2,2'-dipyridyl solution (Fig. 1).

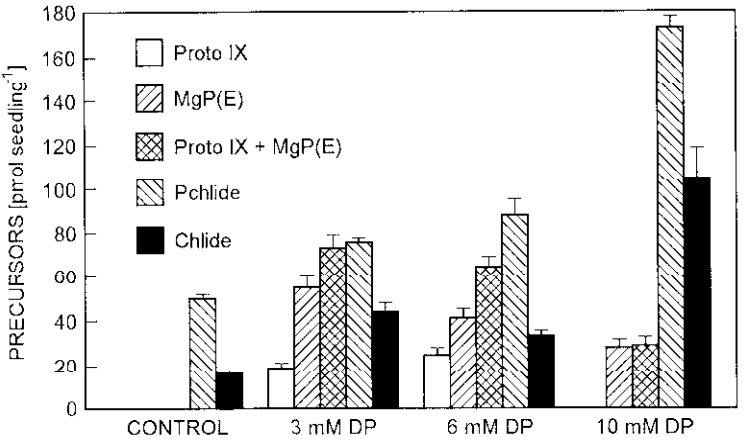

Fig. 1. Changes of the precursor contents [pmol per seedling] in green 7-d-old barley leaves under the influence of different concentrations of 2,2'-dipyridyl (DP), after 17 h in darkness. Chlide - chlorophyllide; MgP(E) - Mg-protoporophyrin IX-monomethyl ester; Pchlide - protochlorophyllide; Proto IX - protoporphyrin IX.

At the end of dark incubation, Chl and Car amounts, as well as the activity of ALAD (the enzyme converting ALA into porphobilinogen), in the control and 2,2'-DP treated plants were practically the same. After 4, 8, and 24 h of irradiation of segments that were incubated with 2,2'-DP, the photodynamic processes were increased, which led to a partial destruction of the Cars, Chl *a,* and Chl *b*, as well as to a decrease of ALAD activity (Fig. 2). After 4 h of irradiation, Chl and Car amounts decreased by 5 and 12 %, respectively, in comparison with control plants, and ALAD activity decreased by 15 %. After 8 h of irradiation, the photodegradation of Chl was 10 % and that of the Cars 21 %. ALAD activity decreased to 67 % as

compared to the control. Subsequent irradiation caused a further decrease in the mentioned parameters, but it was not as fast as that after the 4 and 8 h irradiation.

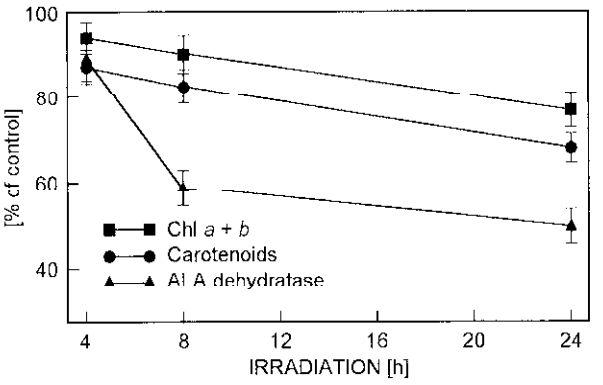

Fig. 2. Changes in the δ-aminolevulinic acid (ALA)-dehydratase activity, and pigment amounts (Chl *a*+*b* and carotenoids) in green barley seedlings treated with 3 mM 2,2'-dipyridyl in light.

The enzyme system for ALA synthesis was more sensitive to the action of 2,2'-DP. The ALA amount in the leaves treated with 2,2'-DP drastically decreased after 4 h of irradiation in the presence of LA. After 8 h of irradiation, ALA synthesis was practically entirely inhibited, amounting to 7 % of the control (Figs. 3 and 4). At the same time, the activity of ALAD remained fairly high - about 85 % after 4 h and 67 % after 8 h. Even after 24 h of irradiation, its activity was about 50 % of the control, while the ALA amount in 2,2'-DP treated leaves was less than 3 % compared with control plants.

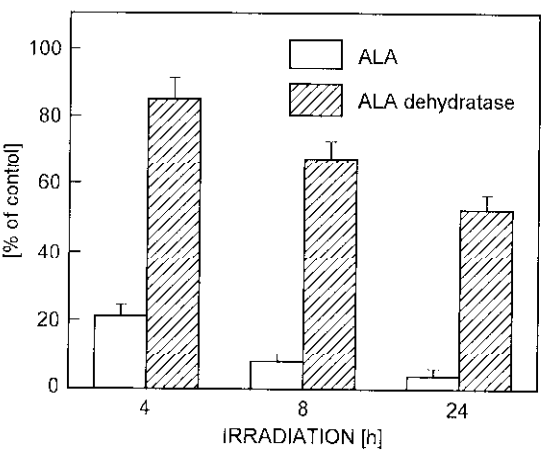

Fig. 3. Comparison of the amounts of δ-aminolevulinic acid (ALA) and the activity of ALA-dehydratase after 4, 8, and 24 h of irradiation of green barley seedlings treated with 3 mM 2,2'-dipyridyl.

In the course of irradiation of the leaves treated with 2,2'-DP, the combined amounts of protochlorophyllide and chlorophyllide as ALA synthesis retroinhibitors (Stobart and Ameen-Bukhari 1984, Averina 1986, Dörneman *et al.* 1989) did not exceed their amounts in the control plants, *i.e.*, the drastic decrease of ALA accumulation was due to photodestruction of the enzyme molecules catalysing the formation of this compound.

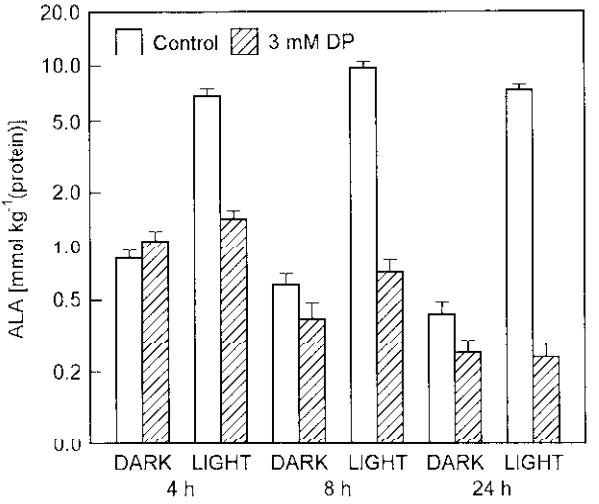

Fig. 4. δ-aminolevulinic acid (ALA) amount in 7-d-old green barley seedlings treated with 2,2'-DP after 4, 8, and 24 h in darkness or in light.

Discussion

The low photodestruction of Chl and Cars included in the pigment-protein complexes of photosynthetic apparatus, against the background of fast porphyrin disappearance and fast degradation of the enzymes involved in the early stages of Chl production, could mean that the photodynamic effect induced by porphyrines accumulated in the presence of 2,2'-DP influenced first the enzymatic system of Chl synthesis and practically did not affect the pool of already synthesized photosynthetic pigments. At the same time, the parallel decrease in Chl *a* and *b* amounts, which did not change the Chl *a/b* ratio in the leaves treated with 2,2'-DP, might testify that the next sensitive target for the singlet oxygen generated by porphyrins could be the LHCP of the photosynthetic apparatus (Mostowska and Siedlecka 1995). The lack of change in the Chl *a/b* ratio under the influence of accumulated protoporphyrins is a proof to it. If the target were some of the photosynthetic systems, there would be a change in that ratio.

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