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Biosynthesis of Chlorophyll of Photosystem II Reaction Centers in Plant Leaves in Early Stages of Etiolation

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Abstract—Spectral methods were used to study the sequences of chlorophyll biosynthesis reactions in etiolated pea, bean, and maize plants in early stages (3-4 days) of growth. For these juvenile plants, along with the reaction chain known for mature (7-9 day-old) plants, a new reaction chain was found which started with phototransformation of the long-wavelength form PChld 686/676 into PChld 653/648. (PChld 653/648 differs from the main known precursor form PChld 655/650). The subsequent photoreduction of PChld 653/648 leads to the formation of Chld 684/676, which is transformed into Chl 688/680 in the course of a dark reaction. After completion of this reaction, fast (20-30 sec) quenching of the fluorescence of the reaction product is observed with the formation of non-fluorescent Chl 680. The reaction accompanied by pigment fluorescence quenching is absent in pea mutants with depressed function of Photosystem II reaction centers. This suggests that the newly found reaction chain leads to the formation of chlorophyll of the Photosystem II reaction center.

Key words: chlorophyll biosynthesis, protochlorophyllide, reaction centers, Photosystem II, etiolated leaves

In studies of the terminal stage of chlorophyll biosynthesis from its precursor PChld 655/650, leaves of dark-grown plants are usually the objects of investigation. As a rule, mature etiolated plants are used, i.e., plants grown in darkness for a long time (7 to 12 days old) so that they could accumulate the necessary amount of the precursor. The character of chlorophyll biosynthesis at early stages of growth, 3-4 days after germination, remained unknown. Such plants are further called juvenile, in contrast to mature 7-12 day-old plants.

In our previous papers [1, 2] it was shown that in mature etiolated leaves after a special treatment (D_2O introduction, heat shock), along with the biosynthesis of the bulk of the antenna chlorophyll from PChld 655/650, a different chain of reactions occurs leading to biosynthesis of the chlorophyll of PS II RC (presumably of the pigment P 680). In the experiments with juvenile etiolated plants containing significantly lower amount of the precursor of antenna chlorophyll PChld 655/650, the reaction chain of PS II RC chlorophyll biosynthesis was observed going under natural conditions, without any of the above-mentioned treatments.

MATERIALS AND METHODS

Leaves of 3-4-day-old etiolated seedlings of maize (Bukovinsky variety), bean (1024), and pea (Capital variety), all henceforth called juvenile plants, were used in this study. Seven-eight-day-old leaves of the same plants were used as the control. In some experiments, leaves of pea core mutants obtained by S. A. Gostimsky (Department of Genetics and Selection, Lomonosov Moscow State University) from pea of Capital variety (line Chl-1) with depressed function of PS II were used. In green leaves of this lethal mutant, photosynthetic oxygen production, delayed fluorescence, the typical ESR signal, and the reduction of dichlorophenolindophenol, $NADP^+$, and of exogenous plastocyanin at the expense of such electron donors as water or diphenylcarbazide were depressed. Analysis of the polypeptide composition of the thylakoid membranes from green leaves of this mutant showed these functional changes to be related to the practically complete absence of typical apoproteins of PS II RC, the content of pigment–protein complexes of the antenna and PS I being normal [3, 4].

Abbreviations: PChld) protochlorophyllide; PChl) protochlorophyll; RC) reaction centers; PS II) Photosystem II; Chld) chlorophyllide; Chl) chlorophyll. In the abbreviations of the native pigment forms the first figure stands for the maximum of the low-temperature fluorescence spectrum, the second figure for the maximum of the long-wavelength absorption band, and the figure in brackets indicates the position of the Soret band in the low-temperature fluorescence excitation spectrum.

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The etiolated plants were grown in a light-isolated thermostat at 26°C. The introduction of exogenous inhibitors and stimulants of chlorophyll biosynthesis was performed through cuts of the stems of the etiolated seedlings, the procedure having been described elsewhere [1].

To initiate greening, the etiolated leaves were illuminated with an incandescent lamp having a SZS-23 light filter transmitting radiation in the range 400-750 nm. In special experiments with initiation of individual photoreactions with monochromatic light, interference light filters with transmittance maxima at 650 and 680 nm were used (Carl Zeiss, Germany; halfwidth of band 8 nm, transmittance at the wavelength of maximum 35%). To illuminate samples at the temperature of liquid nitrogen, a xenon lamp (200 W) in combination with an SZS-23 light filter was used. Light intensity is indicated in figure legends.

At physiological temperatures, illumination of samples was carried out after a preliminary short-term (5 min) cooling to 4°C. Cooling was applied for inhibition of dark reactions involving the intermediates of Chl biosynthesis [5]. Subsequent increase in temperature to a given value (figure legends) allowed observing the course of dark reactions following the photochemical reaction. The cycle including illumination and subsequent dark period was completed by fixing the sample in liquid nitrogen to measure the low-temperature absorption spectra, fluorescence emission and excitation spectra, and derivatives of spectra, the methods for these measurements having been previously described by us [1].

The pigments were extracted from greening leaves with dimethylformamide saturated with $MgCO_3$. To estimate the esterified to non-esterified pigment ratio in the extracts, the methods described in [6] were used.

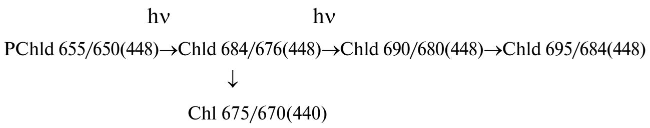
RESULTS

Precursor native forms in leaves of etiolated juvenile plants. Comparing the spectral curves of the non-illuminated leaves (Fig. 1, a-d (curves *1*) and Fig. 2a), one can see that the spectra of both juvenile and mature plants contain fluorescence and absorption bands of three well-known protochlorophyll(ide) forms: PChl 633/628, PChl(d) 642/637, and PChld 655/650 [7, 8]. However, in the spectra of juvenile plants, an additional rather intense absorption band is observed at 676 nm with the corresponding fluorescence band at 686 nm. The spectrum of this pigment *in vivo* resembles that of chlorophyll. Analysis of extracts of this pigment showed these bands belong not to chlorophyll but to a new protochlorophyllide form PChld 686/676. The classification of this pigment form as protochlorophyllide is confirmed by experiments with disaggregating actions: under repeated thawing of juvenile plants (frozen to –196°C) and subsequent heating (to 26°C), and on the heating of leaves to 60°C, a complete transformation of the main precursor form PChld 655/650 and of the new form PChld 686/676 into the short-wavelength PChld 633/628 was observed. This effect is well known for the main native protochlorophyll(ide) form PChld 655/650 and is usually ascribed to its disaggregation [9]. The bands of Chl formed from the precursor do not undergo this shift.

The native long-wavelength protochlorophyll(ide) with spectral characteristics close to those of PChld 686/676 was also previously found in mature etiolated leaves, but only smaller amount, available for analysis only using derivative spectra [10].

PChld 686/676 found in the juvenile plants differs in the fluorescence excitation spectrum from the main precursor form PChld 655/650: its Soret band is located at 440 nm (designated as PChld 686/676(440)), while the Soret band of PChld 655/650 is located at 448 nm (designated as PChld 655/650(448)) (Fig. 3d, curve *3*). This shows the absence of energy migration between these pigment forms, which made it possible to observe their individual photoreactions under monochromatic light (see below).

Sequence of reactions of antenna Chl biosynthesis from PChld 655/650(448) in juvenile plants. In juvenile leaves under illumination with monochromatic light of 650 nm absorbed by the precursor of antenna Chl, PChld 655/650(448) [11], a decrease in the intensity of the spectral bands of this form and the sequential formation of the intermediates in the antenna Chl biosynthesis occurred (Fig. 2). Analysis of the spectra showed that the reaction chain initiated by 650 nm light in juvenile plants completely coincided with the sequence of reactions of antenna chlorophyll synthesis studied for mature etiolated plants [11].



Sequence of the long-wavelength PChld 686/676(440) reactions in etiolated juvenile plants. We found conditions under which a different sequence of reactions takes place starting with the phototransformation of the long-wavelength precursor form PChld 686/676(440) found in the juvenile plants. We illuminated etiolated juvenile plants with monochromatic light of 680 nm in the spectral region where light absorption by the long-wavelength PChld 686/676(440) is rather high, and the known main form of the precursor PChld 655/650(448), giving rise to the antenna chlorophyll, does not absorb radiation.

This illumination leads to the disappearance of the 676 nm band in the absorption spectrum of juvenile leaves (Fig. 3a, curves *1* and *2*) and simultaneous emergence of an unstructured absorption band at 648-650 nm. This

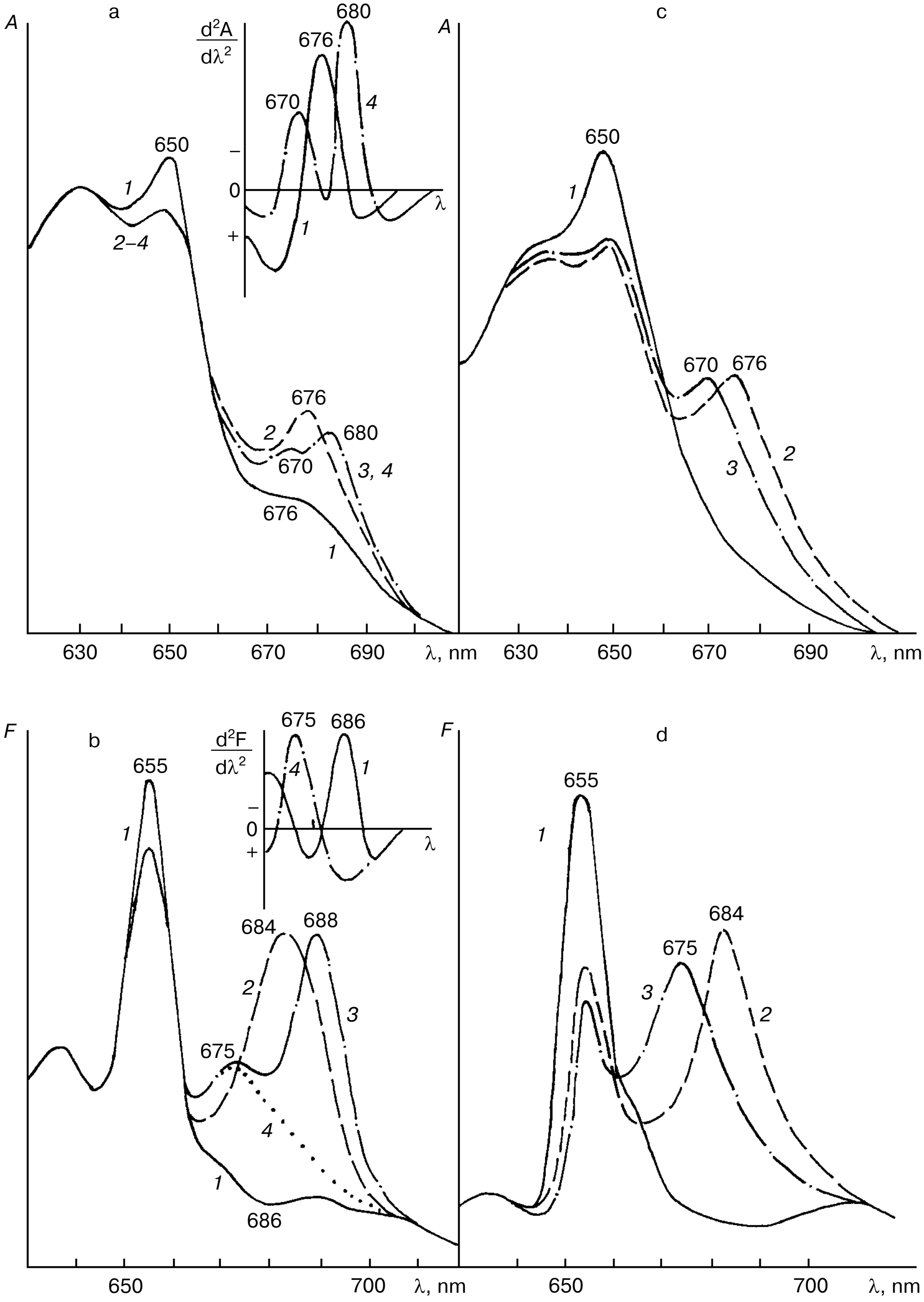


Fig. 1. Initial stages of PChld 655/650 phototransformation and dark reactions of the intermediate Chld 684/676 in juvenile (a, b) and mature (c, d) etiolated maize plants. Low-temperature (–196°C) absorption spectra (a, c) and fluorescence emission spectra (b, d) of etiolated leaves. *1*) Non-illuminated leaf; *2*) sample *1* illuminated with 680 nm monochromatic light (60 sec) at 4°C and additionally with 650 nm monochromatic light (10 sec); *3*) sample *2* plus 5 sec in darkness at 20°C; *4*) sample *3* plus 30 sec in darkness at 20°C. The second derivatives of the spectra are presented in the insets.

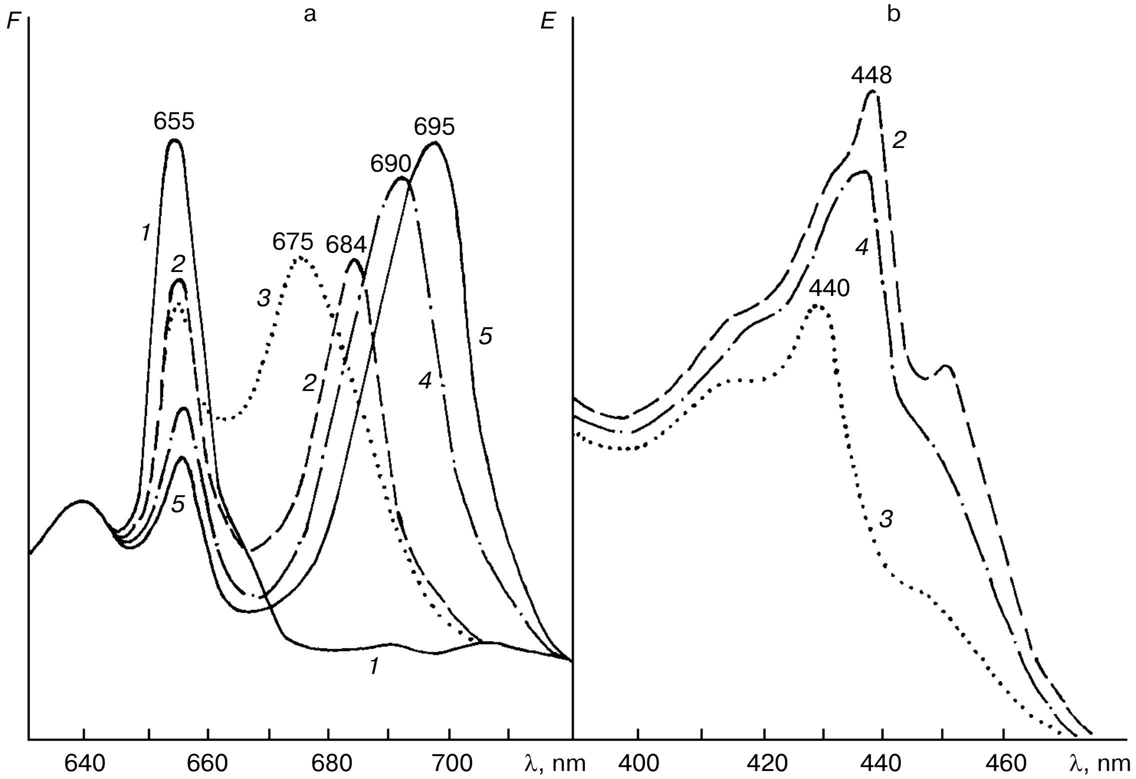


Fig. 2. Reactions of the main Chl biosynthesis pathway in juvenile maize plants initiated by 650 nm monochromatic light. a) Low-temperature (–196°C) fluorescence spectra: *1*) non-illuminated sample; *2*) sample *1* plus 20 sec illumination at 4°C; *3*) sample *2* plus 10 sec in darkness at 20°C; *4*) sample *2* plus 60 sec illumination at 4°C; *5*) sample *4* plus 4 min in darkness at 20°C; b) the corresponding low-temperature (–196°C) fluorescence excitation spectra of the intermediates: *2*) Chld 684/676; *3*) Chl 675/670; *4*) Chld 690/680.

process did not involve changes in the pigment contents of the extracts: neither before nor after illumination with 680 nm light was chlorophyll(ide) observed, and the protochlorophyll to protochlorophyllide ratio remained constant. Thus, it seems reasonable to suggest that a photoinduced transformation of the long-wavelength PChld 686/676(440) into a shorter-wavelength precursor form occurred. In fact, we found by the difference absorption and fluorescence spectra (Fig. 3b) that during illumination with monochromatic light of 680 nm, the decay of the absorption band at 676 nm and fluorescence band at 686 nm was accompanied by an increase in the absorption band at 648 nm and in the fluorescence band at 653 nm. This effect may be accounted for by the phototransformation of the long-wavelength protochlorophyllide into a form close to the main form of the precursor but having a small spectral shift towards shorter wavelengths: PChld 686/676 → PChld 653/648. The photoproduct of this reaction differs from the main photoactive precursor form PChld 655/650 by the presence of a short-wavelength shift of the bands in the blue spectral region. For example, Fig. 3d (curves *1* and *2*) shows that in the excitation spectrum of fluorescence registered at 655 nm, before illumination with 680 nm light (when only PChld 655/650 fluoresces in this region), only one Soret band is detected: the band at 448 nm. After illumination, when at 655 nm both PChld 655/650 and PChld 653/648 fluoresce, in the fluorescence excitation spectrum an additional band at 440 nm emerges. It would be reasonable to relate this band to the formed pigment PChld 653/648. Thus, juvenile plants after illumination with 680 nm light contain both PChld 655/650(448) and PChld 653/648(440) (Fig. 3, a and b).

What was going to happen to these two similar PChld forms was shown by special experiments with additional illumination of the leaves (already containing both PChld 655/650(448) and PChld 653/648(440)) with the monochromatic 650 nm light. As seen from Fig. 3a (curve *3*), the additional illumination led to a decrease in the absorption at 648-650 nm, in fluorescence at 653-655 nm, and simultaneously to the formation of the bands of the photoproduct Chld 684/676. In the fluorescence excitation spectra, the Soret band of this intermediate had a peculiar doublet character; it had maxima at 440 and 448 nm (Fig. 4, curve *2*), while in the spectra of the mature leaves the Soret band has only one component with a maximum at 448 nm (Fig. 4, curve *1*). Thus, it is natural to assume that the other maximum at 440 nm belongs to a new, pre-

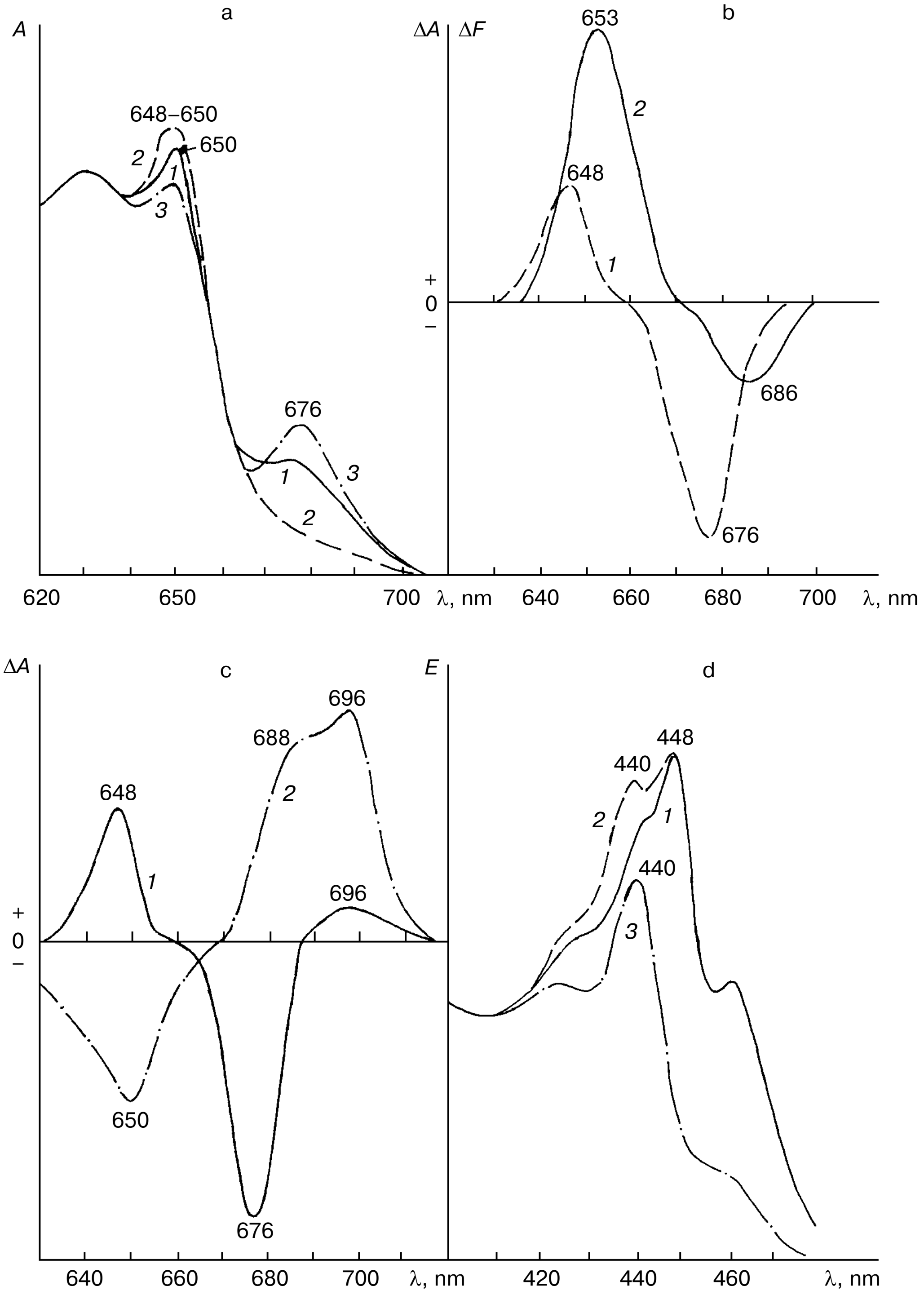


Fig. 3. Sequence of two photoreactions PChld 686/676(440) → PChld 653/648(440) → Chld 684/676 in juvenile maize leaves. a) Changes in low-temperature (–196°C) absorption spectra of leaves: *1*) non-illuminated sample; *2*) sample *1* plus 60 sec illumination with 680 nm monochromatic light at 4°C; *3*) sample *2* plus 10 sec of illumination with 650 nm monochromatic light at 4°C; b) difference (light minus dark) low-temperature (–196°C) absorption spectra (*1*), and difference fluorescence emission spectra (*2*) measured after 680 nm monochromatic illumination during 10 sec at 4°C; c) difference low-temperature (–196°C) absorption spectra measured during illumination of juvenile plants with a xenon lamp $5\cdot10^2$ W/m^2: *1*) the spectrum of the sample illuminated for 10 sec minus spectrum of non-illuminated spectrum; *2*) the spectrum of the sample illuminated for 60 sec minus spectrum of the sample illuminated for 10 sec; d) 655 nm fluorescence excitation spectra before (*1*) and after (*2*) illumination of juvenile plants with 680 nm monochromatic light; *3*) 686 nm fluorescence excitation spectrum (PChld 686/676) in non-illuminated sample.

viously unknown intermediate Chld 684/676(440), different in the position of Soret band from the analogous intermediate Chld 684/676(448) found in the mature leaves. Since PChld 653/648(440) is found in juvenile and not in the mature plants, the formation of the new intermediate Chld 684/676(440) should be related to photoreduction of this precursor form under illumination with 650 nm light.

Thus, under sequential illumination with 680 nm light and 650 nm light, in the juvenile plants not one (as it is the case for mature plants) but two intermediates are formed with similar long-wavelength absorption bands (676 nm) and fluorescence bands (684 nm) but different in the positions of the Soret band. These are the product of PChld 653/648(440) phototransformation—Chld 684/676(440)—and the product of PChld 655/650(448) phototransformation—Chld 684/676(448). This interpretation is also confirmed by the juvenile plants illuminated exclusively with 650 nm monochromatic light. Under such illumination only one intermediate, Chld 684/676(448), is formed, while the intermediate Chld 684/676(440) is not detected (Fig. 2, a and b, curves *2*). This may be accounted for by the fact that the light of 650 nm is not absorbed by PChld 686/676(440); therefore, the sequence of photoreactions PChld 686/676(440) → PChld 653/648(440) → Chld 684/676(440) is not observed.

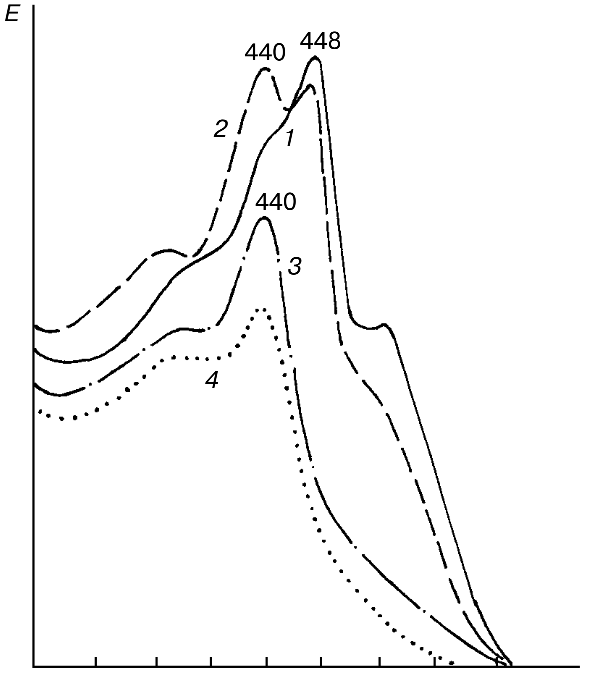


Fig. 4. Fluorescence excitation spectra (–196°C) of the intermediate Chld 684/676 in mature (*1*) and juvenile (*2*) etiolated maize leaves and of the intermediates Chl 688/680 (*3*) and Chl 675/670 (*4*) in juvenile plants. The conditions of Chld 684/676 (curves *1* and *2*) accumulation correspond to the sample presented in Fig. 3a, curve *3*; *3*) sample *2* plus 5 sec in darkness at 20°C; *4*) sample *3* plus 30 sec in darkness at 20°C.

The conclusion about the heterogeneity of Chld 684/676 in juvenile leaves received additional confirmation in studies of its dark reactions.

Dark stages following photoreactions of the main and long-wavelength precursor forms. As seen from curves *2* and *3* of Fig. 1, c and d, and curves *2* in Fig. 2, in the etiolated leaves containing only Chld 684/676(448) after illumination (juvenile plants illuminated with 650 nm light exclusively, or illuminated mature plants; see above) at positive temperatures, only one dark reaction, Chld 684/676(440) → Chl 675/670(440), is observed. In the samples containing post-illumination both intermediates, Chld 684/676(440) and Chld 684/676(448) (that is, in juvenile plants illuminated with 680 nm light and, subsequently, with 650 nm light), along with the known dark reaction leading to the formation of Chl 675/670(440) from Chld with the absorption band at 676 nm and fluorescence emission at 684 nm, an additional dark process occurs. It is manifested in a spectral shift of the Chld fluorescence band from 684 to 688 nm, and of the absorption band from 676 to 680 nm (Fig. 1, a and b, curves *2* and *3*); judging by the analysis of the extracts, a simultaneous esterification of Chld to Chl takes place. The Soret band in the fluorescence excitation spectra of Chl fluorescing at 688 nm was located at 440 nm (Fig. 4, curve *3*); thus, we designated this native Chl form as Chl 688/680(440). The process is completed by quenching of the low-temperature fluorescence of Chl 688/680(440) with the formation of non-fluorescent Chl 680 (Fig. 1, a and b, curves *3* and *4*). Since the new dark process is observed only in the samples containing Chld 684/676(440), we conclude that it involves this intermediate form (not Chld 684/676(448)) and can be described by the following scheme: Chld 684/676(440) → Chl 688/680(440) → fluorescence quenching → Chl 680.

The sequence of reactions leading to the formation of non-fluorescent Chl 680 was observed in juvenile maize, pea, and bean plants. The exception was the pea mutant where the biosynthesis of pigment–protein complexes of PS II RC was significantly depressed in contrast to biosynthesis of pigment–protein complexes of the antenna and PS I (see "Materials and Methods", [3]). The peculiarity of the studied sequence of reaction in these mutants was in the blocking of the final reaction accompanied by Chl 688/680(440) fluorescence quenching (Fig. 5). It can be concluded that the absence of chlorophyll fluorescence quenching in these mutants is because Chld 688/680(440) is not yet included in the PS II RC complex. Considering this, we assume that the studied sequence of reactions is involved in the biosynthesis of Chl of PS II RC. Judging by the position of the maximum of non-fluorescent Chl 680, it is possible that this pigment is identical to the primary donor of PS II, P 680.

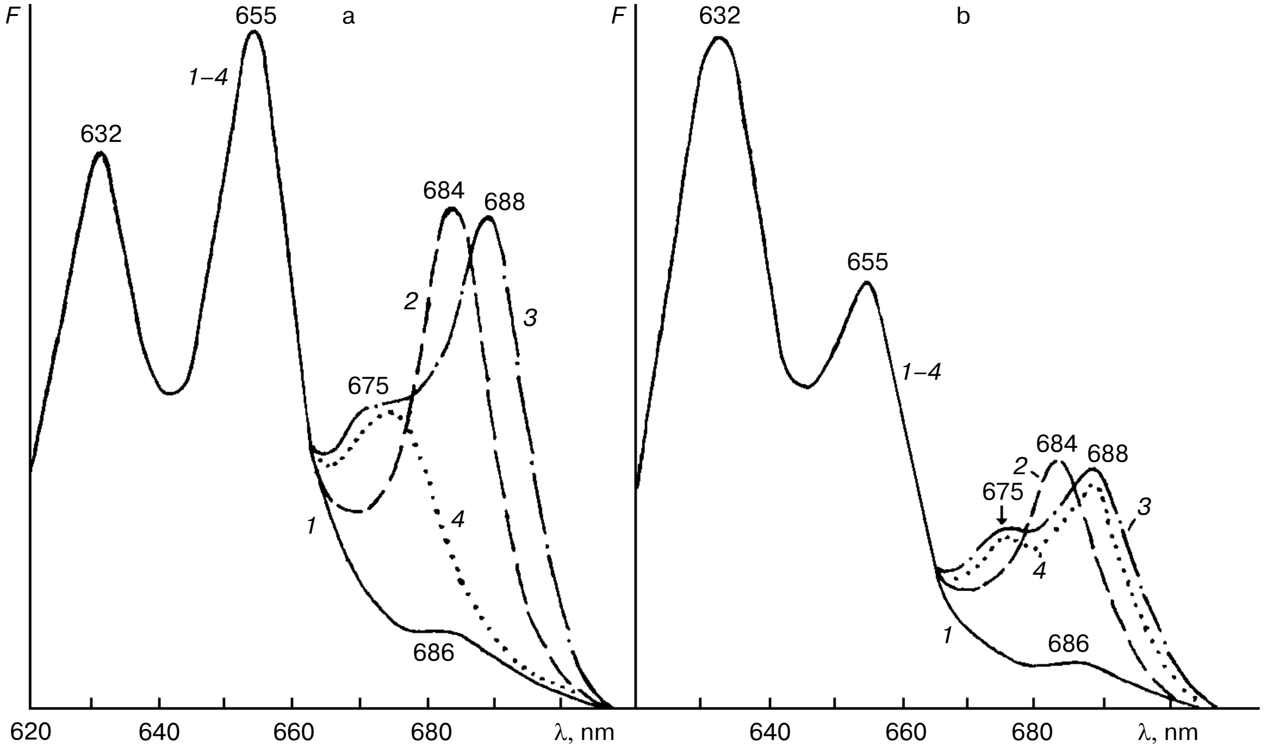


Fig. 5. Dark reactions of Chld 684/676 in juvenile etiolated Capital variety pea leaves (a) and in mutant pea leaves leaves with depressed biosynthesis of PS II RC (b). a, b) Spectra of low-temperature (–196°C) fluorescence of leaves: *1*) non-illuminated sample; *2*) sample *1* plus 4 sec illumination with white light 10^2 W/m^2 at 4°C; *3*) sample *2* plus 5 sec in darkness at 26°C; *4*) sample *3* plus 1 h in darkness at 26°C.

It remained obscure whether the intermediate Chld 684/676(440) participates in the dark reaction with the formation of the short-wavelength Chl 675/670(440) in juvenile leaves. The results presented in Fig. 1 allow the accumulation of this short-wavelength Chl form in juvenile plants to be attributed both to dark transformation of Chld 684/676(440) and to the well-studied dark reaction Chld 684/676(448) → Chl 675/670(440) in the reaction chain of antenna Chl synthesis. This was shown by special experiments on inhibition of the reaction Chld 684/676(440) → Chld 688/676(440) in juvenile leaves (KCN infiltration in the leaves or placing leaves in oxygen-free medium). In juvenile leaves after 8 h of KCN infiltration applied before illumination, the whole pool of intermediate Chld 684/676 formed in the light (Chld 684/676(440) + Chld 684/676(448)) in darkness was transformed into Chl 675/670(440). The same effect was produced by oxygen removal (juvenile leaves were transferred in a Thunberg tube from which air was pumped off or replaced by argon or nitrogen). When the dark transformation of the mixture of intermediates Chld 684/676(440) and Chld 684/676(448) into Chl 675/670(440) was complete, no other reaction products were observed. The complete transformation of both intermediates into Chl 675/670(440) shows that under natural conditions (without external intervention), the reaction proceeding with Chl 675/670(440) formation involves not only Chl 684/676(448) (the side reaction in antenna Chl synthesis) but Chld 684/676(440) as well (in the chain of Chl 680 synthesis).

Thus, our studies of the photochemical and dark reactions in juvenile plants show that the chain of reactions leading to Chl 680 synthesis can be described by the scheme shown in Fig. 6.

The rate ratio of the reactions in the chain of Chl 680 biosynthesis strongly depends on a number of the external factors. Along with the above noted role of O_2 and KCN, significant inhibitory and stimulating factors were heat shock and D_2O/H_2O substitution.

In the experiments with heat shock, etiolated juvenile plants were stored at 38°C during 8 h before illumination. After this treatment the reactions Chld 684/676(440) → Chl 688/680(440) → Chl 680 were stimulated in comparison to the reaction Chld 684/676(440) → Chl 675/670(440).

After 8 h D_2O infiltration of non-illuminated juvenile leaves (D_2O/H_2O-substitution), Chl 680 synthesis was inhibited at the initial, photochemical stage: the reaction PChld 686/676(440) → PChld 653/648(440) was not detected even under long-term illumination with 680 nm

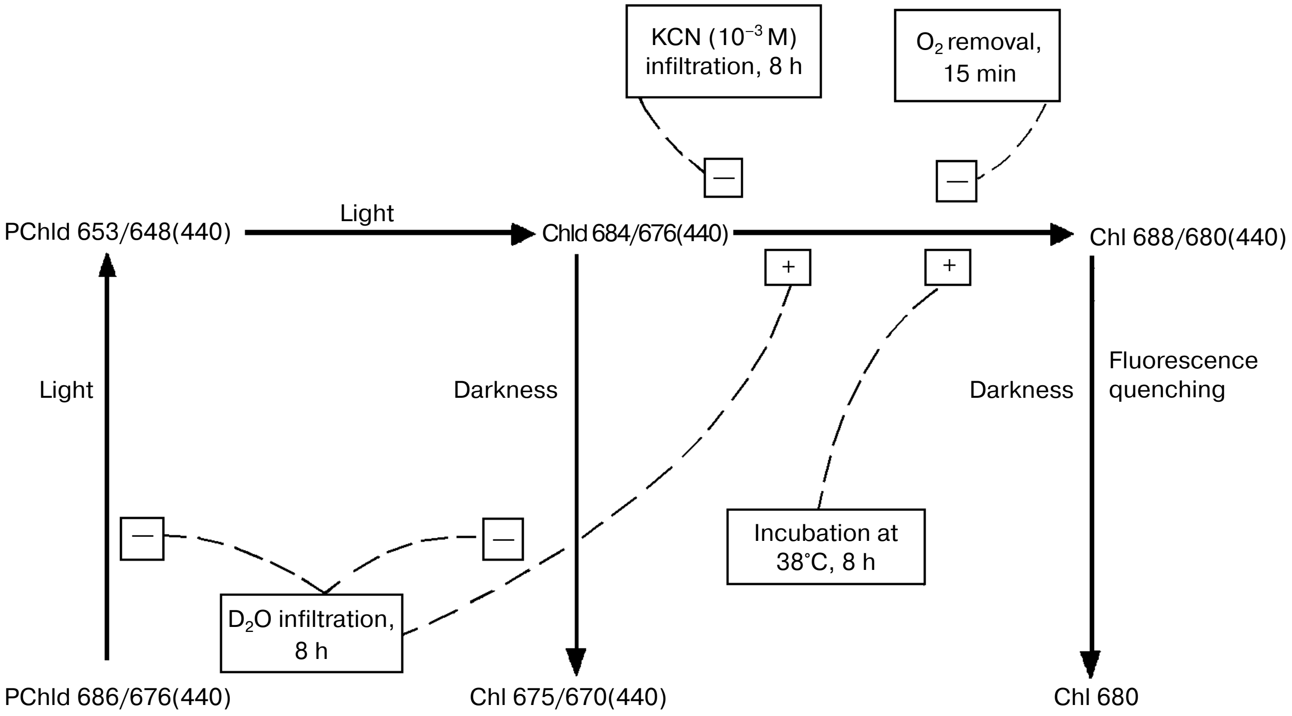


Fig. 6. Sequence of reactions of biosynthesis of non-fluorescent Chl 680 in juvenile etiolated plants. The dashed line shows the effects of inhibitory (minus) and stimulating (plus) external factors.

monochromatic light. The concentration of PChld 686/676(440) was not affected by D_2O/H_2O substitution, but its phototransformation was completely inhibited.

In the course of experiments with D_2O/H_2O-substitution in the etiolated juvenile plants, the switching of the reaction Chld 684/676(448) → Chl 675/670(440) (typical of antenna Chl synthesis) to the reaction Chld 684/676(448) → Chl 688/680(448), typical of the synthesis of Chl 680 occurs. This effect had been studied earlier with mature leaves [1]. In the juvenile leaves this switching was incomplete because no quenching of Chl 688/680(448) fluorescence was observed; the maxima of the Soret bands of Chld 684/676(448) and Chl 688/680(448) also remained unchanged, characteristic of the intermediates of the antenna Chl synthesis.

The data on the effect of the inhibitory and stimulating factors on the reaction sequence of Chl 680 synthesis are presented in Fig. 6.

Photoreactions of the long-wavelength PChld 686/676(440) in etiolated juvenile plants at low temperature. In juvenile etiolated plants under white light, both photoreaction sequences must proceed: antenna Chl synthesis and the synthesis of Chl 680; this must make the picture of spectral changes rather complicated. The sequential transformation of PChld 686/676(440) into PChld 653/648(440) and later into Chld may be observed not only under monochromatic light, but under illumination of juvenile plants with white light at lowered (−196°C) temperature. At this temperature phototransformation of the main precursor form, PChld 655/650(448), is known to proceed with the formation of non-fluorescent intermediates [12] having absorption bands at 690-696 nm (a position different from those of PChld 686/676(440)). This is convenient for studies of Chl 680 synthesis. On the other hand, the photoreactions of PChld 655/650(448) and PChld 653/648(440) at −196°C were considerably inhibited compared to the photoreaction PChld 686/676(440) → PChld 653/648(440). All this made it possible observe in the difference spectra of juvenile plants illuminated with white light at −196°C (Fig. 3c) a decrease in the 676 nm band and the formation of the band at 648 nm (PChld 686/676(440) → PChld 653/648(440)), and under further illumination a decrease in the band at 648-650 nm and the emergence of the bands of intermediates at 696 and 688 nm. The latter bands, judging by their position in the spectrum and the absence of the corresponding fluorescence bands, apparently, belong to the non-fluorescent intermediates R-696 and R-688 (that are assumed to be PChld anion-radicals) known as precursors of Chld 684/676 in mature etiolated leaves [12]. On increasing the temperature to −100°C in darkness, the non-fluorescent intermediates caused the formation of Chld 684/676 (the spectra are not presented because this final reaction was described in detail in our earlier study [12]). The fluorescence excitation spectra of intermediate Chld 684/676 had a doublet Soret band (maxima at 440 and 448 nm). This showed that at low temperature the phototransformation involved two sequences of reactions, the synthesis of antenna Chl and Chl 680 synthesis, the low-temperature intermediates preceding the formation of Chld 684/676(440) from PChld 653/648(440) and of Chld 684/676(448) from PChld 655/650(448) being spectrally similar or identical.

Features of PChld phototransformation kinetics in juvenile etiolated plants under short-term illumination with white light. A short-term (2-4 sec) illumination of juvenile etiolated plants with white light at positive temperature values leads to the appearance of Chld 684/676 spectral bands without any marked decrease in the intensity of PChld 655/650(448) bands in the absorption and fluorescence emission spectra of the leaves (Fig. 5). The bands of the short-wavelength precursor forms PChl 633/628 and PChl(d) 642/637 also remained unchanged. However, the analysis of pigment extracts from the leaves showed the phototransformation of PChld into Chld (estimated by the loss of the precursor and the growth of Chld concentration) in juvenile plants proceeded at the same rate and depth as in the mature plants. Such lag-phase in the decrease in the intensity of absorption and fluorescence bands of PChld 655/650(448) was observed by us at physiological temperatures only in the presence of PChld 686/676(440) (in juvenile plants) and on the condition of simultaneous excitation of photoreactions of PChld 686/676(440) and PChld 655/650(448) by white light. Under illumination with 650 nm monochromatic light, in juvenile leaves the lag-phase was absent (Fig. 2). Taking into consideration the above presented scheme of reactions, one can assume that the delay in the phototransformation is more apparent than real. This delay may be explained by the fact that under illumination with white light the decrease in PChld 655/650(448) bands may be temporarily compensated during antenna Chl synthesis at the expense of accumulation of the intermediate PChld 653/648(440) having fluorescence band and long-wavelength absorption band close to those of the main form of the precursor (the initial photoreaction of Chl 680 synthesis).

This conclusion is confirmed by the analysis of changes in the absorbance at 650 nm for juvenile and mature plants illuminated with white light at 20°C (Fig. 7, curves *1* and *2*). The difference of these two kinetic curves (Fig. 7, curve *3*) has a shape characteristic of the kinetic curve of the intermediate in the sequence of two reactions (in the case of juvenile plants, the reaction sequence PChld 686/676(440) → PChld 653/648(440) → Chld 684/676(440)). It is reasonable to assume this intermediate to be PChld 653/648(440), since this reaction sequence is absent in mature plants. The analysis of the kinetics of these two reactions showed the kinetic curve of PChld 653/648(440) may be well modeled (Fig. 7, points in curve *3*) if the rate constant of the initial reaction PChld 686/676(440) → PChld 653/648(440) is 4-fold greater than the rate constant of the second reaction PChld 653/648(440) → Chld 684/676(440). In the interpretation of this fact we were taking into consideration that the rate constant of the photochemical reaction is determined not only by the quantum yield but also by the absorption cross-section. Judging by the difference absorption spectra (Fig. 2b), the PChld 686/676(440) absorption coefficient for the red spectral region is 2.5-3-fold higher than that of PChld 653/648(440). This can account for the calculated high rate of PChld 686/676(440) phototransformation as compared to photoreaction of PChld 653/648(440), whose quantum yield may be lower than that of PChld 655/650(448), which is up to 40%.

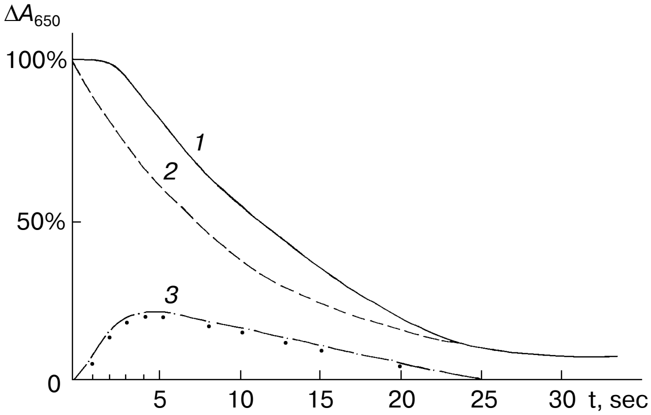


Fig. 7. Changes in absorbance of etiolated leaves at 650 nm during illumination with white light of 10^2 W/m^2 at 4°C: *1*) juvenile maize plants; *2*) mature maize plants. The curves are normalized for non-illuminated samples; *3*) curve *1* minus curve *2*.

DISCUSSION

The nature of the initial photochemical reaction PChld 686/676(440) → PChld 653/648(440) leading to the synthesis of Chl 680 remains obscure. The light-induced shift of PChld bands to shorter wavelengths and the absence of direct PChld 686/676(440) phototransformation into Chld may be connected with the photoinduced changes in PChld pigment–protein complex that provide the conformational conditions required for the subsequent PChld 653/648(440) photoreduction to Chld. Taking into account the weak temperature dependence of the photoreaction PChld 686/676(440) → PChld 653/648(440), the assumed conformational reorganization of the complex may be explained by PChld disaggregation proceeding at low (–196°C) temperature and accompanied by a shift of its spectral bands towards shorter wavelengths [13]. In this connection, it seems interesting that in our studies (data not presented) the photoreaction PChld 686/676(440) → PChld 653/648(440) was blocked in the presence of D_2O, which is known to increase the rigidity of pigment–protein complexes [14] in greening leaves as well [1].

The data show that in juvenile etiolated plant leaves, along with the earlier known complex of the photoactive precursor PChld 655/650(448) starting the biosynthesis of antenna Chl [11], a long-wavelength complex PChld 686/676(440) is formed, the precursor of the non-fluorescent Chl 680 (presumably, the pigment of PS II RC, P 680; see above). It is the early formation of the precursor for RC (PChld 686/676(448)) during plant germination that determines the earlier formation of the PS II RC complex as compared to the other PS II components and pigment–protein complexes of the antenna [15].

We previously showed [2] that in mature etiolated leaves after 8 h heating to 38°C, branching of the reaction chain of antenna Chl synthesis was observed at the level of the intermediate Chld 684/676(448): in those samples a new reaction chain was found, Chld 684/676(448) → Chl 688/680(448) → Chl 680, analogous to the terminal stage of Chl 680 synthesis in juvenile plants. In spite of the spectral similarity of the processes leading to Chl 680 formation in the juvenile and mature plants after heat shock, their equal dependences on temperature and on the external stimulating and inhibitory actions, different precursor complexes of these reaction chains should be noted: PChld 686/676(440) and PChld 653/648(440) for juvenile plants and PChld 655/650(448) for mature plants. Besides, in juvenile plants all the intermediates of Chl 680 synthesis have the Soret band at 440 nm in the fluorescence excitation spectra, while in the corresponding reaction proceeding in mature plants exposed to heat shock the Soret band is located at 448 nm [2]. This shows that the compared reaction chains occur in different pigment–protein complexes. Only the reaction chain of Chl 680 synthesis in juvenile plants occurs naturally, without external actions. Our measurements of the relative PChld concentrations (per unit of wet weight) and the analysis of the spectral curves for etiolated leaves showed that further growth of the etiolated plants in darkness (from 3-4- to 8-10-day-old) led to 10- to 15-fold enhancement of the precursor concentration. However, this enhancement was mainly due to the increase in PChld 655/650(448), not in PChld 686/676(440), i.e., at the expense of the predominant accumulation of the precursor of the antenna Chl [11]. This suggests that the precursor of PChld 686/676(440) is not PChld 655/650(448) but a different pigment complex formed at an earlier stage of biogenesis. This precursor may be, for instance, one of the known short-wavelength PChld forms, PChld 642/637(444) or PChld 633/628(440).

It was shown that, in juvenile etiolated plants, the reaction chain of Chl 680 synthesis involving the intermediate Chld 684/676(440) can be observed separately. The relative roles of two precursors of this intermediate, PChld 686/676(440) and PChld 653/648(440), are not quite clear. Experiments with etiolated plants showed that the process proceeds with the participation of the long-wavelength PChld 686/676(440). However, without etiolation, under continuous illumination, this form must not accumulate due to its rapid photoreaction. It remains obscure whether the dark synthesis and subsequent photoactivation of the long-wavelength form are necessary for PChld photoreduction or the long-wavelength form serves as an extra reservoir for PChld 653/648(440) and should be considered the key component of Chl 680 synthesis. These problems require further investigation.

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REFERENCES

1. Ignatov, N. V., and Litvin, F. F. (1998) *Photosynth. Res.*, **56**, 83-89.
2. Ignatov, N. V., Gostimsky, S. A., Satina, L. Ya., and Litvin, F. F. (1998) *Biochemistry (Moscow)*, **63**, 1319-1327.
3. Matorin, D. N., Grosser, M., Borner, T., Herrman, F. N., and Gostimsky, S. A. (1982) *Biol. Nauki*, **1**, 35-38.
4. Roshchina, V. V., Bozhok, G. V., and Gostimsky, S. A. (1982) *Biokhimiya*, **47**, 1512-1521.
5. Litvin, F. F., and Belyaeva, O. B. (1971) *Photosynthetica*, **5**, 200-209.
6. Vorobyova, L. M., Bystrova, M. I., and Krasnovsky, A. A. (1963) *Biokhimiya*, **28**, 524-534.
7. Kahn, F., Boardman, N. K., and Thorne, S. W. (1970) *J. Mol. Biol.*, **48**, 85-101.
8. Virgin, H. I. (1975) *Photosynthetica*, **9**, 84-92.
9. Butler, W. L., and Briggs, W. R. (1966) *Biochim. Biophys. Acta*, **112**, 45-53.
10. Litvin, F. F., and Stadnichuk, I. N. (1980) *Fiziol. Rast.*, **27**, 1024-1032.
11. Litvin, F. F., Belyaeva, O. B., and Ignatov, N. V. (1998) *Biol. Membr. (Moscow)*, **15**, 490-502.
12. Ignatov, N. V., Belyaeva, O. B., and Litvin, F. F. (1993) *Photosynth. Res.*, **38**, 117-124.
13. Ignatov, N. V., and Litvin, F. F. (1987) *Biofizika*, **32**, 692-695.
14. Venediktov, P. S., and Krivoshejeva, A. A. (1984) *Planta*, **160**, 200-203.
15. Henningsen, K. W., and Boardman, N. K. (1973) *Plant. Physiol.*, **51**, 1117-1126.