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Biosynthesis of Chlorophyll from Protochlorophyll(ide) in Green Plant Leaves

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Abstract—Using spectral methods, the biosynthesis of protochlorophyll(ide) and chlorophyll(ide) in green plant leaves was studied. The main chlorophyll precursors in the green leaves (as in etiolated leaves) were photoactive photocholorophyll(ide) forms Pchl(ide)655/650(448) and Pchl(ide)653/648(440). The contributions into Chl biosynthesis of the shorter-wavelength precursor forms, which were accumulated in darkened green leaves as well, were completely absent (of Pchl(ide) 633/628(440)) or insignificant (of Pchl(ide) 642/635(444)).

Key words: biosynthesis of chlorophyll, chlorophyll(ide), etiolated leaves, green leaves, protochlorophyll(ide), protochlorophyllide oxidoreductase

In studies of the final stage of Chl biosynthesis, the objects of investigation are usually leaves of etiolated dark-grown (during 4-12 days) plant seedlings, where the Chl precursor, protochlorophyll(ide) is accumulated. The main precursor form in the etiolated leaves is Pchlide 655/650(448) [1]. The photoreduction of this form to chlorophyllide starts the chain of biosynthesis of the total of antenna chlorophyll. The shorter-wavelength precursor forms Pchlide 633/628(440) and Pchlide 642/635(444) are accumulated in smaller amounts; apparently, they also can participate in Chl synthesis, though their role is not clear [2].

In juvenile (3-4-day-old) etiolated plants one more reaction chain is observed: it starts with the phototransformation of the long-wavelength form Pchlide 686/676(440) into a shorter-wavelength form Pchlide 653/648(440), non-identical to the main precursor form Pchlide 655/650(448). Its further photoreduction to Chlide 684/676(440) gives start to the biosynthesis of chlorophyll of PS II RC [3].

Abbreviations: Pchlide) protochlorophyllide; POR) protochlorophyllide oxidoreductase; Pchl) protochlorophyll; Pchl(ide)) the form of chlorophyll precursor with phytol presence or absence not established; Chlide) chlorophyllide; Chl) chlorophyll. The first number after pigment symbol corresponds to the position of the maximum of low-temperature fluorescence spectrum (nm), the second number to the position of the long-wavelength absorption band, and the number in brackets to the position of the Soret band in the excitation spectra of low-temperature fluorescence of the native pigment form.

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The bulk of chlorophyll under natural conditions is accumulated in plant leaves not at the stage of the above-mentioned de-etiolation, but during plant growth and leaf-formation in the light. On the other hand, it has long been known that in mature green leaves an intensive Chl biosynthesis constantly takes place, compensating for the dark destruction and photodestruction of the pigment [4]. It remains unclear whether the knowledge of Chl biosynthesis obtained for the etiolated leaves can be extrapolated to that in green plants. The difficulties in the studies of Pchlide and Chl biosynthesis in green leaves arise from the fact that the amounts of the precursor and the intermediates of its phototransformation are by two or three orders of magnitude smaller than the amount of chlorophyll already accumulated in the leaves. Besides, the spectroscopicc measurements are impeded by a high background of fluorescence absorption and fluorescence emission of chlorophylls *a* and *b* in the region where the native precursor forms and the products of their phototransformations absorb and fluoresce.

In the earlier studies of low-temperature fluorescence spectra of green bean leaves, it was shown [5] that in the plants kept in darkness for several hours, the form of the precursor spectroscopically identical to the main photoactive precursor form found in the etiolated leaves (fluorescence at 655 nm) was accumulated and rapidly disappeared under subsequent illumination. The estimation of the concentration of the accumulated precursor (by the spectra of the extracts) showed that through this form the renewal of Chl occurred in green leaves, its rate had been earlier measured by the isotope

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method [4]. Later, the accumulation in green leaves of protochlorophyllide with a fluorescence maximum at 655 nm and phototransformation of this form were confirmed [6-9].

In studies [10-12], it was ascertained that constant Chl synthesis in green leaves was realized not through Pchlide 655/650(448), photoactive in the etiolated leaves, but through the shorter-wavelength precursor forms fluorescent at 633 or 642 nm. However, this opinion was based not on direct measurements of green leaves spectra, but on the comparative study of protochlorophyllide oxidoreductase (POR) catalyzing Pchlide to Chlide photoreduction in normal and mutant etiolated plants. In the normal etiolated plants where Pchlide 655/650(448) is photoactive, two protochlorophyllide oxidoreductases are found, POR-A and POR-B, while in the mutant etiolated plants, where phototransformation of the shorter-wavelength precursor forms is observed, only one form of the enzyme, POR-B, is present [10]. Having correlated these data with the observation of only POR-B being present in green leaves, the authors concluded that in green leaves Chl biosynthesis must occur through the shorter-wavelength precursor forms fluorescent at 633 or 642 nm [10-12].

To elucidate the contributions of different precursor forms in Chl biosynthesis, spectra of green leaves of different plant species were studied under conditions of preliminary darkening and subsequent illumination.

MATERIALS AND METHODS

The objects of investigation were the most mature, the first and second (by order of formation) leaves of green 14-20-day-old plants: bean (*Phaseolus vulgaris* L.) 1024, pea (*Pisum sativum* L.) of Kapital variety, maize (*Zea mays* L.) Bukovinsky variety, and barley (*Hordeum vulgare* L.) of Vizit variety grown on vermiculite and tap water under the light of luminescent lamps (illuminance 30 W/m^2, light period duration 16 h per day, at 24-28°C). In some experiments green leaves of the perennial houseplant hibiscus (*Hibiscus rosa-sinensis* L.) grown under natural illumination were used.

To observe protochlorophyll(ide) synthesis, the plants with their roots immersed in water, or individual leaves of the perennial plant with the cuttings immersed in water were placed in a thermostat and there incubated in darkness at 27°C for several hours to several days (see figure legends). Then, pieces of the leaves were cut symmetrical relatively to the midrib, one piece being illuminated to observe the phototransformation of the resynthesized protochlorophyll(ide), the other being the dark control.

To stimulate Pchl(ide) biosynthesis, in some experiments the roots of the annual plants were submerged in solution of Δ-aminolevulinic acid (10 mM) [13] and the plants were dark-incubated for several hours to several days, cuttings of hibiscus leaves being submerged in this solution.

Illumination of the temporarily darkened leaves was performed at room temperature with an incandescent lamp supplied with a SZS-23 light filter transmitting light at 400-750 nm. In some experiments interference light filters were used (Carl Zeiss, Germany) with transmittance maxima at 650 and 680 nm (transmittance in the maximum was 35%, the half-width of the transmittance band was 8 nm).

The dark resynthesis of Pchl(ide) and its subsequent phototransformation into Chl were studied by measuring low-temperature (−196°C) fluorescence spectra and fluorescence excitation spectra on a spectrofluorimeter [14] with two monochromators (slit spectral widths of both did not exceed 1.5 nm). Fluorescence of the leaves was measured from the front at an angle of about 45° to the fluorescence exciting beam.

For the selective excitation of the fluorescence of Pchl(ide) and Chl *a* in green leaves, the monochromatic exciting light of 440 nm was applied (the wavelength within the Soret band of the native forms of these pigments). This allowed (see below) to exclude completely Chl *b* fluorescence which was known to be spectrally close [7, 8] to that of Pchlide 655/650(448).

To estimate the Pchl(ide)/Chl(ide) ratio, the pigments were extracted from the leaves with dimethyl formamide saturated with $MgCO_3$, the fluorescence spectra of the extracts were measured, fluorescence being excited at 430 nm.

RESULTS AND DISCUSSION

The fluorescence spectra of green leaves taken from plants growing in the light (10-16 h) and the spectra of the extracts from these leaves did not show the presence of Pchl(ide). Only the intensive bands of Chl fluorescence at 670-735 nm were observed. The quantitative analysis showed that at the available sensitivity of the measurements, this result was possible if the relative Pchl(ide) concentration (in respect to Chl) did not exceed 0.01%. When these samples were placed in darkness, Pchl(ide) synthesis in the leaves was observed; that could be seen by the appearance of the fluorescence typical of Pchl(ide) in the fluorescence spectra of the leaf extracts (the fluorescence maximum at 628 nm, the Soret band in the fluorescence excitation spectrum at 438 nm). The first signs of Pchl(ide) synthesis in the extracts spectra were seen after 16-20 h of dark incubation of the plants, as dependent on plant species; Pchl(ide)/Chl(ide) ratio was not higher than 0.3-0.5%. By the end of the second day of dark incubation the ratio reached 1-1.5%, by the end of the fourth day it was 3%. The further dark accumulation of Pchl(ide) slowed down (Fig. 1, curve *1*).

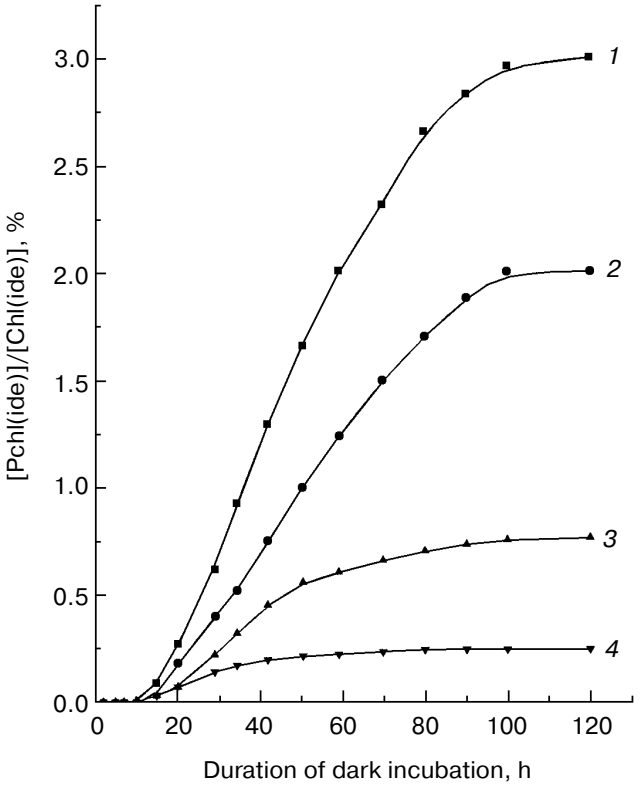

Fig. 1. Accumulation of Pchl(ide) in green leaves of 3-week-old barley plants during dark incubation: *1*) Pchl(ide)/Chl(ide) concentration ratio for the extracts from dark-incubated leaves; *2-4*) the same values measured *in vivo* for the native forms Pchlide 655/650(448) (*2*), Pchl(ide) 633/628(440) (*3*), and Pchl(ide) 642/635(444) (*4*).

The measurements of the fluorescence spectra of green leaves *in vivo* showed that at the earliest stage of dark incubation (16 h) the fluorescence bands at 631-633, 642, and 655 nm were formed (Fig. 2, curves *1* and *2*). A comparison with the spectra of the leaf extracts (Fig. 1, curves *1-4*) showed that these bands belonged to the Chl precursor synthesized in darkness. Of these bands two (633 and 642 nm) belonged to the native short-wavelength forms of protochlorophyll(ide) analogous to the short-wavelength Pchl(ide) forms found in the etiolated leaves: Pchl(ide) 633/628(440) and Pchl(ide) 642/635(444). This conclusion is confirmed by the comparison of the fluorescence excitation spectra of the fluorescence bands with the maxima at 633 and 642 nm of the etiolated leaves to those of the temporarily darkened green leaves (Fig. 3). In our experiments, the dominance of 642 nm band (which is usually not much pronounced in fluorescence spectra of the etiolated leaves) was achieved by the treatment of the plants with Δ-aminolevulinic acid [13] (Fig. 4b, curves *1* and *2*); this allowed the measurements of the individual fluorescence excitation spectrum for the fluorescence at 642 nm, through minimizing the contributions of the adjacent bands at 633 and 655 nm. As seen from Fig. 3 (curves *2* and *3*), the positions of the maxima and the general shape of the fluorescence excitation spectrum (the ratios of the intensities of the main band to that of the short-wavelength satellite and of the band half-widths) are practically equal for the etiolated and darkened green leaves. The Soret band in the fluorescence excitation spectrum for the fluorescence at 633 nm is located at 440 nm and in the excitation spectrum for the 642 nm fluorescence at 444 nm both for etiolated and darkened green leaves.

In the range of 600-660 nm in the fluorescence spectra of the darkened green leaves, the most intense was the 655 nm band, it would be reasonable to associate this band with the main photoactive form of the precursor in the etiolated leaves, Pchl(ide) 655/650(448). In favor of this assumption is not only the position of the fluorescence emission maximum but also the fluorescence excitation spectra for the fluorescence at 655 nm that were identical in the etiolated and the darkened green leaves (Fig. 3, curve *1*) (the main maximum of the fluorescence excitation spectrum was located at 448 nm, the longer-wavelength satellite at 460 nm).

Nevertheless, to draw the conclusion that the 655 nm fluorescence band in the spectra of the darkened green leaves belonged to protochlorophyllide, we must prove that

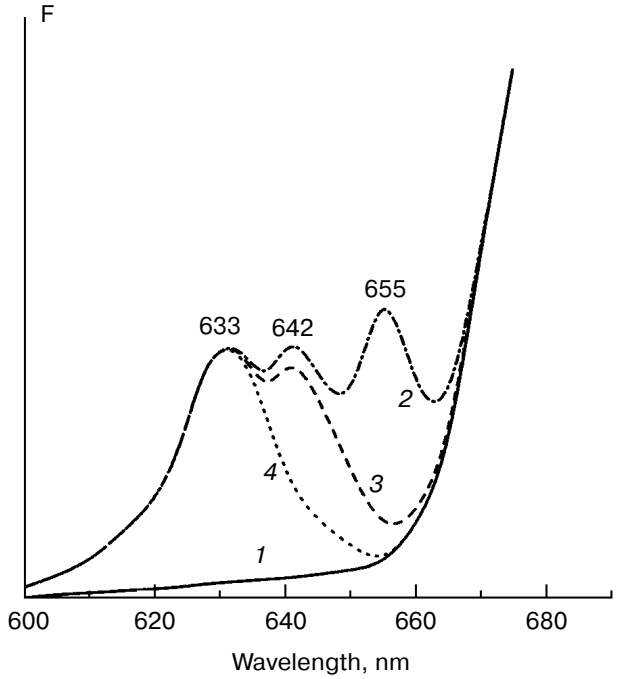

Fig. 2. Low-temperature (−196°C) fluorescence spectra (F) of hibiscus green leaves: *1*, *2*) spectra measured before and after 16-h dark incubation of the sample; *3*) sample *2* plus 15-sec illumination with white light (10 W/m^2); *4*) sample *3* plus 10-min illumination with white light (10^3 W/m^2).

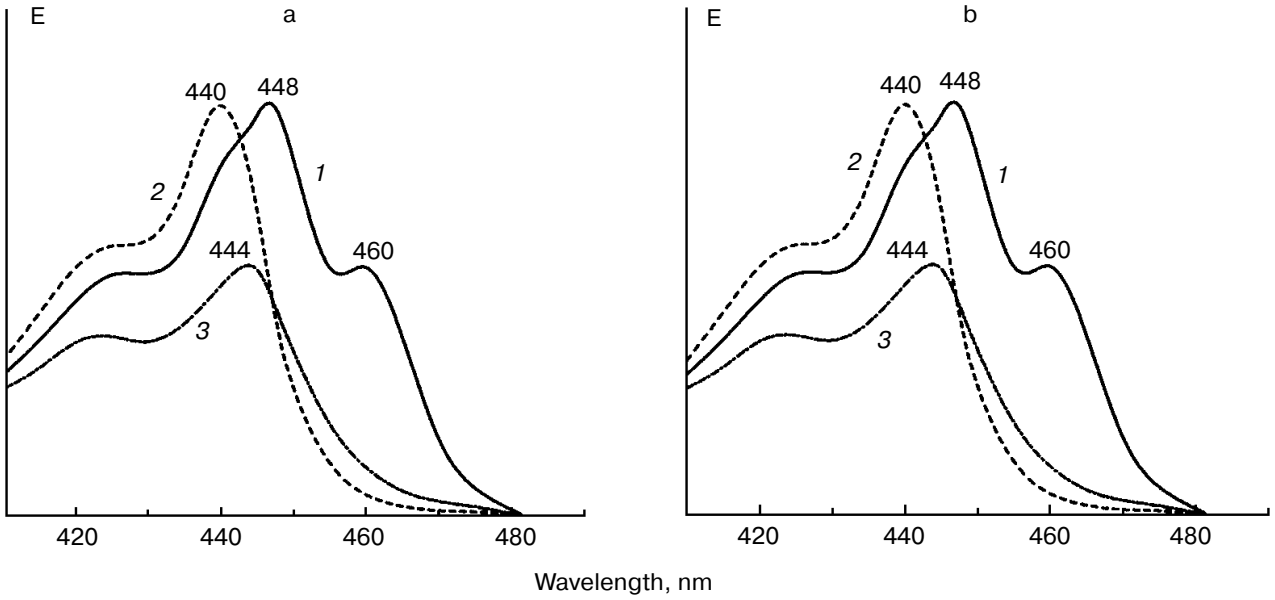

Fig. 3. Low-temperature (−196°C) fluorescence excitation spectra (E) of hibiscus green leaves dark-incubated for 16 h (a) and of etiolated bean leaves (b). Fluorescence was registered at 655 (*1*), 633 (*2*), and 642 nm (*3*). The spectrum (b), curve *3* was measured for etiolated bean leaf after 2 days incubation in Δ-aminolevulinic acid solution (10 mM). For the fluorescence spectra corresponding to the fluorescence excitation spectra in panel (a), see Fig. 2; for those corresponding to the fluorescence excitation spectra in panel (b), see Fig. 4b.

the fluorescence at 655 nm was not even partially due to Chl *b*. The fact that the 655 nm fluorescence band is not connected with Chl *b* (whose fluorescence may be manifested at 655 nm in spite of the efficient energy migration from Chl *b* to Chl *a* [7, 8]) may be proved by the position of the Soret band in the fluorescence excitation spectra of the 655-nm fluorescence band. It is located (Fig. 3) at 448 nm, i.e., in the shorter-wavelength region than the Soret band in the fluorescence excitation spectrum of Chl *b in vivo* (465-470 nm [7]). Besides, even on a short-term

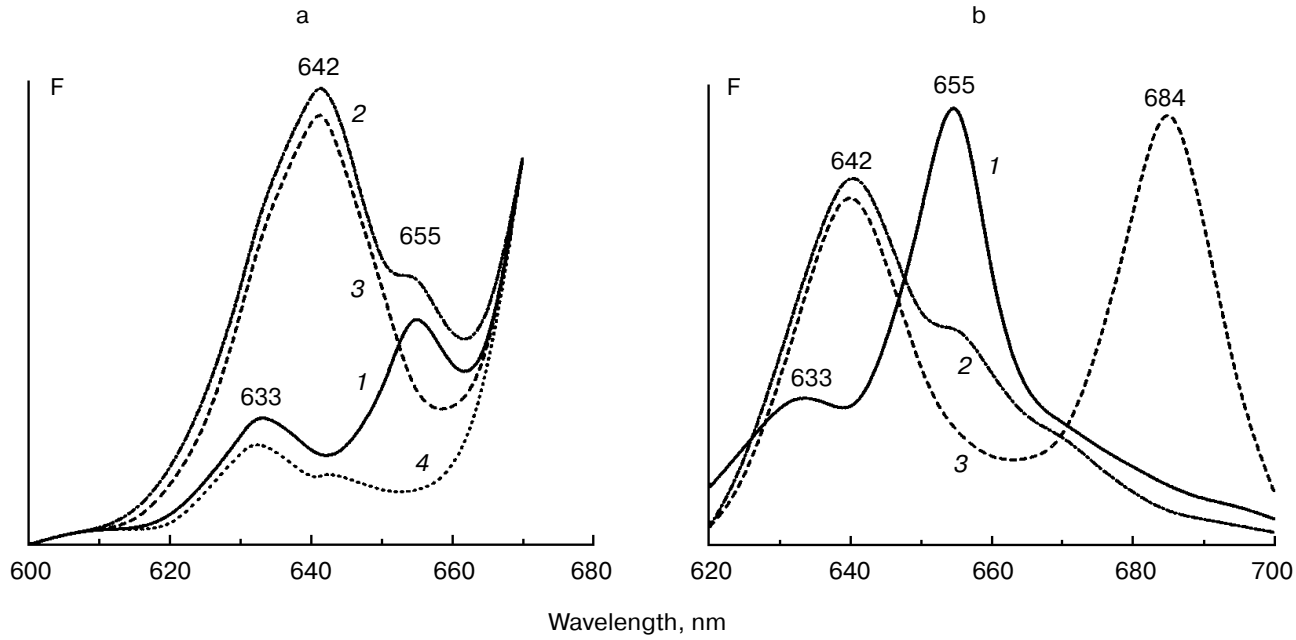

Fig. 4. Low-temperature (−196°C) fluorescence spectra (F) of green leaves of 3-week-old bean plants pre-incubated for 2 days in darkness (a) and of 7-day-old etiolated bean leaves (b): *1*) control sample pre-incubated for 2 days in darkness in water; *2*) the sample pre-incubated for 2 days in darkness in Δ-aminolevulinic acid solution (10 mM); *3*) sample *2* plus 15-sec illumination with white light (10 W/m^2); *4*) sample *3* plus 30-min illumination with white light (10^3 W/m^2).

(several seconds) illumination of the darkened green leaves with the low intensity (10 W/m^2) light, the 655 nm fluorescence band completely disappeared from the fluorescence and derivative spectra of the leaves (Figs. 2, 4, and 5) as could be expected due to Pchlide phototransformation to Chlide. Fast photoinduced disappearance of 655-nm band from the fluorescence spectra was observed not only at the positive but at the low temperature values as well, up to −70°C. This excludes the possible supposition of the photoinduced rebuilding of the pigment Chl *b*–Chl *a* complex being the cause of the quenching of Chl *b* fluorescence due to an increased energy migration to Chl *a*.

The similarity of the native Pchlide form fluorescent at 655 nm found in the green leaves to the precursor form Pchlide 655/650(448) contained in the etiolated leaves was manifested not only in the form of their spectra. These forms had close temperature limits of the reactions of phototransformation into Chlide: both in etiolated and green leaves this photoreaction occurred not only at room temperature but also at the low temperature values (to −80°C). Another common parameter for these forms was practically the same phototransformation halftime measured during the illumination of the samples with monochromatic light of 650 nm. Taking into consideration the screen effect of Chl *b* at 650 nm, characteristic of the green leaves, one can conclude that the quantum yield of Pchlide phototransformation in the green leaves was at least not lower than that of Pchlide 655/650(448) in the etiolated leaves (where it was 40%).

This relates the 655 nm fluorescence band of the darkened green leaves to the photochemically active Pchlide form spectrally identical to the main precursor form Pchlide 655/650(448) of the etiolated leaves [1, 2, 5]. Thus, in the green leaves during dark incubation Pchl(ide) is synthesized in three native forms, spectrally close to the native forms known for the etiolated leaves: Pchl(ide) 633/628(440), Pchl(ide) 642/635(444), and Pchlide 655/650(448) [1, 2].

To what extent do these three protochlorophyll(ide) forms participate in Chl biosynthesis in green leaves? The photochemical activity of the Pchl(ide) form with fluorescence at 655 nm is beyond question because its fluorescence band disappears completely from the spectra even on a short-term illumination of the sample with a low-intensity light. The short-wavelength Pchl(ide) form with fluorescence at 631-633 nm of the darkened green leaves is stable even under their illumination with intensive light (10^3 W/m^2) for 30 min (Figs. 2 and 4a). It disappears on a longer exposure of the darkened leaves to light—during 10-16 h (see above, and Fig. 2, curve *1*). No Pchl(ide) was found in the extracts of these leaves. This phenomenon could have been explained by the destruction of the short-wavelength form under a long-term illumination. However, its disappearance is observed after a long-term exposure to even very weak light (0.1 W/m^2), this contradicts to the supposition of photodestruction. In

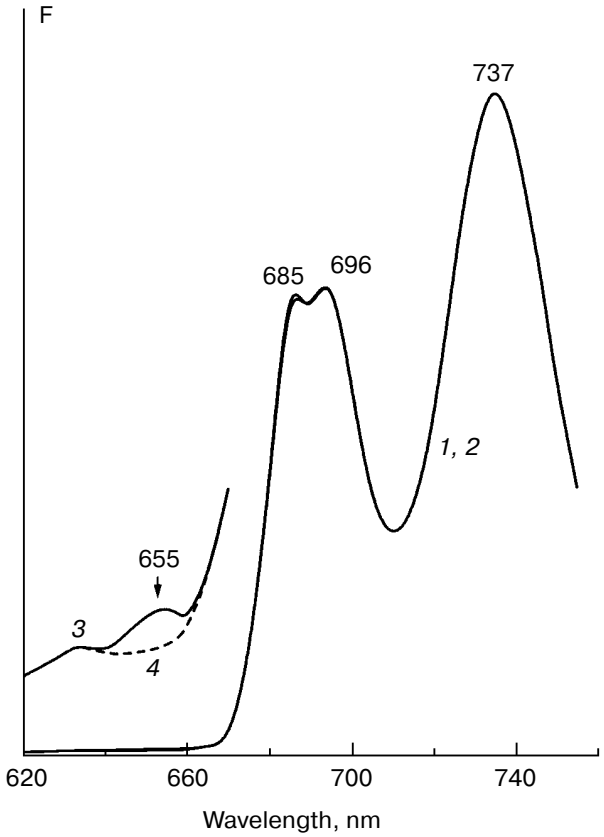

Fig. 5. *1*, *3*) Low-temperature (−196°C) fluorescence spectra (F) of green leaves of 3-week-old maize plants pre-incubated in darkness for 4 days; *2*, *4*) the same sample plus 15-sec illumination with white light (10 W/m^2); *3*, *4*) the same as *1* and *2*, respectively, but enlarged by a factor of 20.

the interpretation of the results of these experiments, one should take into account the data obtained on etiolated leaves: the short-wavelength Pchl(ide) form is the precursor of the main photoactive form, Pchlide 655/650(448) in the chain of dark pigment synthesis [1, 2, 15]. Under a constant long-term illumination of the darkened green leaves, very low stationary concentrations not only of the fast transformable photoactive Pchlide 655/650(448) but also of its precursor, Pchl(ide) 633/628(440) are formed. The rate constant of the dark reaction Pchl(ide) 633/628(440) $\rightarrow$Pchlide 655/650(448) must be 10^{-3}-10^{-4} sec^{-1}, in the absence of the product. Under the dark incubation its value is considerably decreased, which can be accounted for by inhibition of the precursor biosynthesis by the reaction product, Pchlide 655/650(448).

The most complicated is the question of photochemical activity of the precursor form with fluorescence at 642 nm, because 642-nm fluorescence band is observed in the spectra as an individual not very pronounced maximum only at the earliest stages of darkening—16 h after the start of dark incubation (Fig. 2). Longer dark incubation leads to an increase exclusively of 633 and 655 nm

bands fluorescence. On the background of their fluorescence, the photoactivity and even the presence of the form fluorescent at 642 nm are not easily recognizable (Fig. 4a, curve *1*; Fig. 6a). Illumination of the samples adjusted for the investigation of fluorescence at 642 nm (dark-incubated during 16 h) caused a complete disappearance of 655 nm fluorescence band from the spectra after several seconds; at the same time, the decrease in fluorescence at 642 nm was insignificant and slow as compared to the changes in the 655 nm band (Fig. 2, curves *2* and *3*). For example, the complete disappearance of 642-nm band occurred not after several seconds of illumination with a low-intensity light but after 20-30-min illumination with intense (10^3 W/m^2) light (Fig. 2, curve *4*). However, the light-induced disappearance of 642-nm fluorescence band was also observed at the low temperature values (to −70°C) that, in our opinion, is indicative of the photochemical nature of the process. Apparently, in this case Pchl(ide) photoreaction with a very low quantum yield occurs. It cannot be excluded that this effect is connected not with Pchlide photoreduction to Chlide but with the pigment photodestruction. The same conclusions were drawn by us for the experiments that showed an increase in accumulation of the precursor fluorescent at 642 nm in green leaves pre-incubated in darkness in 10 mM solution of Δ-aminolevulinic acid (Fig. 4a). This is known [13] to result in the predominant accumulation of Pchl(ide) with fluorescence at 642 nm in comparison to the other precursor forms (Fig. 4b).

Unfortunately, we did not succeed in measuring *in vivo* the fluorescence spectra of the products of phototransformation of Pchl(ide) accumulated during the dark incubation of green leaves. As known from the studies of the etiolated leaves ([1] and Fig. 4b), the first product must be Chlide with fluorescence at about 684 nm. But in the darkened green leaves this fluorescence was not manifested on the background of the fluorescence of Chl *a* (maximum at 685 nm), which is at least three orders of magnitude more intense than fluorescence of Chlide synthesized *de novo*. Besides, the fluorescence of the synthesized Chlide is effectively reabsorbed by chlorophyll *a* of green leaves. However, the fact that in green leaves the synthesized Pchlide in the light is transformed in Chl(ide) and is not decomposed was demonstrated with the spectra of pigment extracts from green leaves pre-incubated in darkness during 4 days when Pchl(ide)/Chl(ide) ratio reached 3 to 4%. In this case in the extracts of leaves a change in the Pchl(ide)/Chl(ide) ratio in favor of Chl(ide) was registered. In the fluorescence spectra of the leaves, this change was accompanied by a decrease only of 655-nm fluorescence band (Fig. 5). This shows that the native Pchlide form fluorescent at 655 nm is the main precursor of Chl(ide) in the green leaves as well. After 4-day incubation of green leaves, no degradation of the photosynthetic apparatus was observed; this was indicated by the shape of the longer-wavelength part of the fluorescence spectrum, typical of the normal green leaves (Fig. 5). Green plants exposed to light after a long-term dark incubation preserved their photosynthetic activity.

Previously, in studies of etiolated juvenile plants, we found, alongside with the main photoreaction of the precursor transformation Pchlide 655/650(448) → Chlide 684/676(448), one more chain of Pchlide phototransformations in Chlide starting with phototransformation of the newly found longer-wavelength Pchlide 686/676(440) into the Pchlide 653/648(440), non-identical to the main form Pchlide 655/650(448) [3]. The existence and photoactivity of Pchlide 653/648 in etiolated leaves were recently confirmed [9]. Does this reaction proceed in the darkened green leaves? To observe directly the formation of the minor longer-wavelength form Pchlide 684/676(440) is difficult due to the intensive fluorescence of Chl in green leaves. However, one can try to register the phototransformation of this form, its product being Pchlide 653/648(440), judging by the studies of etiolated leaves.

To do this, green leaves pre-incubated in darkness for 3 days were illuminated with 650-nm monochromatic light until the fluorescence of the main precursor form, Pchlide 655/650(448) completely disappeared from the spectra of the leaves (Fig. 6a, curves *1* and *2*). Such illumination, as it followed from our experiments with etiolated juvenile plants, did not lead to phototransformation of the long-wavelength Pchlide 686/676(440) [3]. The subsequent illumination of the sample with 680-nm monochromatic light (by the following scheme: darkened green leaf → 650-nm light → 680-nm light) caused the formation of fluorescence band at 653 nm in the spectra (Fig. 6a, curve *3*), the maximum of the fluorescence excitation spectrum of the newly formed 653-nm fluorescence band was located not at 448 nm (as that of the 655-nm band lost after the first illumination) but at 440 nm (Fig. 6b, curves *1* and *3*), which is typical of Pchlide 653/648(440). When the sample, with the 653-nm band already present in the fluorescence spectrum, was additionally illuminated with monochromatic light of 650 nm (by the scheme: darkened green leaf → 650-nm light → 680-nm light → 650-nm light), 653-nm band was observed to disappear from the spectra (Fig. 6a, curve *4*). These results, in our opinion, are evidence of the fact that in green leaves, alongside with the main reaction Pchlide 655/650(448) → Chlide, one more sequence of Chl biosynthesis reactions takes place, initiated by another precursor form: Pchlide 686/676(440) →680-nm light → Pchlide 653/648(440) →650-nm light → Chl(ide). It is similar to the chain of reactions leading to the formation of Chl of the reaction centers of PSII in etiolated leaves [3].

Summing up the data of the above-described experiments, we can make a number of conclusions. Our results obtained with monocotyledonous, dicotyledonous, annual, and perennial plants confirm the existing data [5-9] of the constant chlorophyll synthesis in the green leaves proceeding through the main photoactive form Pchlide 655/650(448), like in the etiolated leaves. The

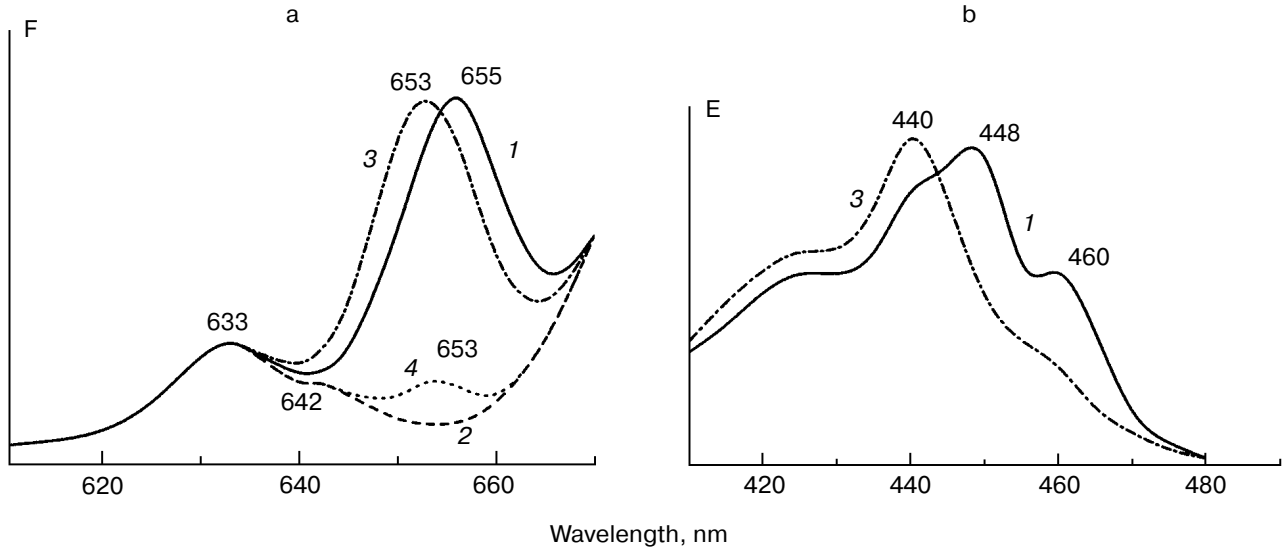

Fig. 6. Low-temperature (−196°C) fluorescence spectra (a) and 654-nm fluorescence excitation spectra (b) measured for green leaves of 3-week-old barley plants pre-incubated in darkness for 3 days: *1*) non-illuminated sample; *2*) sample *1* plus 60-sec illumination with monochromatic light of 650 nm; *3*) sample *2* plus 120-sec illumination with monochromatic light of 680 nm; *4*) sample *3* plus 60-sec illumination with monochromatic light of 650 nm.

supposition of Lebedev and coworkers about the dominant role of the shorter-wavelength precursor forms fluorescent at 633 and 642 nm in Chl biosynthesis in green leaves [10-12] was not confirmed in our studies. The contribution of these forms in Chl biosynthesis in green leaves is either absent or considerably smaller than that of Pchlide 655/650(448). It cannot be excluded that the short-wavelength forms of the precursor are involved in Chl synthesis but indirectly, through the main photoactive form fluorescent at 655 nm, in the same way that is supposed for etiolated leaves [15]. In Chl biosynthesis in green leaves, like in etiolated leaves, another form of the precursor, Pchlide 653/648(440) is involved. It is the product of photochemical reaction of the longer-wavelength protochlorophyllide form Pchlide 686/676(440). On the whole, the final stage of Chl(ide) biosynthesis in green leaves may be described by the following scheme, close to that proposed by us for etiolated leaves [3]:

Pchlide 655/650(448) → light → Chlide;

Pchlide 686/676(440) → light → Pchlide 653/648(440) → light → Chlide.

As noted, in the etiolated leaves two forms of protochlorophyllide oxidoreductase were found. In the green leaves dark-incubated for 20 h, only one of them, POR-B, was present [10]. Correlating these facts with the results of the above-described spectral studies it can be concluded that POR-B catalyzes photoreactions of two types of chlorophyll precursor complexes: Pchlide 655/650(448) and Pchlide 653/648(440).

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