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A New Pathway of Photoinactivation of Photosystem II. Irreversible Photoreduction of Pheophytin Causes Loss of Photochemical Activity of Isolated D1–D2–Cytochrome b_{559} Complex

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Abstract—A new pathway of photoinactivation of photosystem II (PS II) connected with irreversible photoaccumulation of reduced pheophytin (Ph) in isolated D1–D2–cytochrome b_{559} complexes of reaction center (RC) of PS II was discovered. The inhibitory effects of white light illumination on photochemical activity of D1–D2–cytochrome b_{559} complexes of RCs of photosystem II, isolated from pea chloroplasts, have been compared under anaerobic conditions in the absence and in the presence of sodium dithionite, electron transfer from which to the oxidized primary electron donor $P680^+$ results in the photoaccumulation of anion-radical of the primary electron acceptor, Ph^{-}. In both cases, prolonged illumination (1-5 min, 120 W/m^2) led to a pronounced loss of the photochemical activity as it was monitored by measuring the amplitude of the reversible photoinduced absorbance changes at 682 nm related to the photoreduction of Ph. The extent of the photoinactivation depended on the illumination time and pH of the medium. At pH 8.0, the presence of dithionite during photoinactivation brought about a protective effect compared to that in a control sample. In contrast, lowering pH to 6.0 increased the sensitivity to photoinactivation in the dithionite-containing samples. For 5 min irradiation, the photochemical activity in the absence and in the presence of dithionite decreased by 35 and 72%, respectively (this was accompanied by an irreversible bleaching of the pheophytin Q_x absorption band at 542 nm). Degradation of the D1 and D2 proteins was not observed under these conditions. A subsequent addition of an electron acceptor, potassium ferricyanide, to the illuminated samples restored neither the amplitude of the signal at 682 nm nor absorption at 542 nm. It is suggested that at pH < 7.0 the photoaccumulated Ph^{-} is irreversibly converted into a secondary, most probably protonated form, that does not lead to destruction of the RCs but prevents the photoformation of the primary radical pair [$P680^+Ph^{-}$]. A possible application of this effect to photoinactivation of PS II *in vivo* is discussed.

Key words: photosystem II, reaction center, pheophytin, photoinactivation

According to the current view, photoinactivation of the electron-transfer reaction of photosynthesis, called photoinhibition, first involves the destruction of pigment–protein complex of PS II [1]. This phenomenon is connected with the high sensitivity of PS II to photoinduced degradation due to its ability to form a very active biological oxidizer, the anion-radical of chlorophyll *P*680, which leads to the oxidation of water (redox potential of $P680^+/P680$ is +1.12 V) [2]. The degradation is known to be inside the reaction center of PS II. The D1-protein (which is the most important component of this complex) undergoes continuous degradation and resynthesis in the light and more rapid *de novo* synthesis than other proteins of the thylakoids membrane [3]. The quick renovation of D1-protein is one of many mechanisms reducing the negative effects of light. The injured RC can be repaired due by synthesis of new protein D1 [4]. However, notwithstanding the protection, the destruction of reaction centers of PS II can occur under rather low intensity of light even without any other factors of stress. When the velocity of degradation exceeds the velocity of reparation, the reduction of photochemical activity or photoinactivation is observed; this can lead to destruction and degradation of the reaction center of PS II.

Isolated D1–D2–cytochrome b_{559} complexes of photosystem II RC is a convenient experimental system

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for investigation of primary and secondary processes of electron transport, and also for the investigation of sensitivity of PS II to photodestruction. Studies conducted with this complex can be important for understanding the molecular processes happening under conditions of physiological stress. These complexes consist of D1 and D2 proteins, α- and β-subunits of cytochrome b_{559}, and the product of the *psbI* gene [5, 6]. Highly purified RC complex samples are known to bind four-six molecules of chlorophyll *a*, two molecules of pheophytin *a*, and one-two molecules of β-carotene [5, 7, 8].

The two main mechanisms of photoinactivation have been described based on investigations using isolated D1–D2–cytochrome b_{559} complexes with artificial donors and acceptors of electrons as a model system for studying mechanisms leading to the destruction of PS II under the influence of light. It was shown that without additions isolated RC is stable against photodestruction if oxygen is removed from the medium. The photochemical activity of isolated RC is the photoinduced formation and recombination of ion-radical pair [$P680^+Ph^-$]. When oxygen is present in the medium, RC is subject to photodestruction. The mechanism of this destruction involves the influence of singlet oxygen ($^1O_2$), which is formed when a molecule of triplet oxygen ($^3O_2$) reacts with the triplet excited state of chlorophyll *P*680 ($^3P680$). Singlet oxygen is highly active and causes irreversible bleaching of chlorophyll *P*680 [9] and destruction of some amino acid residues [10]. Under these conditions, decrease in photochemical activity and protein conformation changes [11] and degradation [12, 13] are observed. Protein D1 degrades to fragments with molecular masses of 23 and 10 kD. This mechanism of photoinduced destruction is attributed to the acceptor side.

In the case of artificial electron acceptor (silicomolybdate or 2,5-dibromo-3-methyl-1,6-isopropyl-*p*-benzoquinone) added to isolated RC, electrons from Ph^- with be able to pass on to these exogenous acceptors, and the life-time of chlorophyll $P680^+$ will be increased [6]. Under these conditions RC also become sensitive to inhibition by light whether or not oxygen is present [14, 15]. Irreversible changes in absorption caused by light under these conditions differ from those of the oxygen-dependent photodestruction on the acceptor side. In this case the high oxidizing potential of $P680^+$ leads to the irreversible oxidation of β-carotene and accessory chlorophyll *P*670 [10, 16]. They are unstable in their cationic forms and can degrade. Together with this phenomenon, the degradation of D1-protein, forming fragments with molecular masses of 24 and 9 kD is observed [12, 17]. This mechanism of the photoinduced destruction of RC, caused by accumulation and destructive influence of cation-radical of $P680^+$, is called the mechanism of the donor side.

These two mechanisms are supposed to occur in intact systems. The acceptor side mechanism becomes possible under intensive illumination, when the double reduction of primary quinone acceptor of electrons, plastoquinone Q_A, happens and double reduced plastoquinone (Q_AH_2) is formed [18]. This may enlarge the possibility of the recombination of ion-radical pair [$P680^+Ph^-$] and generate an excited triplet state of *P*680, which can lead to the formation of $^1O_2$. An excited triple state of *P*680 can appear when recombination of long-living states, as $S_2Q_B^-$ occurs [19]. The donor side mechanism can also occur when the destabilization of the reactions of water oxidation is observed, for example, under cold stress conditions [20].

In our opinion, in the studies discussed above the possibility of one or the other pathway of inactivation of RC of photosystem II, which could be connected with photoaccumulation of long-living state [$P680Ph^-$], has not investigated yet. When exogenous electron donor (e.g., sodium dithionite) is added to isolated RC and all of the electron-accepting substances that are capable to oxidize Ph^- are absent, such state can be obtained under light conditions. This article describes our investigation of isolated D1–D2–cytochrome b_{559} complexes as a model for assay of the photoinduced inactivation of PS II in the presence of exogenous donor of electrons (sodium dithionite), when the photoaccumulation on anion-radical Ph^- occurs and RC is present in the [$P680Ph^-$] state.

MATERIALS AND METHODS

Tris and Triton X-100 were obtained from Reanal (Hungary); *n*-dodecyl-β-D-maltoside and SDS were purchased from Sigma (USA). Sodium dithionite and all other reagents of "chemically pure" grade were obtained from domestic sources.

Reaction centers of PS II were taken from pea (*Pisum sativum*) leaves by a method described previously [21]. The concentration of samples was 60 μg chlorophyll per milliliter. Preparations of RC contained polypeptides of D1 (32 kD), D2 (34 kD), cytochrome b_{559} (9 kD), and traces of antennae protein with a molecular mass 43 kD. There were six-eight molecules of chlorophyll *a* and one-two molecules of β-carotene per two molecules of pheophytin *a*. For calculation of quantities of reduced pheophytin, the extinction coefficient $0.32 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ (λ = 682 nm) was used [22].

The experiments were conducted under anaerobic conditions. For this purpose, the system containing glucose (20 mM), glucose oxidase (0.1 mg/ml), and catalase (0.039 mg/ml) was used [23]. Initial samples of RC were suspended in different buffer systems (50 mM Tris-HCl, pH 8.0; 50 mM Tris-HCl, pH 7.0; 50 mM Mes-NaOH, pH 6.0) until the concentration of chlorophyll attained 5 μg/ml. Then sodium dithionite (3 mg/ml) was added to the experimental solutions, and samples lacking dithionite were used as controls. These preparations containing 15 mM NaCl and 0.1% *n*-dodecyl-β-D-maltoside (DM)

without dithionite were placed into a closed glass cuvette (1 × 1 × 2 cm) and were illuminated by white light (120 W/m^2) using a LETI-60 light source (Russia) with a heat filter at room temperature. Then the photochemical activity of experimental and control samples of RC was measured. It was determined using a phosphoroscopic device using the amplitude of the reversible decrease of absorption at 682 nm (ΔA_{682}), connected with photoaccumulation of pheophytin in the presence of sodium dithionite (3 mg/ml) and methyl viologen (10^{-6} M) [24]. When measuring samples containing sodium dithionite of the required concentration, the methyl viologen was not added to the solutions.

Spectral changes were measured with a UV-1601PC spectrophotometer (Shimadzu, Japan).

The proteins were analyzed by gradient electrophoresis in 12-18% polyacrylamide gel in the presence of 6 M urea and 0.1% SDS [25]. The upper electrode Tris-glycine buffer (150 mM, pH 8.3) contained 0.1% SDS. Proteins in the gels were stained with 0.23% Coomassie G-250. Densitograms were scanned with a Specord M-40 spectrophotometer (Germany) with a scanning device after electrophoresis.

RESULTS AND DISCUSSION

Nanba and Satoh [5] indicated that isolated D1–D2–cytochrome b_{559} complex of PS II does not contain quinone acceptors Q_A and Q_B, thus the primary photochemical reaction in this RC complex is restricted to electron transfer from excited primary donor of electrons *P*680* to one of the pheophytin *a* (Ph) molecules [6]. The primary ion-radical [$P680^+Ph^-$] formed has a rather short lifetime (30-40 nsec at room temperature) and the system can easily recombine into the initial state [*P*680Ph] or into the triplet state [26]. However, in the presence of exogenous electron donor (e.g., dithionite), which is capable to the effective electron transfer to $P680^+$, competing with a recombination of charges between $P680^+$ and Ph^-, the state [$P680Ph^-$] can be accumulated under anaerobic conditions in light [24]. In this case the amplitude of reversible changes of absorption (ΔA), connected with a reversible photoreduction of Ph to Ph^- can be used as a measure of the photochemical activity of RC (the differential absorption spectrum of "light minus dark", corresponding to the spectrum of "Ph^- minus Ph"). In this work we studied the influence of the inhibitory light in the presence of exogenous electron donor, dithionite, on the amplitude of reversible reduction of absorption at 682 nm (ΔA_{682}) (in the maximum of ΔA spectrum connected with a photoaccumulation of Ph^-), or in other words the influence of inhibiting light in the presence of exogenous electron donor on the quantity of chemically active and capable of photoseparation changes of RC was studied. A sample of RC under light without dithionite was used as a control. The experiment was conducted in the following manner: the initial samples of RC was resuspended in three different buffer systems with different pH values (8.0, 7.0, 6.0) and containing 15 mM NaCl, 0.1% DM and a system providing the removal of oxygen from the medium. Then they were illuminated by white light (120 W/m^2) in the presence of dithionite (3 mg/ml) (experimental sample) and without dithionite (control sample). For every light exposure a new sample was used. Values of photoinduced ΔA (at 682 nm) were with a phosphoroscopic device, using white light illumination during 4 sec. Since the only one reaction of photoaccumulation of anion-radical Ph^- was used in the experiment and in the measurement of the photochemical activity, it is important to note that the short time of illumination (4 sec) and the weak light intensity (0.15 W/m^2) used in photochemical activity measurements did not influence the result of the experiment. The dark incubation of RC samples for 10 min in the 50 mM Tris-HCl buffer (pH 8.0), 50 mM Tris-HCl buffer (pH 7.0), and 50 mM Mes-NaOH buffer (pH 6.0) was found to cause no significant changes in their absorption spectra and photochemical activity.

Figure 1 (a, b, c) shows the dependence of amplitude of the reversible photoinduced change of absorption (at 682 nm) of RC samples on the time of illumination at different pH values. It can be concluded that when D1–D2–cytochrome b_{559} complex is illuminated at weakly basic pH (8.0) the presence of dithionite caused a stabilizing effect during the photoinactivation process. In 5 min of photoinactivation, with dithionite the photochemical activity was reduced by only 37%; at that time without dithionite 61% of activity is lost (Fig. 1a). The opposite situation was revealed under conditions when the experiment was carried out at weakly acidic pH (6.0) (Fig. 1b); from the beginning of the illumination a considerable difference in curves of the decrease of photochemical activity of samples (control and experimental) is observed. The presence of dithionite and illumination considerably increased the photoinactivation of D1–D2–cytochrome b_{559} complex. Within 5 min of illumination the amplitude of the reversible photoinduced decrease of absorption of Ph (at 682 nm) decreased by 35% for the control sample, and by 72% for the experimental sample. At neutral pH (7.0) (Fig. 1c) the photochemical activity of RC decreased for the first 3 min identically for experimental and control samples. With further illumination the velocity of photoinactivation in the presence of dithionite is higher than without it.

These data show that photoinactivation of isolated D1–D2–cytochrome b_{559} complex in the presence of dithionite depends on pH of the medium. At pH > 7.0 the presence of dithionite in the sample provides a stabilizing effect, but at pH < 7.0 the addition of dithionite to the sample causes an increase in its sensitivity to the light. Illumination by the inhibitory light should be noted to cause the decrease of the photochemical activity in the

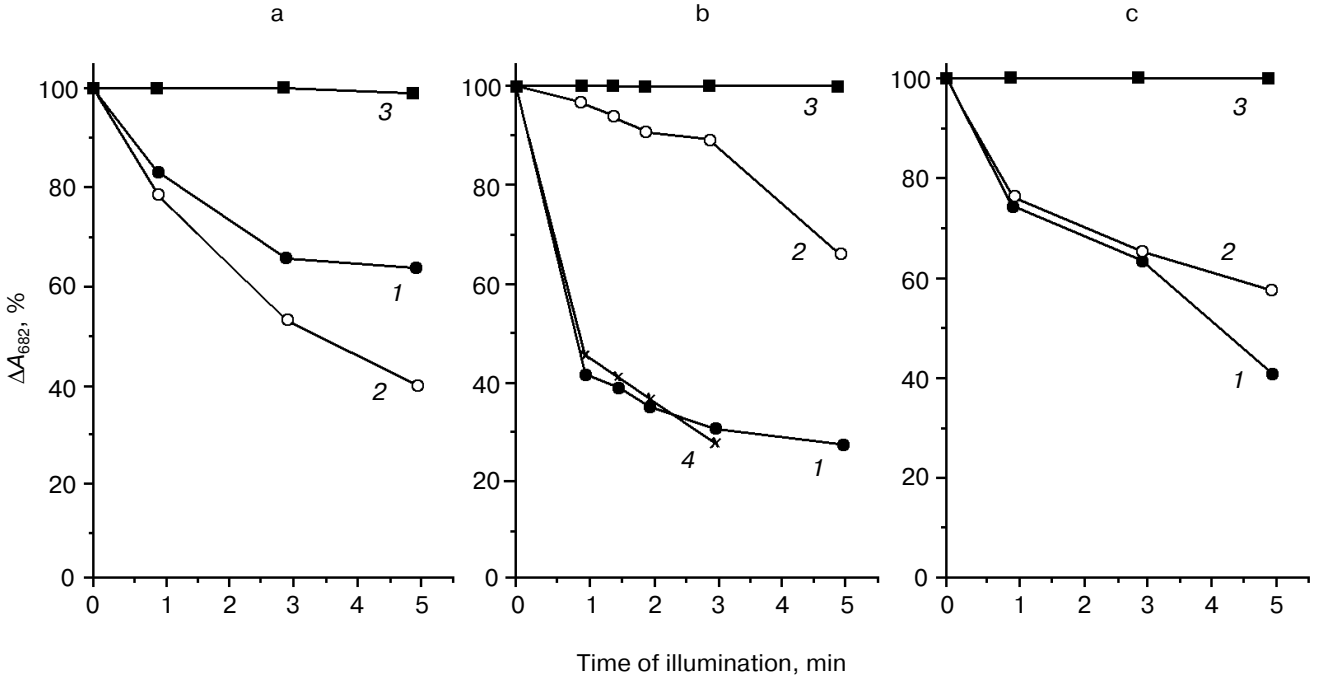

Fig. 1. Dynamics of loss of photochemical activity of RC of photosystem II monitored by the values of reversible photoinduced changes of absorption at 682 nm, connected with photoreduction of primary electron acceptor pheophytin (Ph) to anion-radical Ph^{-} in the presence of sodium dithionite (3 mg/ml) and 10^{-6} M methyl viologen in D1–D2–cytochrome b_{559} complexes during white light photoinactivation (120 W/m^2) under anaerobic conditions in the presence (*1*) and without (*2*) sodium dithionite at pH 8.0 (a), 6.0 (b), and 7.0 (c); *3*) dark control; *4*) dynamics of photobleaching of Q_x band of Ph.

control sample containing no dithionite. It can be suggested that under anaerobic conditions the photoinactivation of RC could go by through another unknown mechanism, or the sample could be contaminated by traces of oxygen even in the presence of the "oxygen trap". From the literature there are some data about photoinactivation of isolated RC due to illumination under anaerobic conditions [27]. However, there is no explanation of the molecular mechanism of inactivation in this article. The triplet state of *P*680 (^{3}*P*680) is known to form under anaerobic conditions with illumination [18]. It can easily react with the traces of oxygen in the sample, forming singlet oxygen ($^1O_2$), which is able to cause a well-known destruction influence on RC. Then the stabilizing effect of sodium dithionite under conditions of pH 8.0 with light (Fig. 1a) can be explained in the following manner: on one hand, traces of oxygen are removed in the presence of dithionite, and on the other hand the transfer of electrons towards $P680^+$ competes with recombination of charges between $P680^+$ and Ph^{-} and the state of [$P680Ph^{-}$] accumulates. The excited triplet state of *P*680 does not occur in this situation. It seems to be that the anion-radical Ph^{-} does not have a destructive action on RC in the absence of other substances, so samples subjected to the inhibiting light in the presence of dithionite at pH 8.0 are more stable than controls.

The opposite situation is observed on illumination at pH 6.0. In this case the increase of RC sensitivity to the inhibiting light in the presence of dithionite in comparison with the control could be explained as the possibility of irreversible transition of anion-radical Ph^{-} to the reduced and protonated form under conditions of higher proton concentration. Thus, Ph^{-} can transfer a part of the RC into the "closed" state (when RC loses the ability for photoseparation of charges) (Fig. 1b).

When RC is illuminated at neutral pH (7.0) the result is intermediate. The photochemical activity decreases identically in the presence of dithionite and without it in the beginning of the experiment, but if the time of illumination is rather long the sensitivity of RC to the light will increase in the presence of the exogenous donor of electrons.

The data obtained in this manner differs from the results published in [10, 14], where the illumination of isolated RC in the presence of exogenous donor of electrons (Mn^{2+}) did not lead to the suppression of the photochemical activity.

For the possible irreversible changes of absorption during the photoinactivation of isolated D1–D2–cytochrome b_{559} in the presence of dithionite at pH 6.0 to be monitored, the absorption spectra were measured. Figure 2 (a, b) demonstrates the comparison between the absorp-

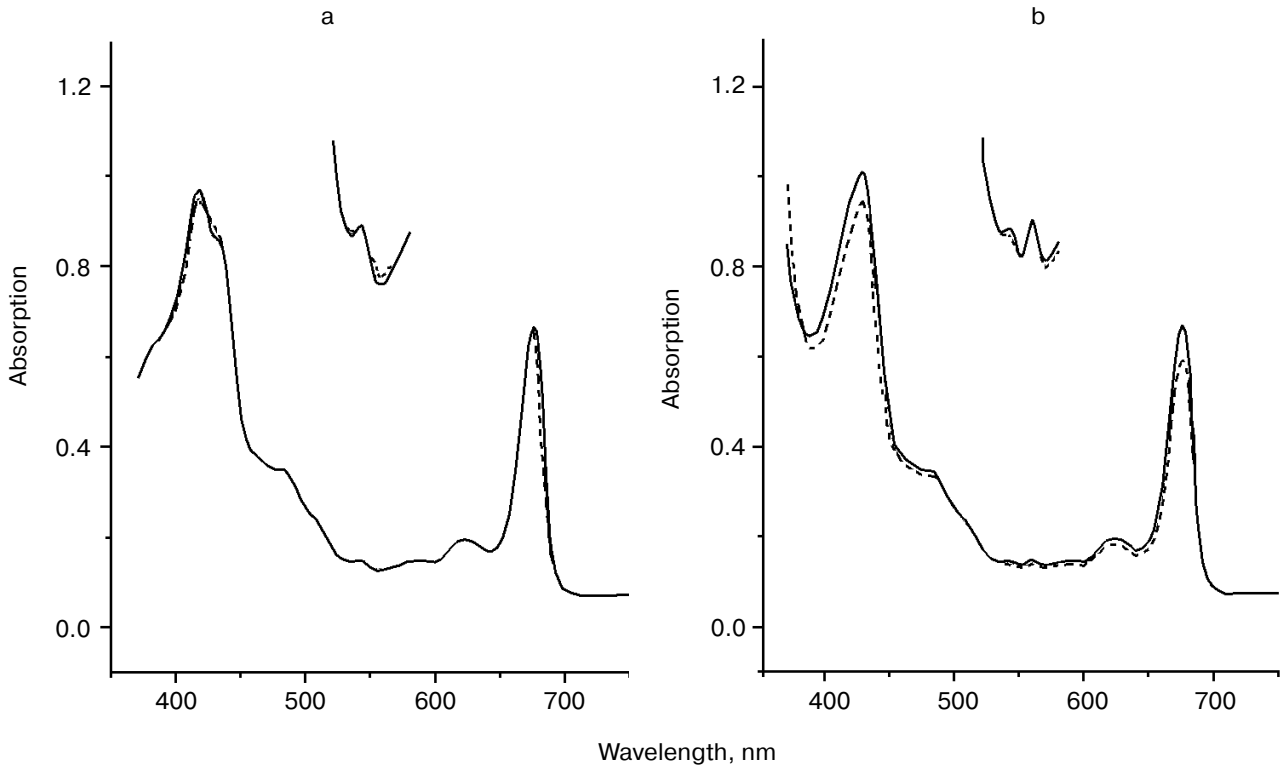

Fig. 2. Comparison of absorption spectra of RC samples in Mes-NaOH buffer, pH 6.0, containing 15 mM NaCl and 0.1% *n*-dodecyl-β-D-maltoside, before (continuous line) and after white light inactivation (120 W/m^2) during 3 min illumination (dotted line) under anaerobic conditions in the absence (a) and in the presence (b) of sodium dithionite. Q_x band of Ph is shown on the inset with a fourfold magnification.

tion spectra of RC samples that were illuminated under anaerobic conditions in the presence of dithionite (Fig. 2b) and without it (Fig. 2a). The illumination of the D1–D2–cytochrome b_{559} complex is seen from the figure to lead to a decrease of the absorption of Q_x band of Ph with a maximum at 542 nm and to the decrease of Q_y band at 680 nm. When the samples were illuminated without dithionite, there was no observed change of Q_x region of Ph, although a small change in the Q_y region at 680 nm was observed. Thus, in the case of the photoinhibiting illumination at pH 6.0 in the presence of dithionite an irreversible decrease of the Q_x band is observed, which did not appear in the case of the photoinactivation of the control samples. The spectra of absorption in the region of Q_x band of Ph before and after illumination in the presence of dithionite are compared in Fig. 3 (10-time magnification).

According to the literature, only one of two molecules of Ph (bound with D1 protein) is photoactive. This occurs in the active branch of the electron-transfer chain. The other molecule of Ph, bound with D2 protein, is located in the inactive branch of the RC and could not participate in the photoseparation of charges. One group of the authors supposes that the absorption of the active and inactive form of Ph in Q_x- and Q_y-band differs slightly [28-31]: the absorption of the active form of Ph in Q_y region is moved towards longer wavelength part in the spectrum by ~2 nm. Other authors consider the absorption of the active and inactive forms of Ph to differ in the Q_y region [32, 33], the active form absorbing at 680 nm and the inactive at 672-674 nm. The analysis of the spectral properties of the RC samples of PS II is made difficult by the considerable overlapping of the spectra of the chromophores composing the RC. It is difficult to determine the contributions from each of the chromophores because in the Q_y region (680 nm) the primary electron donor (chlorophyll *P*680) absorbs as well as the active form of Ph. Since the observed reduction of the Q_y band of Ph at 542 nm in the presence of dithionite decreases at most after 3 min illumination that corresponds to the loss of the photochemical activity by 70%, it seems that mainly the active form of Ph is bleaching. Figure 1b shows that the kinetics of decrease of the photochemical activity and photobleaching of the Q_x band of Ph during photoinactivation at pH 6.0 in the presence of dithionite are practically the same. Because both of the molecules of Ph (active and inactive) absorb in the Q_x band, the values of photobleaching of this band were compared to the 50%

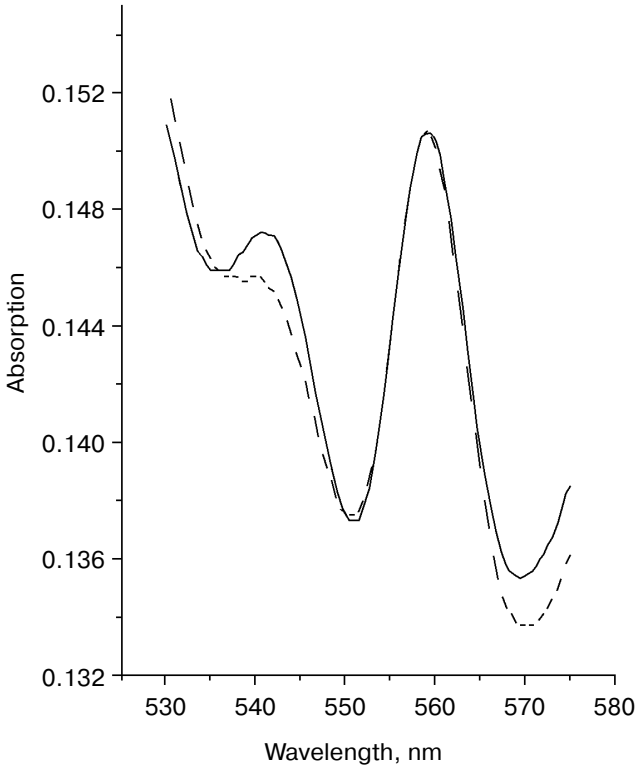

Fig. 3. Dynamics of the decrease of Q_x band of absorption of pheophytin (10-time magnification) before (continuous line) and after (dotted line) white light inactivation for 3 min of samples of RC of photosystem II in Mes-NaOH buffer, pH 6.0, containing 15 mM NaCl and 0.1% *n*-dodecyl-β-D-maltoside in the presence of sodium dithionite.

amplitude of the Q_x band, remembering that we deal with the active form of Ph. As pointed out before [34], the protonated product of the anion-radical $Ph^{\cdot-}$ can be obtained if PS II is illuminated. The velocity of protonation of the reduced form of Ph is considerably higher than that of the reduced form of chlorophyll [34]. Also, in this article the mechanism of the electrochemical reduction of Ph in dimethyl formamide was studied on the basis of polarograms and cyclic volt–ampere curves under the conditions of different values of pH and the dependence of this process on pH of the medium was revealed. In our experiments on the photoaccumulation of long-living states of RC with reduced anion-radical $Ph^{\cdot-}$ at pH 6.0 the formation of long-living protonated forms can occur. The following products of pheophytin reduction form: $Ph^{\cdot-}$, Ph^{2-}, PhH^{-}, PhH_2 [34]. Transition states of Ph^{2-} and PhH^{-} have redox potentials of –0.44 and –0.97 V, accordingly, and the double-protonated form of PhH_2 has the potential of +0.33 V. The double-reduced form of Ph (PhH_2) is called a "red" reduced form of Ph, because it has an absorption maximum in the region of 520 nm, and the anion-radical $Ph^{\cdot-}$ with middle transition states can have such maxima at 430-480 nm. There are no reliable data about spectral description of protonated forms of Ph in the literature. The difficult observation of maxima of absorption under photoinactivation of RC of photosystem II is connected with the difficulty of the spectrum and with rather low values of extinction of these forms. However, in spite of this fact anion-radical $Ph^{\cdot-}$ might be suggested to be transformed irreversibly to one of the protonated forms under illumination in the presence of dithionite and weakly acidic pH. Our experiments showed that an addition of potassium ferricyanide to the samples after illumination in the presence of dithionite did not give any changes in absorption spectra and did not restore the photochemical activity (data not shown). This suggests that ferricyanide cannot reoxidize the long-living form of Ph obtained during illumination.

Thus since the decrease of the photochemical activity in isolated D1–D2–cytochrome b_{559} complex as a result of photoinactivation in the presence of an exogenous donor of electrons (dithionite) depends on the pH of the medium (it occurs only at pH < 7.0) and is accompanied by an irreversible photobleaching of the Q_x band of Ph. Also if we consider the ability of reduced Ph to transform to one of the protonated forms [34], it way be suggested that the photoinactivation of isolated RC under these conditions is connected with the photoaccumulation of one of the long-living reduced protonated form of Ph.

The results of the electrophoresis of RC samples illuminated in the presence of with and without dithionite are compared in Fig. 4 (a and b). The data show that photoinactivation in the presence of dithionite leading to the irreversible photobleaching of pheophytin does not cause the degradation of the D1 and D2 proteins of RC of photosystem II. Judging by the decrease of absorption at 280 nm of the proteins bands on the electrophoregrams, there is only a decrease in the band corresponding to cytochrome b_{559} (by 8% for 5 min of illumination). However, the degradation of the control samples of D1 and D2 proteins occurs under anaerobic conditions without dithionite. According to the literature, fragmentation of D1 and D2 proteins of PS II is observed when photoinactivation occurs without traces of oxygen [26, 27], although the mechanism of such destruction has not been clarified. This photodegradation might be suggested to be connected with the existence of an unknown mechanism of destruction of PS II under anaerobic conditions or in the presence of traces of oxygen, which are sufficient for the beginning of the destruction through the well-known pathway [9, 10, 12, 13]. Earlier we studied the influence of white light on the proteins structure of isolated RC complexes [35]. A strict dependence between the reduction of the photochemical activity in the samples and the degradation of the protein complex was noted. According to the literature, the same tendency is seen [13, 36]. The changes of conformation of the pigment–protein com-

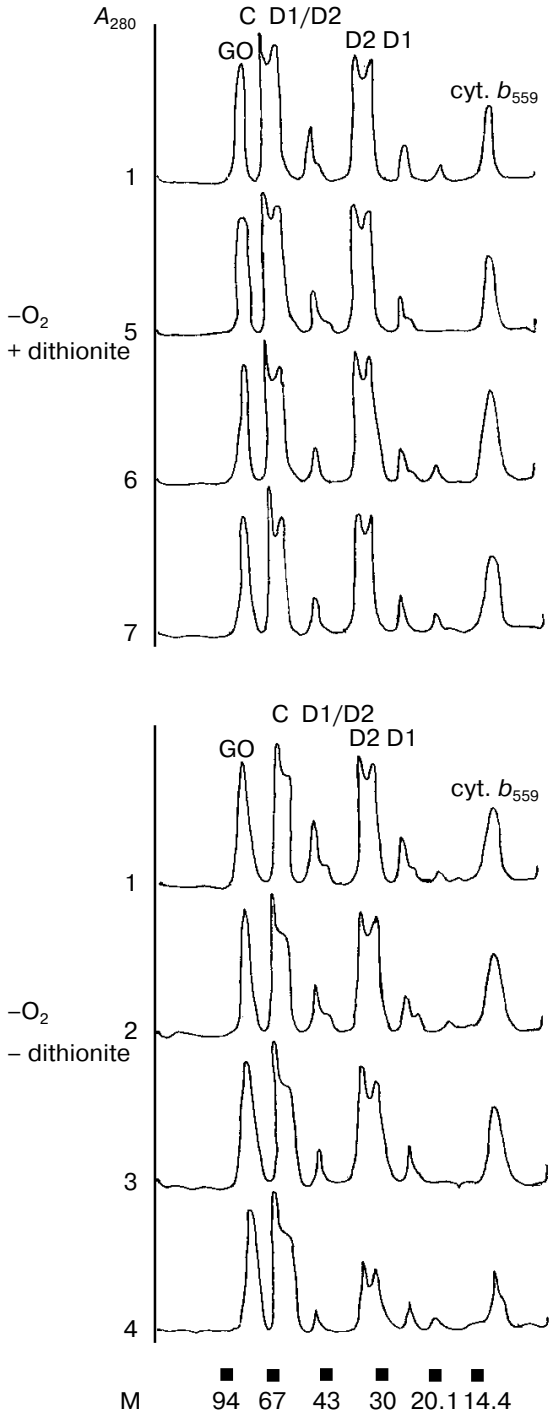

Fig. 4. Profiles of RC of photosystem II samples after SDS-PAGE before (*1*) and after photoinactivation under anaerobic conditions without sodium dithionite for 1 (*2*), 3 (*3*), and 5 min (*4*) and in the presence of sodium dithionite for 1 (*5*), 3 (*6*), and 5 min (*7*). Abbreviations: GO) glucose oxidase; C) catalase; D1/D2) heterodimer of D1 and D2 proteins; cyt. b_{559}) cytochrome b_{559}; D1) D1 protein; D2) D2 protein; M) marker proteins (Sigma, USA), their molecular masses (kD) are shown below (phosphorylase *b*, albumin, ovalbumin, carboanhydrase, trypsin, α-lactalbumin).

plex of RC are supposed to occur after the breach of electron-transport chain components [11], and then the fragmentation of its main proteins follows [12-15, 17]. These fragments of degradation under photoinduced destruction differ in the gap of D1 and D2 proteins chains in accordance with the two possible ways (donor-side and acceptor-side). Both are connected with the oxidizing influence as cation-radicals of $P680^+$ and Y^+ [12, 17], as singlet oxygen [12, 13]. In our case, the destruction of proteins of the complex of RC does not occur when photoinactivation is conducted in the presence of dithionite at pH 6.0. Apparently, one of the long-living forms of Ph obtained during illumination cannot participate in the primary damage of RC, leading to the cleavage of the polypeptide chain of D1 and D2 proteins. Thus, under these conditions of illumination only the loss of photochemical activity happens, but the photoinduced destruction of D1–D2–cytochrome b_{559} complex (that is degradation and fragmentation of its proteins components) does not occur. The irreversible formation of the long-living reduced and protonated form of Ph under these conditions of illumination of isolated RC might be suggested to be reaction centers incapable of photoseparation of charge or to the "closed" state.

Thus, based on the data, we suggest a new pathway of the photoinactivation of PS II connected with photoaccumulation of reduced Ph. It can be characterized by the dependence on pH and occurs at pH < 7.0 with irreversible loss of the photochemical activity. The loss of the photochemical activity in the process of the photoinactivation of isolated D1–D2–cytochrome b_{559} complex in the presence of dithionite is supposed to be connected with a photoaccumulation of anion-radical $Ph^{\cdot-}$, which can be transformed to one of the long-living protonated forms at pH < 7.0. This form may be presented as PhH^- or as the "red" reduced form PhH_2 (in the case of the double reduction of Ph). With such a type of photoinactivation the degradation of D1 and D2 proteins of RC is not observed, this distinguishing the pathway from all of the known mechanisms of photoinactivation. An irreversible generation of protonated forms of Ph under these conditions of illumination make the reaction center "closed", that is to be unable to show separation of charge, but the RC itself remains with no damage. The mechanism of the photoinactivation of RC of photosystem II, where no considerable changes of the structure is observed, may be used for the directed modification of the structural-functional organization of PS II samples. It cannot be ruled out that the pathway observed in our study may occur in intact systems. For example, it may be possible when intense illumination acts at low concentration of O_2, or with a deficiency of CO_2, the substrate for the Calvin cycle, when the rereduction of the photosynthetic electron-transport chain occurs and when the double photoreduction of the primary electron acceptor Q_A [19] with the increase of pos-

sibility of the photoaccumulation of anion-radical Ph^{-} may take place.

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