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Predominant Protection of D2-Protein against Photodestruction in Isolated D1/D2/Cytochrome b_{559} by K15, a Phenolic-Type Inhibitor of Electron Transfer in Photosystem 2

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Abstract—A protective action of K15 (4-[methoxy-bis(trifluoromethyl)methyl]-2,6-dinitrophenylhydrazone methyl ketone), an inhibitor of electron transport in photosystem 2 (PS 2), against photoinactivation of the PS 2 reaction center (RC) D1/D2/cytochrome b_{559} complex, isolated from pea chloroplasts, by red light (0.7 mmol photons/sec per m^2) has been investigated under aerobic conditions. The inhibitor K15 causing cyclic electron transfer around PS 2 and thus prohibiting stabilization of separated charges has been shown to effectively protect RC both against the loss of photochemical activity (measured as reversible photoinduced absorbance changes related to photoreduction of pheophytin) and aggregation and degradation of the proteins D2 and D1 during photoinactivation. Comparison of the protective action of K15 and of another inhibitor of electron transfer in PS 2, diuron, against light-induced destruction of proteins D1 and D2 shows that diuron stabilizes protein D1 and K15 stabilizes protein D2. The preferential protection of D2 against photoinduced destruction revealed in our work is in accord with the concept of a specific binding of K15 with this protein. It is proposed that this binding site may be that of the primary quinone electron acceptor Q_A located on the D2 protein (in contrast to diuron, which is known to replace the secondary electron acceptor Q_B from its binding site on D1).

Key words: photosystem 2, pigment–protein complex, reaction center, photoinactivation, photosystem 2 electron transport inhibitor

According to the current conception, photoinactivation of electron-transport reactions and consequent photoinduced destruction of the photosynthetic pigment–protein complexes (designated as photoinhibition) influences initially photosystem 2 (PS 2) [1]. High vulnerability of PS 2 to inhibiting action of light is determined by its unique capability to generate surprisingly high redox potential (the E_0 for the P^+_{680}/P_{680} pair is 1.12 V [2]) required for water oxidation and formation in PS 2 of molecular oxygen, which can be easily converted to its various toxic species. The majority of investigators believe that the site most vulnerable to light is located inside the reaction center (RC) of PS 2. The fact that a key component of the RC, the D1 protein, is synthesized *de novo* much faster than other proteins of the thylakoid membrane [3] favors this assumption. Plants have a complex system of mechanisms protecting the photosynthetic apparatus from dangerous action of light, as well as a reparation system neutralizing consequences of such damages. Nevertheless, destruction of PS 2 can occur not only at high but also at relatively low light intensities [4]. If the rate of destruction exceeds the reparation rate, then a decrease in photochemical activity (or photoinactivation) occurs. It can ultimately lead to photoinduced degradation of the pigment–protein complexes of PS 2. At present two basic mechanisms for explanation of the phenomena of the PS 2 photoinhibition are proposed. They are connected with photoinduced breach of regular functioning of the components of the electron transport chain on the acceptor or donor sides of the photosystem [5]. These mechanisms are conditionally denoted as "acceptor" and "donor" mechanisms.

Photoinhibition according to the "acceptor mechanism" occurs during the regular functioning of the oxygen-evolving complex (OEC) under aerobic conditions at high intensities of the actinic light. Illumination by highly intensity light leads to reduction of the whole plastoquinone membrane pool and as a consequence to formation of doubly reduced primary quinone electron acceptor Q_A (Q_AH_2) [5, 6]. In this case, the photosynthetic

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charge separation in the PS 2 reaction center is confined by the formation of the primary ion-radical pair [P^+_{680}Pheo$^-$]. As a result of subsequent recombination of charges of pair [P^+_{680}Pheo$^-$] a triple state of the chlorophyll P_{680}, $^3P_{680}$ can be generated [7]. Under aerobic conditions interaction of the triplet state of chlorophyll P_{680} with molecular oxygen lead to the formation of highly active singlet oxygen ($^1O_2$) able to induce redox destruction of chlorophyll [8] and certain amino acid residues [9]. The next step is proposed to be conformational changes of the proteins D1 and D2 [10] and their subsequent degradation with formation of fragments with molecular weight 23, 16-18, 10 kD and 29, 21 kD [11-14].

Under conditions when the supply of electrons from the PS 2 oxygen-evolving complex to the primary donor, chlorophyll P_{680}, is limited photoinhibition occurs according to the "donor mechanism". In contrast to the "acceptor mechanism", the "donor mechanism" of photoinhibition can occur both at high and low intensity of the actinic light [4]. A decrease in the efficiency of the electron donation from oxygen evolving complex to reaction center results in accumulation of the radicals P^+_{680} and Y^+_Z (the oxidized secondary electron donor, tyrosine 161 of D1 protein [15]) which can cause irreversible destruction of β-carotene and accessory chlorophyll P_{670} [16-18]. Under these conditions the degradation of polypeptides D1 and D2 with formation of fragments with molecular weight 24, 17, 10 kD and 29, 21 kD [19, 20], respectively, is revealed.

We recently identified a group of highly efficient inhibitors of PS 2 electron transfer, derivatives of perfluoroisopropylldinitrobenzene [21]. A compound designated as K15 is one of the most active inhibitor of these. The mechanism of the inhibitory action of these agents is based on the redox interaction with the components of the PS 2 reaction center: the reduced intermediary electron acceptor, pheophytin (Pheo$^-$) and the oxidized primary electron donor, chlorophyll P^+_{680} and formation of cyclic electron transfer around this photosystem [22, 23] leading to upsetting of the primary reaction of the photoseparation of charges. It has also been proposed that these inhibitors displace the primary plastoquinone electron acceptor Q_A from its binding site [24, 25]; nevertheless, direct proofs of the proposition have not yet been obtained. It has been established on subchloroplast membrane fragments of PS 2 and isolated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers [23-25] that K15 inhibitor efficiently protects PS 2 against photoinduced suppression of the photochemical activity. However, the question on the influence of the inhibitor on structural changes of the isolated pigment–protein complexes D1/D2/cytochrome b_{559} of the PS 2 reaction centers and degradation of the D1 and D2 polypeptides under photoinhibition remained open.

The traditional inhibitors of the electron transfer in PS 2, the derivatives of urea (diuron) and triazine (atrazine), are the best studied. It is known that the presence of these agents in thylakoids and the PS 2 subchloroplast membrane fragments decreases selectively the rate of photoinduced degradation of the D1 protein [26, 27]. These inhibitors block the electron transfer between the primary and secondary plastoquinone electron acceptors of PS 2—Q_A and Q_B. The binding site of the secondary quinone electron acceptor, Q_B, located on the D1 protein is their binding site. A number of investigators believe that the Q_B binding site is just the primary place of the destruction under photoinactivation. It is assumed that redundant light induces photochemical processes causing chemical modification of the Q_B binding site. This in turn leads to disturbance of the electron transport and change in the sensitivity of the D1 protein to the action of proteases [28, 29].

The isolated complexes of the PS 2 reaction centers serve as convenient experimental systems for investigating the mechanisms of photoinhibition and influence of various agents on this process. They comprise the D1 and D2 proteins, the α- and β-subunits of cytochrome b_{559}, as well as the *psbI* gene product [30]. They bind 4-6 molecules of chlorophyll *a*, 1-2 molecules of β-carotene, and 2 molecules of pheophytin [31].

In this work we investigated a protective effect of the novel inhibitor of the electron transfer in PS 2—K15 (4-[methoxy-bis(trifluoromethyl)methyl]-2,6-dinitrophenylhydrazone methyl ketone)—on structural-functional changes of the isolated D1/D2/cytochrome b_{559} complexes of the PS 2 reaction centers under photoinactivation as compared to protective effect of the known inhibitor of the PS 2 electron transfer, diuron.

MATERIALS AND METHODS

In this work we used Tris and Triton X-100 from Reanal (Hungary); *n*-dodecyl-β-D-maltoside and sodium dodecyl sulfate (SDS) from Sigma (USA); Fractogel TSK DEAE Toyopearl 650S from Toyo Soda (Japan); Coomassie G-250 as well as other domestic chemicals of chemically pure grade.

The D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers were isolated from pea leaves (*Pisum sativum*) as described earlier [32]. Photochemically active preparations of RC of PS 2 comprised the D1 (34 kD) and D2 (32 kD) polypeptides, cytochrome b_{559} (9 kD), and heterodimer of D1 and D2 polypeptides (58 kD). There are six molecules of chlorophyll *a* and 0.9-1.4 molecules of β-carotene for two pheophytin *a* molecules.

Photoinhibition of the isolated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers was conducted as follows: solution of the reaction centers (final chlorophyll concentration was 5 μg/ml) in medium containing 50 mM Tris-HCl buffer (pH 7.5), 150 mM NaCl, 0.1% *n*-dodecyl-β-D-maltoside, and 10% glycerol was placed in

an open quartz cuvette (1 × 1 × 2 cm) and illuminated by red light (0.7 mmol photons/sec per m^2) which was provided by filtering through a heat-absorbing filter and glass filter (KS-11) transmitting light with $\lambda > 600$ nm. The thickness of the irradiated layer was 10 mm. The distance between the lamp axis and the cuvette with the preparation was 2 cm.

The polypeptide composition of the investigated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers was analyzed by gradient electrophoresis in 12-18% polyacrylamide gel under denaturing conditions (in the presence of 6 M urea and 0.1% SDS) [33]. The upper electrode Tris-glycine buffer (150 mM, pH 8.3) contained 0.1% SDS. Polypeptides in the polyacrylamide gels were stained after electrophoresis with 0.25% solution of Coomassie G-250.

Photochemical activity of the PS 2 reaction center preparation was measured by the photoinduced absorbance changes at 682 nm (ΔA_{682}) related to photoreduction of the PS 2 intermediary electron acceptor, pheophytin [34], in the presence of dithionite (1 mg/ml) and methyl viologen (1 μM) in the buffer described above, at pH 8.0.

Densitograms after electrophoresis were obtained on a Specord M-40 (Germany) using a scanning device. The experiments were performed in fivefold biological and sevenfold analytical trial.

RESULTS

1. Dependence of protective action of the inhibitor K15 upon its concentration during photoinhibition of isolated PS 2 reaction centers. The isolated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers do not contain plastoquinone electron acceptors Q_A and Q_B [30] hence the primary photochemical reaction in these complexes is restricted to electron transfer from the excited primary electron donor P^*_{680} to the intermediary electron acceptor pheophytin *a* (Pheo) [35]. In this case the amplitude of reversible photoinduced absorbance changes with maximum at 682 nm (ΔA_{682}) related to photoaccumulation of pheophytin in reduced form $Pheo^-$ in the presence of dithionite (1 mg/ml) and methyl viologen (1 μM) can be used as a measure of the reaction center photochemical activity. The characteristic for the PS 2 reaction center photoreaction was selected by us for monitoring the functional activity of the isolated complexes D1/D2/cytochrome b_{559} under photoinhibition. For our experimental conditions, it was necessary to determine the concentration of the inhibitor K15 at which the agent protected functional activity of RC against photoinhibition with maximum efficiency. For this purpose, we investigated the dependence of the loss of functional activity of the complexes D1/D2/cytochrome b_{559} under illumination by red light (0.7 mmol photons/sec per m^2) for 3 min

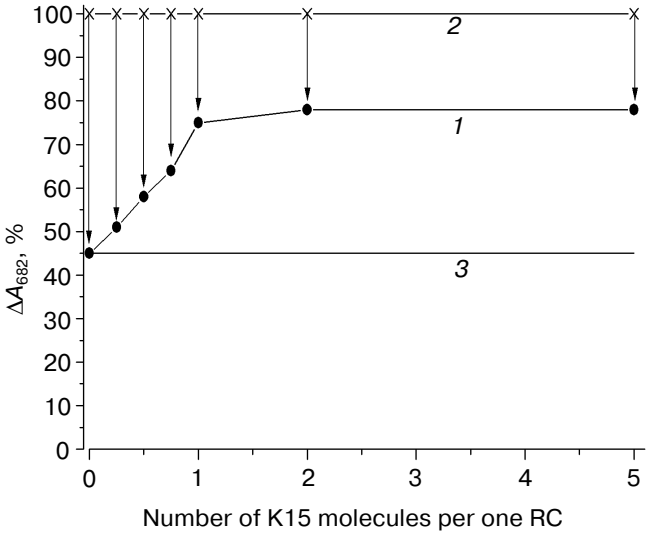

Fig. 1. Dependence of functional activity of isolated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers recorded after 3 min of photoinactivation by red light (0.7 mmol photons/sec per m^2) under aerobic conditions (*1*) and after 3 min of dark incubation (*2*) on concentration of the inhibitor K15 (arrows show the loss of photochemical activity as a results of photoinactivation); *3*) the level of functional activity of the RC after 3 min of illumination by red light (0.7 mmol photons/sec per m^2) without addition of K15. Functional activity was estimated from the value of the reversible photoinduced absorbance changes at 682 nm (ΔA_{682}), related to photoreduction of the PS 2 primary electron acceptor, pheophytin, in the presence of dithionite (1 mg/ml) and methyl viologen (1 μM). Chlorophyll concentration was 5 μg/ml. It is necessary to note that during measurement of functional activity inhibitor K15 was practically absent in any variant shown because, as shown earlier [21], dithionite destroys the K15 chemically (evidently by a redox mechanism). One molecule of the K15 per PS 2 reaction center corresponds to the addition of 0.25 μM K15.

(Fig. 1, curve *1*) upon concentration of the inhibitor K15. It should be stressed that dithionite (1 mg/ml) and methyl viologen (1 μM) were added to the sample before measurement of functional activity in each variant. During measurement of the activity the inhibitor K15 was absent in any samples investigated since, dithionite chemically destroys K15 as shown earlier [21] (evidently by a redox mechanism).

Figure 1 (curve *1*) shows that protective action of K15 increases on its increasing concentration in the region of 0.1-0.5 μM, saturating at the concentration of 0.5 μM corresponding to the addition of one molecule of the inhibitor per reaction center. At higher concentrations of K15 (corresponding to 2-5 molecules of the inhibitor per a reaction centre of PS 2) the protective action of K15 does not change. Functional activity of the RC complexes that were not subjected to photoinhibition (that is they were incubated in the dark during the same time in the presence of the same increasing concentra-

tions of the inhibitor K15) remains practically constant (Fig. 1, curve *2*).

Following the data obtained in the previous investigations, we selected the concentration of the inhibitor K15 equaling two molecules per reaction center. This choice was also determined by experimental data [21] showing that the presence of a molecule of the inhibitor per reaction center is enough for the maximum inhibitory action on PS 2 activity.

2. Influence of K15 on rate of photoinactivation of functional activity of PS 2 reaction center. For the conditions selected by us to carry out the experiments, namely to reveal the capability of inhibitor K15 for protection of native structural functional organization of PS 2 under photoinhibition (two molecules of the inhibitor per reaction center), it was necessary to investigate the dynamics of decrease of photochemical activity of PS 2. These data would also allow us to answer the question on the relationship between degradation of polypeptides under photoinduced destruction of the isolated pigment–protein complexes D1/D2/cytochrome b_{559} and the loss of functional activity.

As shown in Fig. 2, illumination of isolated complexes D1/D2/cytochrome b_{559} of PS 2 reaction centers by red light (0.7 mmol photons/sec per m^2) in the absence of the inhibitor K15 results in significant suppression of the reaction of photoreduction of pheophytin (–K15). The amplitude of photoinduced ΔA at 682 nm was suppressed by 50 and 80%, respectively, after 2 and 10 min of photoinactivation, which is in agreement with the data obtained earlier [36]. In the presence of K15 (1 μM, which corresponds to two molecules K15 per RC) photoinactivation is considerably slowed down: a 50% loss of the activity is observed only after 5 min of illumination, and after 10 min of photoinactivation about 40% of the activity is retained (+K15). Thus, K15 effectively protects the isolated reaction centers of PS 2 against photoinactivation as mentioned earlier [22, 23]. The loss of functional activity of the sample for an indicated period of photoinhibition as a result of other causes can be excluded since the amplitude ΔA_{682} of the sample in the dark does not decrease (dark control).

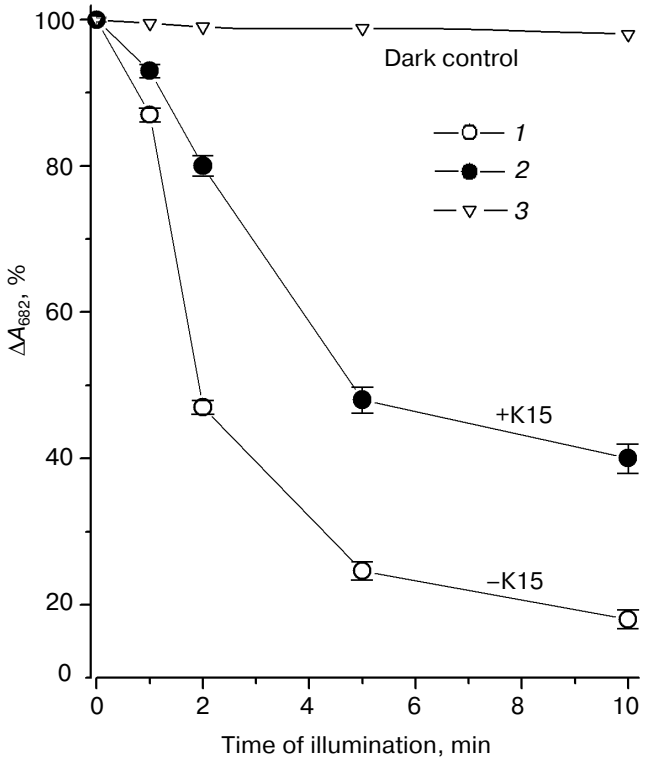

Fig. 2. Dynamic decrease in photochemical activity of isolated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers under photoinhibition by red light (0.7 mmol photons/sec per m^2) under aerobic conditions in the absence (*1*) and presence (*2*) of inhibitor K15 at the concentration of 0.5 μM corresponding to two molecules of the inhibitor per RC. Curve *3* is dark control in the presence of 0.5 μM K15. Photochemical activity was estimated from the value of the reversible photoinduced absorbance changes at 682 nm (ΔA_{682}) related to photoreduction of the PS 2 primary electron acceptor, pheophytin, in the presence of dithionite (1 mg/ml) and methyl viologen (1 μM). Chlorophyll concentration was 5 μg/ml.

3. K15 protects polypeptides D2 and D1 against photoinduced aggregation and degradation. It was shown earlier [11-14, 19, 20] that under photoinhibition of the isolated complexes D1/D2/cytochrome b_{559} of PS 2 reaction centers, in addition to the loss of functional activity a degradation of the constituting integral polypeptide components occurs: polypeptide D1 and to a lesser extent polypeptide D2. The degradation of polypeptides under photoinhibition is supposed to be due to the loss of functional activity and not vice versa. Based on data that the inhibitor K15 protects the functional activity of the isolated complexes D1/D2/cytochrome b_{559} under photoinhibition, it is reasonable to expect that photodestruction of polypeptides D1 and D2 also will slow down in this case. At the same time, it is of interest to elucidate whether the inhibitor K15 protects both of the polypeptides to an equal extent under photoinhibition. The results of such experiments are presented below.

Figure 3 shows the influence of the inhibitor K15 on the dynamics of decrease in the amount of D1 and D2 proteins registered as absorbance at 280 nm of polypeptide bands 32 kD (polypeptide D1) and 34 kD (polypeptide D2) as a result of photoinhibition of the D1/D2/cytochrome b_{559} complexes.

Photoinhibition under aerobic conditions leads to fast and considerable degradation of the D1 and D2 polypeptides (Fig. 3a) as noted earlier [11-14, 36]. After 10 min of photoinhibition in the absence of compound K15 only 42% of the polypeptide D1 and 57% of the polypeptide D2 is saved, which is in agreement with known data about higher sensitivity of the D1 protein to photodegradation [3, 36]. As mentioned earlier [36], during photoinhibition under aerobic conditions polypeptide

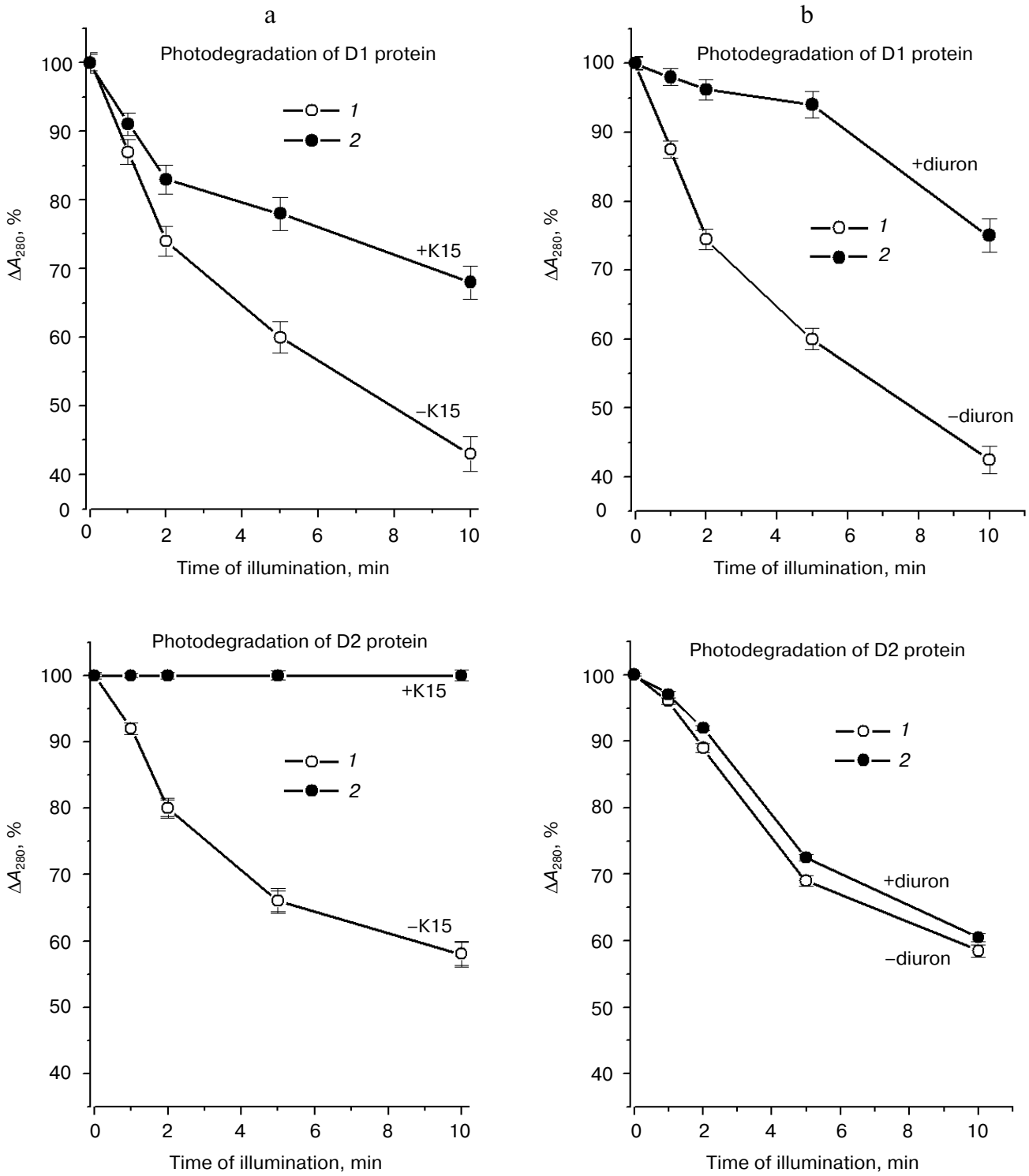

Fig. 3. a) Dynamic decrease in the amount of the D1 and D2 proteins (estimated from the value of absorbance at 280 nm of polypeptide bands corresponding to these polypeptide) as a result of photoinhibition of the D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers under aerobic conditions by red light (0.7 mmol photons/sec per m^2) in the absence (*1*) and presence (*2*) of inhibitor K15 at the concentration of 0.5 μM corresponding to two molecules of the inhibitor per one reaction center. Chlorophyll concentration was 5 μg/ml. b) Dynamic decrease in the concentration of the D1 and D2 proteins (estimated from the value of absorbance at 280 nm of polypeptide bands corresponding to these polypeptide) as a result of photoinhibition of the D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers under aerobic conditions by red light (0.7 mmol photons/sec per m^2) in the absence (*1*) and presence (*2*) of diuron at the concentration of 0.5 μM corresponding to two molecules of the inhibitor per one reaction center. Chlorophyll concentration was 5 μg/ml.

aggregates with molecular weight higher 100 kD are produced, which are delayed on the boundary of the separation gel during electrophoresis. Figure 4 shows densitograms obtained after denaturing electrophoresis of the isolated RC illuminated both with and without K15 under aerobic conditions.

The presence of the inhibitor K15 (at the ratio of two inhibitor molecules per RC) during photoinhibition of the D1/D2/cytochrome b_{559} complexes under aerobic conditions effectively protects the polypeptides of the reaction center against photodegradation. The greatest protective effect of K15 is observed for polypeptide D2: in the presence of K15 the protein is not destroyed at all even after 10 min of inhibiting illumination. The degradation of D1 protein is retarded though to a lesser extent than the degradation of the D2 protein: in the presence of K15 about 68% of the protein is saved (versus 42% in the absence of the inhibitor) (Figs. 3a and 4).

4. Comparison of the action of diuron and K15 on degradation of the D1 and D2 polypeptides under photoinhibition. It was shown earlier [26-29] that the known inhibitors of electron transfer in PS 2 selectively protected polypeptide D1 against degradation under photoinhibition (just as inhibitor K15 protects polypeptide D2 with greater selectivity than polypeptide D1). The so-called traditional inhibitors of the electron transfer in PS 2, the derivatives of urea (diuron) and triazine (atrazine) possess such capability. The presence of these agents in PS 2 preparations under photoinhibition decreases considerably the rate of photodegradation of the D1 polypeptide. These inhibitors block electron transfer between the primary Q_A and the secondary Q_B plastoquinone electron acceptors of PS 2. The so-called herbicide-Q_B-quinone binding site located on the D1 polypeptide is known to be a place of their binding [27].

Since diuron protects mainly the D1 polypeptide against degradation, it was reasonable to compare the action of diuron and K15 (differing in main by their mechanism of effect on the electron transport and, as proposed [22], by their binding sites) on photoinduced degradation of the D1 and D2 polypeptides of the isolated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers. Diuron, like K15, was added at the concentration of two molecules of the inhibitor per PS 2 reaction center. The results obtained are presented in Figs. 3b and 4.

The presence of diuron during photoinhibition of the isolated D1/D2/cytochrome b_{559} complexes under aerobic conditions decreases considerably the degradation of polypeptide D1. After 5 min of photoinactivation in the presence of diuron, about 95% of protein D1 is saved, whereas in the absence of the inhibitor only approximately 60% of the protein is saved for the same period. After 10 min of illumination, these values are 75 and 42%, respectively. At the same time, the maximal protecting effect of diuron on photodegradation of the D2 protein is not more than 5% (Fig. 3b). These data indicate high selectivity for the D1 polypeptide capability of diuron to protect the polypeptide against degradation under photoinhibition, as was repeatedly noted in earlier investigations of photoinhibition of thylakoid membrane and membrane fragments enriched in PS 2 [26-29].

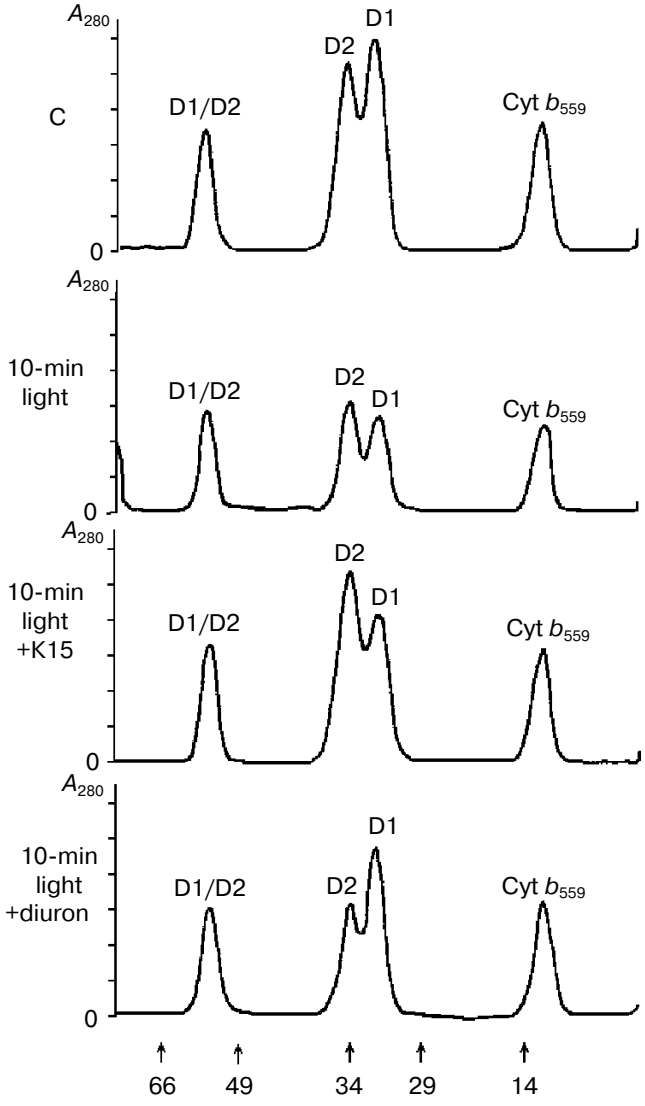

Fig. 4. Densitograms obtained after denaturing electrophoresis of the isolated D1/D2/cytochrome b_{559} complexes of PS 2 reaction centers before (C) and after illumination by red light (0.7 mmol photons/sec per m^2) under aerobic conditions at 4°C for 10 min without additions ("10-min light") and in the presence of the inhibitors: K15 ("10-min light + K15") and diuron ("10-min light + diuron"). Inhibitors were added at the concentration of two molecules of the inhibitor per PS 2 reaction center.

DISCUSSION

It was shown earlier [21] that the presence of one molecule of the inhibitor K15 per reaction center was

enough to achieve the maximum effect on electron transport in PS 2. On one hand, these data indicate high affinity of the K15 compound relative to the components of PS 2 reaction center. On the other hand, they indicate that PS 2 reaction center bears the only binding site having high specificity for K15. Binding of this highly specific site is responsible for all basic inhibiting effects of the agent on the structural functional state of PS 2.

Within this context the data presented in Fig. 1 are important because they show that the K15 protects functional activity of PS 2 under photoinhibition at the concentration of one molecule of the inhibitor per reaction center, i.e., the protecting effect is provided with highly specific binding of K15. In other words, both for suppression of the PS 2 characteristic photochemical reactions and for manifestation of the capability to protect PS 2 against photoinhibition it is quite enough for binding of K15 to a highly specific site of the reaction center, and in both cases maximum effect is observed at the concentration of one molecule of the inhibitor per reaction center. From this it follows that in both cases the inhibitor K15 binds to the same binding site, evidently to the same polypeptide of the PS 2 reaction center.

These data also allow us to propose that the same mechanism of action described by us earlier [21-25] lies at the basis of the ability of K15 to protect functional activity of PS 2 under photoinhibition and its ability to inhibit functional activity of PS 2.

The fact that the level of functional activity of the isolated pigment–protein complexes D1/D2/cytochrome b_{559} incubated in the dark for the same period of time is practically unchanged serves an indicator for high stability of these complexes (Fig. 1, curve *2*). It is necessary to note that the absence of effects of suppression of PS 2 functional activity by inhibitor K15 at increasing concentrations of the agent can be explained by the fact that before measurement of the activity dithionite (1 mg/ml) and methyl viologen (1 μM) are added to the sample in each variant, which results in chemical destruction of the inhibitor as shown earlier [21].

What lies in the basis of the ability of the inhibitor, type K15, to protect functional activity of the PS 2 under photoinhibition? The mechanism of photoinhibition of the PS 2 subchloroplast membrane fragments is not only determined by the conditions in which photoinhibition takes place, but also, to a greater extent, by the state of the PS 2 water-oxidizing complex (WOC). In the case of isolated D1/D2/cytochrome b_{559} pigment–protein complexes, the situation is simplified because the WOC is absent. In isolated D1/D2/cytochrome b_{559} pigment–protein complexes one of the first events of photoinhibition is formation of the triplet state of chlorophyll P_{680}. This occurs as a result of recombination of charges in primary ion-radical pair $[P^{+}_{680}Pheo^{-}]$ leading to transition of a molecule of primary electron donor, chlorophyll P_{680}, into the excited singlet state P^{*}_{680}, which in its turn can be transformed with certain probability to the excited triplet state $^3P_{680}$. Under aerobic conditions, the interaction of the triplet state $^3P_{680}$ with molecular oxygen results in formation of its first excited singlet state ($^1O_2$) [37]. Singlet oxygen induces destruction of the chlorophyll P_{680} [8] and certain amino acid residues [9], and conformational changes of the D1 and D2 proteins [10] and their subsequent degradation [11-14]. The inhibitor K15 forming cyclic electron transfer around PS 2 decreases considerably the possibility of formation of triplet state of chlorophyll, $^3P_{680}$. This, in turn, diminishes the possibility of formation of the basic damaging agent during the process of photoinhibition under aerobic conditions, singlet oxygen. The inhibitor K15 can protect PS 2 reaction center against photoinhibition under aerobic conditions by such mechanism. As was shown earlier the efficiency of the inhibiting action of the K15 on the electron phototransfer in PS 2 does not depend on the presence of oxygen in the medium [21]. This suggests a direct interaction of inhibitor K15 with oxygen to be excluded.

It is proposed that degradation of the D1 and D2 polypeptides under photoinhibition of the isolated D1/D2/cytochrome b_{559} complexes occurs as a result of the loss of functional activity of reaction centers and the D2 polypeptide is damaged to a lesser extent than the D1 polypeptide [3, 5, 36]. The experimental data obtained by us previously confirms these prepositions. Indeed, a decrease in photochemical activity under photoinhibition retards the process of degradation of the D1 and D2 polypeptides. Thus, after 10 min of treatment the functional activity of PS 2 is decreased by more than 80%, whereas the content of the D1 and D2 polypeptides decreases by only 57 and 42%, respectively. This confirms that destruction of pigment–protein complexes of PS 2 depends directly on the state of its electron-transport chain.

The fact that the inhibitor K15 protects the isolated D1/D2/cytochrome b_{559} complexes against the loss of functional activity under photoinhibition suggests that the D1 and D2 polypeptides composing the RC integral polypeptide components should be also protected by the inhibitor against photodegradation. This is the case. However, if the ability of K15 to protect these polypeptides against photodegradation was only determined by general protecting action of the inhibitor under photoinactivation of PS 2, then equal efficiency of the protecting action of K15 for both polypeptides should be expected. Furthermore, taking into account data about larger vulnerability of the D1 polypeptide to photoinhibition and the data on its higher rate of renewal *in vivo* [3], it should be expected that the D1 polypeptide will be protected against photoinhibition by compound K15 even to a greater extent than the D2 polypeptide. In fact, as our data show, inhibitor K15 protects the D2 polypeptide more effectively than the D1 polypeptide. There is no doubt that for the D2 polypeptide in the presence of

inhibitor K15 a novel, additional, but no less effective protecting against photodegradation mechanism arises, leading to the situation when the D2 polypeptide is protected by inhibitor K15 more efficiently than the D1 polypeptide.

What distinguishes the D2 polypeptide versus the D1 polypeptide with respect to inhibitor K15 providing the additional property to protect the D2 polypeptide against photodegradation? It is known that derivatives of urea (diuron) and triazine (atrazine) protect the D1 polypeptide against degradation under photoinhibition with larger selectivity [26-29]. Protection of the D1 polypeptide occurs as a result of their binding with the so-called herbicide-quinone binding site located on the D1 polypeptide [27] and consequently protects this polypeptide against degradation under photoinhibition. The possibility in principal that the inhibitor can protect a polypeptide against degradation under photoinhibition occurs only at the expense of its binding to this polypeptide was proved for the traditional inhibitors of the electron transfer in PS 2 [26-29]. It is reasonable to propose that in our case the higher stability of the D2 polypeptide under photoinhibition in the presence of inhibitor K15 is also a result of the binding of inhibitor K15 to this polypeptide. The data obtained earlier that diuron and compound K15 differ principally not only by the mechanism of inhibition action on the electron transfer in the PS 2, but also have different places of binding that is revealed as a lack of competition between diuron and the K15 for the binding sites in PS 2 [24]. Moreover, according to the data obtained by us earlier on the thylakoid fragments of the PS 2 [25], the binding of inhibitor K15 occurs immediately to D2 polypeptide.

Based on the results of denaturing electrophoresis one can conclude that the inhibitor K15 not only protects the D1 and D2 polypeptides against photoinduced degradation but also impedes the formation of aggregates from these polypeptides in the process of photoinhibition under aerobic conditions. Oxygen-dependent aggregation of polypeptides was observed earlier [36, 38], where it was shown that the light-induced formation of active species of oxygen which modified amino acids of proteins, promoting formation of bonds between them. The inhibitor K15 probably prevents formation of $^1O_2$ and thereby decreases the process of formation of large aggregates during photoinhibition under aerobic conditions.

Thus, inhibitor K15 forming cyclic electron transfer around PS 2 and probably impeding formation and accumulation of free radicals on the donor and acceptor sides of the PS 2 effectively protects the PS 2 reaction center not only against suppression of photochemical activity, as shown earlier [21-23], but also against destruction of the D1 and D2 polypeptides in the process of photoinhibition under aerobic conditions. Suppression of photochemical activity slows the process of degradation of the D1 and D2 polypeptides. This confirms that destruction of pigment–protein complexes of PS 2 depends directly on the state of the electron-transport chain. The fact that K15 protects selectively the D2 polypeptide against destruction under photoinhibition is in good agreement with supposition that its binding site is located on this polypeptide. From the practical point of view, these data suggest the possibility to achieve regulated photoinhibition in the presence of compound K15 of complexes of PS 2 in which D1 polypeptide will be removed but D2 polypeptide will be practically totally retained. This can be used in a series of works directed to investigation of the mechanisms of reconstruction of native and modified complexes of PS 2.

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