Photoreduction of $NADP^+$ in Photosystem II of Higher Plants: Requirement for Manganese

Suleyman I. Allakhverdiev and Vyacheslav V. Klimov

Institute of Soil Science and Photosynthesis, U.S.S.R. Academy of Sciences, Pushchino, Moscow Region, 142292, U.S.S.R.

Z. Naturforsch. **47c**, 57–62 (1992); received August 6, 1990/July 9, 1991

Photoreduction of $NADP^+$, Photosystem II, Pheophytin, Manganese, Reaction Center

The effects of reversible manganese extraction on $NADP^+$ photoreduction were studied with higher plant subchloroplast preparations of photosystem II (PS II). Under anaerobic conditions, when the reaction centers (RCs) of PS II are "closed" (*i.e.* in the state [P680 Pheo] Q_A^-), and in the presence of ferredoxin–ferredoxin–$NADP^+$ reductase, $NADP^+$ reduction is observed at a rate of 0.8–1.1 μmol/mg × chlorophyll × h. After complete removal of manganese from PS II, the rate of $NADP^+$ reduction is reduced 40–50-fold. Upon the addition of Mn at a concentration of approx. 4 Mn atoms per reaction center, the $NADP^+$ reduction is restored up to 85–90% of the initial value. When half of this amount of Mn is combined with about 40 times of the equivalent concentration of other divalent ions (Ca^{2+}, Sr^{2+}, Mg^{2+} etc.) the reaction is also reactivated. Dinoseb (10^{-6} M) an inhibitor of electron transfer in PS II prevents $NADP^+$ photoreduction. It is concluded that under conditions when the first quinone acceptor, Q_A, is in its reduced state (Q_A^-), electrons are transferred from reduced pheophytin ($Pheo^-$) to $NADP^+$, indicating that PS II can reduce $NADP^+$ without the participation of PS I. On the basis of these and literature data, an alternate pathway for electron phototransfer in PS II reaction centers of higher plants is suggested. Some problems concerning the Z-scheme are discussed.

Introduction

The Z-scheme for photosynthetic electron transport is generally accepted and is in accordance with the necessity for the absorption of 8 quanta in order to release one O_2 molecule. Four quanta are absorbed by each of the two photosystems which promote the transfer of 4 electrons from 2 water molecules to two $NADP^+$ molecules ($E_0' = -0.32$ V) [1–5]. Normally electrons from reduced ferredoxin (Fd) are transferred to $NADP^+$ *via* ferredoxin-$NADP^+$ reductase. This specific flavoprotein mediates the one-electron reaction of ferredoxin and the two-electron reaction of $NADP^+$.

The mid-point redox-potential of the intermediate electron acceptor of PS II, pheophytin (Pheo), is −0.61 V [6, 7]. Theoretically, this potential is sufficent to allow PS II to photoreduce electron acceptors with $E_0' \simeq -0.4$ V (ferredoxin, $NADP^+$, methylviologen, benzylviologen, etc.) typical for PS I [8, 9]. This idea has been supported by data on the acceleration of the dark oxidation of $Pheo^-$ after its reduction in the light in PS II-enriched subchloroplast particles of higher plants [8, 10] and in mutants of the green alga *Chlamydomonas reinhardii* lacking PS I [9, 10] when ferredoxin, $NADP^+$, MV^{2+}, BV^{2+}, etc. are added to the samples.

Recently, we have shown that PS II particles can photoreduce $NADP^+$ at a low rate [11]. This was confirmed by using PS II reaction center preparations [12], although the relation of this photoreaction to the intact PS II was not quite clear. In this paper, further evidence in presented for $NADP^+$ photoreduction in PS II enriched in subchloroplast particles as directly related to electron transfer within PS II.

Materials and Methods

Chloroplasts were isolated from 10 to 14 day old pea seedlings according to [13] with some modifications [14]. Isolation of the "heavy", oxygen-

Abbreviations: Chl, chlorophyll; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; DCMU (diuron), 3-(3′,4′-dichlorophenyl)1,1-dimethylurea; Dinoseb, 2,4-Dinitro-6-(2-butyl)phenol; MV^{2+}, methylviologen; BV^{2+}, benzylviologen; PPC, protein-pigment complexes; ΔA, photoinduced absorbance changes; ΔF, photoinduced changes of Chl fluorescence yield; PS, photosystem; P680, the primary electron donor in PS II; Q_A and Q_B, primary and secondary electron acceptors of PS II; Pheo, pheophytin; ETC, electron transport chain.

Reprint requests to S. Allakhverdiev.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/92/0100–0057 $ 01.30/0

evolving chloroplast fragments enriched in PS II was carried out as described [7, 15] by centrifugation of a chloroplast suspension, treated with 0.4% digitonin and 0.1% Triton X-100, at 20,000 × *g*. These particles are designated as DT-20 and evolve O_2 at a rate of 100–120 μmol/mg × Chl × h with the saturating light in the presence of 0.5 mM ferricyanide. PS II particles isolated by the treatment with Triton X-100 (T-20) alone according to the method as published in [16] evolved O_2 at a rate of 250–300 μmol/mg × Chl × h. Analysis of the T-20 and DT-20 samples showed that they contained 220–250 and 80–100 chlorophyll molecules per one molecule of P680 or Pheo, as well as 6–8 thousand and 15–15.500 chlorophyll molecules per one molecule of P700, respectively. The removal of Mn from the PS II preparations (≃99%) was carried out as described earlier [7, 17–19]. The Mn content was assayed with a flame atomic-absorption spectrophotometer AAS-1 (Carl Zeiss "Jena") using calibrated solutions of $MnCl_2$ [7, 19]. The activity of PS II was determined by changes in the variable Chl fluorescence yield (ΔF) related to photoreduction of the primary electron acceptor Q_A, and by the rate of 2,6-dichlorophenol indophenol (DCPIP) photoreduction [7, 20]. The kinetics of photoinduced absorption changes of chlorophyll P680 (ΔA_{678}), DCPIP (ΔA_{590}), NADPH (ΔA_{340}) and ΔF was measured in a 10 mm path length cuvette at 20 °C by means of a phosphoroscope set-up [6–10, 17–20]. The concentration of PS II RCs was calculated from the value of ΔA_{685} related to photoreduction of pheophytin as the intermediate electron acceptor [21] and ΔA_{678} related to photooxidation of the primary electron donor P680 [22]. The molar extinction coefficient for NADPH of 6.3×10^3 M^{-1} cm^{-1} at 340 nm was used in calculations [23]. Anaerobic conditions were produced by adding glucose (10 mM), glucose oxidase (about 50 units/ml) and catalase (about 1000 units/ml) into a stoppered cuvette [8–11, 24]. The measurements were carried out in a medium containing 20 mM Tris-HCl (pH 7.8), 35 mM NaCl and 2 mM $MgCl_2$ at 20 °C. Chlorophyll concentration was 10 μg/ml.

Triton X-100 and digitonin were purchased from Merck, Germany; Tris buffer from Serva, Germany; glucose oxidase and ferredoxin-$NADP^+$ reductase from Sigma, U.S.A.; $NADP^+$ from Reanal, Hungary. All other reagents were of analytical or chemical grade from the U.S.S.R.

Results and Discussion

Illumination of untreated PS II particles under aerobic conditions by intense actinic light induces a 3-fold increase in chlorophyll fluorescence yield up to the level of F_{max}, ($F_o + \Delta F = F_{max}$) related to the photoreduction of Q [6–11] (Fig. 1, curve 1). Under anaerobic conditions, the increase in the chlorophyll fluorescence yield up to the level of F_{max} also occurs, indicating that the state Q_A^- is accumulated [8–11, 24], but in the absence of actinic light (Fig. 1, curve 2). In this case reaction centers of PS II are "closed", *i.e.* they are in the state [P680 Pheo] Q_A^- (Fig. 1). After F_{max} is reached the actinic light induce a decrease in fluorescence yield (Fig. 1, curve 2). This is due to the pheophytin photoreduction [6–11, 17–21]. Photoreduction of $NADP^+$ was investigated in the DT-20 particles under anaerobic conditions, when Q_A was in the reduced state Q_A^- (see K in Fig. 1, curve 2).

When 3 mM $NADP^+$ and the catalytic system ferredoxin (10 mM), ferredoxin-$NADP^+$ reductase (*ca.* 4 μg/ml) are added to DT-20 particles under anaerobic conditions, a photoinduced absorbance change at 340 nm related to the reduction of $NADP^+$ is observed (Fig. 2, curve 1). The rate of $NADP^+$ photoreduction in this case was equivalent to 0.8–1.1 μmol/mg × Chl × h.

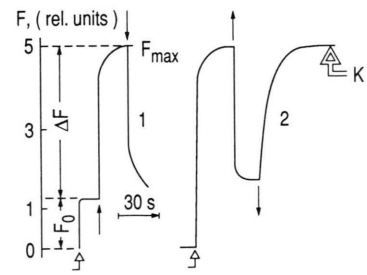

Fig. 1. Kinetics of photoinduced ΔF in chloroplast fragments DT-20 under aerobic (1) and anaerobic (2) conditions. Chlorophyll concentration is about 10 μg/ml. (K-) indicates addition of the system ferrodoxin (Fd) (10 mM) – Fd/$NADP^+$ reductase (*ca.* 4 μg/ml) – $NADP^+$ (3 mM); ⇳ – switch on of the measuring light (approx. 480 nm; ≃0.15 J × m^{-2} × s^{-1}) exciting chlorophyll fluorescence ($\lambda > 650$ nm). ↑ and ↓ -actinic light on and off, respectively ($\lambda > 600$ nm; ≃ 10^{-2} J × m^{-2} × s^{-1}).

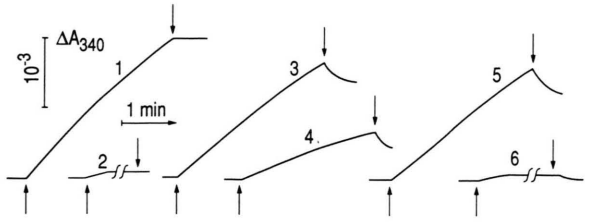

Fig. 2. Kinetics of photoinduced absorbance changes (ΔA) at 340 nm (ΔA_{340}) related to $NADP^+$ photoreduction (added at a concentration of 3 mM) in the presence of 10 mM ferredoxin and ferredoxin-$NADP^+$ reductase (4 μg/ml) by Mn-depleted DT-20 fragments under anaerobic conditions before (1) and after the complete removal of Mn (2–6); in the absence of divalent metals (2) and after addition of 0.4 μM $MnCl_2$ (3); 0.2 μM $MnCl_2$ (4); 0.2 μM $MnCl_2$ plus 4 μM $CaCl_2$ (5); 6 μM $CaCl_2$ (6). Chlorophyll content, 10 μg/ml, 20 °C. ↑ and ↓ -actinic light on and off, respectively.

If $NADP^+$ is reduced by PS II electron transport components, the rate of its photoreduction (similar to photoreduction of DCPIP, Pheo or Q_A and P680 photooxidation [7, 17–20, 22, 24]), should be changed as a result of extraction and subsequent addition of Mn^{2+}. Indeed, upon complete removal of Mn from DT-20 (or T-20) particles, the rate of $NADP^+$ photoreduction is inhibited by a factor of 40–50 (Fig. 2, curve 2). Subsequent addition of 4×10^{-7} M Mn^{2+} to the Mn-depleted DT-20 particles (Fig. 2, curve 3) leads to a marked activation of this photoreaction (up to 85–90% of the initial value). The addition of Mn^{2+} at a concentration of 2×10^{-7} M (Fig. 2, curve 4) has only a slight effect on the rate of $NADP^+$ photoreduction, but this photoreaction can be dramatically activated when 4 μM Ca^{2+} (Fig. 2, curve 5), Sr^{2+} or Mg^{2+} are added along with the Mn^{2+}. However, with Mn^{2+} absent, the addition of any other divalent metal cation does not activate the reaction of $NADP^+$ photoreduction (Fig. 2, curve 6). Na^+ ions up to 0.1 M with or without 2×10^{-7} M Mn^{2+} has no effect on the value of the ΔA_{340} change due to $NADP^+$ photoreduction.

Fig. 3 shows the dependence of $NADP^+$ photoreduction rate (ΔA_{340}) in Mn-depleted DT-20 particles on Mn^{2+} concentration in the medium. This dependence exhibits some peculiarities. The reactivation is observed at extremely low concentrations of Mn^{2+}. It appears at a concentration of 10^{-7} M and is at maximum at 4×10^{-7} M, which corresponds to about 4 Mn^{2+} atoms per RC (Fig. 3, curve 1). When cations like Ca^{2+}, Sr^{2+}, Mg^{2+}, Co^{2+}, *etc.* are present in the medium (40–60 atoms per RC), the maximum reactivation of ΔA_{340} is observed at even lower concentrations of Mn^{2+} (*ca.* 2×10^{-7} M), when no more than two Mn^{2+} atoms per RC are added to the medium (Fig. 3, curve 2). This dependence of the reactivation of the $NADP^+$ photoreduction capacity on the concentration of Mn^{2+} added in combination with Ca^{2+} or other divalent metal cations is similar to that observed earlier under the same conditions for the reactivation of photoreduction of Q_A, Pheo and DCPIP [7, 17, 18, 20] and P680 photooxidation [22].

The decrease of the ΔA_{340} rate after complete removal of Mn from PS II preparations and its increase after subsequent addition of Mn^{2+} indicate that the functional activity of the donor side PS II is required for the photoreaction resulting in $NADP^+$ reduction. This strongly suggests that the donor side of the PS II RC provides the source of the electrons. The effect of the extraction and subsequent addition of Mn^{2+} at extremely low concentrations on $NADP^+$ photoreduction as well as on Q_A, Pheo, DCPIP photoreduction and P680 photooxidation (see ref. above) supports the conclusion that $NADP^+$ photoreduction under these conditions occurs directly *via* the PS II electron transport.

The suggestion that $NADP^+$ can be photoreduced by PS II is supported by inhibition analysis

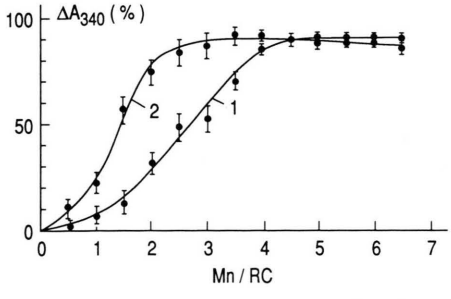

Fig. 3. Dependence of $NADP^+$ photoreduction in Mn-depleted DT-20 particles on concentration of $MnCl_2$ added to the medium in the absence (1) or in the presence of 4 μM $CaCl_2$ (2). Vertical bars show a mean root-square error of ΔA_{340} measurements (8 experiments). A concentration of 1 Mn/RC corresponds to the addition of 10^{-7} M $MnCl_2$ to the suspension of DT-20 particles at a chlorophyll concentration of 10 μg/ml.

of PS II electron transfer. When 10^{-5} M diuron is used to block electron transfer between the primary and secondary acceptors (Q_A and Q_B) in DT-20 particles reactivated by Mn^{2+}, the rate of $NADP^+$ photoreduction was practically unchanged (data not shown). This indicates that $NADP^+$ photoreduction is not connected with electron donation from Q_A or Q_B which are kept in their reduced state during our experiments. In contrast, dinoseb (2,4-dinitro-6-(2-butyl)phenol), a well-known inhibitor of PS II reactions which is capable of accepting an electron from $Pheo^-$ [24, 25] caused a decrease in the $NADP^+$ photoreduction by 15–20% at a concentration of 2×10^{-7} M under anaerobic conditions. At a concentration of about 10^{-6} M dinoseb, a considerable (>80%) decrease of the ΔA_{340} change was observed (data not shown).

When pheophytin photoreduction (ΔA_{685} and ΔA_{545}) and P680 photooxidation (ΔA_{678}) were assayed simultaneously under the same inhibitory conditions the degree of inhibition for these activites was found to correspond to that of the $NADP^+$ photoreduction.

Thus, in PS II preparations the electrons from the photoactive $Pheo^-$ can be transferred to $NADP^+$ in the presence of the exogenous catalytic system: ferredoxin–ferredoxin–$NADP^+$ reductase employing anaerobic conditions when Q_A is in the state Q_A^-. It was shown earlier that in the presence of 3 mM $NADP^+$ whole cells of the *Chlamydonomas reinhardii* mutant ACC-1 (which contains only chlorophyll-α-protein complex of PS II and no photosystem I and light-harvesting complex) a considerable (20–30-fold) acceleration of dark relaxation of the photoinduced ΔF related to Pheo photoreduction [9] occurs. In other words, $NADP^+$ added to the mutant ACC-1 without an exogenous catalytic system resulted in acceleration of dark oxidation of Pheo that is reduced in the light. Apparently, electrons are transferred from $Pheo^-$ to $NADP^+$ [9] and the corresponding reductase is present in these mutants (in contrast to the DT-20 and T-20 particles [9, 10] and $Pheo^-$ is accessible to these enzyme(s). Additionally, it was found [9, 10] that typical PS I electron acceptors like MV^{2+}, BV^{2+}, NO_2^-, NO_3^-, SO_4^{2-} can functionally interact with $Pheo^-$ produced in PS II.

Thus, electron acceptors typical for PS I ferredoxin, $NADP^+$, MV^{2+}, BV^{2+}, NO_2^-, NO_3^-, SO_4^{2-} ($E_0' \simeq -400$ mV) can accept electrons from reduced pheophytin ($E_0' = -610$ mV [6, 7] of PS II ($\Delta E_0' \simeq 200$ mV; $\Delta G^O \simeq 4.7$ kcal/mol). It should be noted that such a change in free energy is not sufficient for ATP synthesis [26–28].

On the other hand, our results support once more the conclusion made earlier [7, 17, 18, 20, 22] that Mn-containing active centers functioning at the donor side of PS II have 4 Mn atoms per 1 RC. Two of them are obligatory in the redox reactions linked with H_2O oxidation to O_2. Two other Mn atoms can be replaced by other divalent metal cations. Probably, the two other Mn atoms perform some structural function.

Taking into account the high rate of electron transfer from $Pheo^-$ to Q_A (200–250 ps [3, 29, 30]), it cannot be expected that $NADP^+$ (or other PS I electron acceptors) will be photoreduced with high efficiency in PS II under conditions when Q_A is in the oxidized (functionally active) form. Instead, this process must likely take place as an artificial side pathway (alternate or reserve way) of electron transfer only when Q_A is accumulated in the reduced form and incapable of accepting electrons. In other words, if normal electron transport is blocked at Q_A level, *i.e.* when the electron influx exceeds the efflux (*e.g.*, under the effect of various stress factors) there may appear an alternative way of electron transfer from the reduced pheophytin to $NADP^+$ without PS I participation.

Earlier, Arnon [31] developed the idea that long-wave and short-wave photosystems function in parallel but not consecutively. According to this author's opinion, the long-wave system provides cyclic and the short-wave one non-cyclic electron flow. It is suggested that each functions independently and each includes a single type of photoreaction. Since 1969 Arnon *et al.* [32] has suggested, and even more recently developed a scheme [33–35], that three photochemical reactions occur within the two photosystems. One photoreaction is involved in the long-wave system of cyclic photophosphorylation, whereas the short-wave noncyclic system includes two photoreactions, II_a and II_b. Photoreaction II_b is suggested to be responsible for H_2O photooxidation to O_2, while photoreaction II_a – for cytochrome b_{559} oxidation and $NADP^+$ reduction.

It was reported [36, 37] that two different types of protein-pigment complexes (PPC) of PS II RC

can be isolated. The study of the functional properties of these complexes showed that the PS II RC from one these protein-pigment complexes lacks P680 [38]. An irreversible bleaching with a maximum of 670 nm, *i.e.* different from the position of P680 band was observed. However, there are indirect data indicating that this PPC must contain RC though it can be of a type quite different from P680 [37, 38].

Hiyama *et al.* [39] suggested that there are two types of P700 present, one of which acts under high light intensities, and is photooxidized in connection with general electron flow, while the second operates only under low light. Breton indicated that the population of PS I RC was heterogenous which was manifested by different sensitivity of signal decay of the centers in the presence of ferricyanide [40]. The authors of ref. [41] propose two types of PS I, being present but only one of them should be subjected to cation regulation at the level of excitation energy distribution. Unfortunately, direct evidence is still absent. Our data and that in the references cited indicate that the Z-scheme can not be considered as a rigidly fixed electron transport chain (ETC) of photosynthesis. Probably, under most conditions this is the best available route for electrons.

Thus, on the basis of literature data and our experimental material it can be suggested that there may exist a side path of electron transport in PS II (Fig. 4).

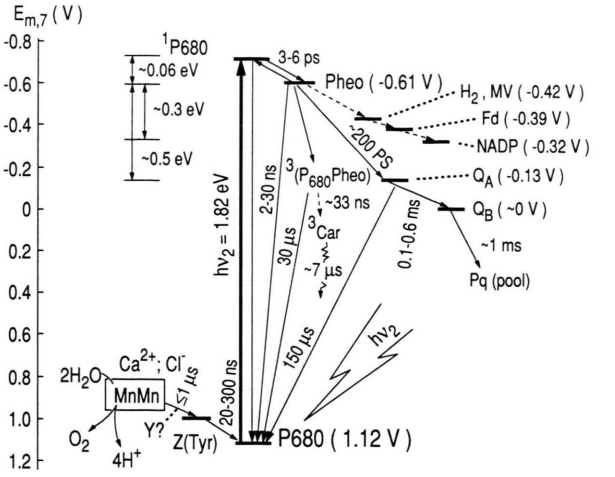

Fig. 4. The supposed scheme of the electron transfer reactions in the reaction centers of photosystem II of plants (supplementing the scheme suggested by us earlier, see [6, 7, 11, 17]).

Acknowledgements

We thank Prof. L. N. Bell and Dr. T. Wydrzynski for valuable comments and fruitful discussion of the results obtained.

[1] R. Clayton, Photosynthesis, Physical mechanisms and chemical patterns, p. 350, Mir, Moscow 1984 (in Russian).
[2] Govindjee, Photosynthesis, pp. 8–108, Mir, Moscow 1987 (in Russian).
[3] V. V. Klimov and V. A. Shuvalov, Biofizika **32,** 814–829 (1987) (in Russian).
[4] A. B. Rubin and V. P. Shinkarev, Electron transport in biological systems, p. 319, Nauka, Moscow 1984 (in Russian).
[5] V. P. Skulachev, Energetics of biological membranes, p. 564, Nauka, Moscow 1989 (in Russian).
[6] V. V. Klimov, S. I. Allakhverdiev, S. Demeter, and A. A. Krasnovsky, Dokl. Acad. Nauk S.S.S.R. **249,** 227–230 (1979) (in Russian).
[7] S. I. Allakhverdiev, Ph.D. Thesis, Inst. of. Biol. Phys., Pushchino, p. 169 (1983) (in Russian).
[8] V. V. Klimov, V. A. Shuvalov, and U. Heber, Biochim. Biophys. Acta **809,** 345–350 (1985).
[9] V. V. Klimov, S. I. Allakhverdiev, and V. G. Ladygin, Photosynth. Res. **10, 3,** 209–219 (1986).
[10] S. I. Allakhverdiev, V. V. Klimov, and V. G. Ladygin, Biofizika **33, 3,** 442–447 (1988) (in Russian).
[11] S. I. Allakhverdiev and V. V. Klimov, Biologicheskie membrany **7, 5,** 497–505 (1990) (in Russian).
[12] D. I. Arnon and J. Barber, Proc. Natl. Acad. Sci. U.S.A. **87,** 5930–5934 (1990).
[13] F. R. Whatley and D. I. Arnon, in: Methods in Ensymology, **Vol. 8,** pp. 308–313, Academic Press, New York 1963.
[14] R. M. Bekina and A. A. Krasnovsky, Biokhimiya **33, 1,** 178–181 (1968) (in Russian).
[15] V. V. Klimov, S. I. Allakhverdiev, N. I. Shutilova, and A. A. Krasnovsky, Fiziologiya rastenii **27, 2,** 315–326 (1982) (in Russian).
[16] D. A. Berthold, G. T. Babcock, and Ch. F. Yocum, FEBS Lett. **134, 2,** 231–234 (1981).
[17] V. V. Klimov, S. I. Allakhverdiev, V. A. Shuvalov, and A. A. Krasnovsky, FEBS Lett. **148, 2,** 307–312 (1982).
[18] V. V. Klimov, S. I. Allakhverdiev, V. A. Shuvalov, and A. A. Krasnovsky, Dokl. Acad. Nauk S.S.S.R. **263, 4,** 1001–1005 (1982) (in Russian).
[19] S. I. Allakhverdiev, V. V. Klimov, and I. I. Proskuryakov, Biofizika **33, 4,** 600–603 (1988) (in Russian).
[20] S. I. Allakhverdiev, A. V. Klevanik, V. V. Klimov, V. A. Shuvalov, and A. A. Krasnovsky, Biofizika **28, 1,** 5–8 (1983) (in Russian).
[21] V. V. Klimov, E. Dolan, and B. Ke, FEBS Lett. **112, 1,** 97–100 (1980).
[22] S. I. Allakhverdiev, M. A. Shafiev, and V. V. Klimov, Photobiochem. Photobiophys. **12, 1,** 61–65 (1986).
[23] Handbook on biochemistry, p. 324, Naukova Dumka, Kiev (1971) (in Russian).
[24] S. I. Allakhverdiev, S. K. Zharmukhamedov, V. V. Klimov, S. S. Vasil'ev, B. N. Korvatovsky, and V. Z. Pashchenko, Biologicheskie membrany **6, 11,** 1147–1153 (1989) (in Russian).
[25] V. V. Klimov, S. I. Allakhverdiev, and S. K. Zharmukhamedov, Fiziologiya rastenii **36, 4,** 770–778 (1989) (in Russian).
[26] M. G. Goldfeld and L. A. Blumenfeld, Bull. Magn. Reson. **1,** 66–104 (1979).
[27] A. G. Volkov, Uspekhi sovremennoi biologii **105, 3,** 467–487 (1988) (in Russian).
[28] A. N. Tikhonov and A. A. Timoshin, Biologicheskie membrany **2,** 349–362 ibid. 608–622 (1985) (in Russian).
[29] V. A. Shuvalov, V. V. Klimov, E. Dolan, W. W. Parson, and B. Ke, FEBS Lett. **118, 1,** 279–282 (1980).
[30] A. M. Nuijs, Ph.D. Thesis, Leiden (1986).
[31] D. Arnon, H. Y. Tsujimoto, and B. Mc. Swain, Nature **207,** 1367–1369 (1965).
[32] D. Knaff and D. Arnon, Proc. Natl. Acad. Sci. U.S.A. **59,** 1527–1530 (1969).
[33] D. Arnon, H. Y. Tsujimoto, and G. M.-S. Tang, Proc. Natl. Acad. Sci. U.S.A. **78,** 2942–2946 (1981).
[34] D. Arnon, H. Y. Tsujimoto, and G. M.-S. Tang Biochem. Biophys. Res. Commun. **106,** 450–45[illegible] (1982).
[35] D. Arnon and G. M.-S. Tang, FEBS Lett. **253,** 253–256 (1989).
[36] K. Satoh, H. Y. Nakatani, and K. F. Steinback, Biochim. Biophys. Acta **724,** 142–150 (1983).
[37] S. M. Kochubey, T. M. Shadchina, and V. N. Lashenko, Photosynthetica **18,** 240–247 (1984).
[38] H. Y. Nakatani, B. Ke, E. Dolan, and C. J. Arntzen, Biochim. Biophys. Acta **765,** 344–352 (1984).
[39] T. Hiyama, B. D. Mc. Swain, and D. Arnon, Biochim. Biophys. Acta **460,** 76–84 (1977).
[40] J. Breton, Biochim. Biophys. Acta **459,** 65–75 (1976).
[41] S. Bose and P. Ramanujam, Biochim. Biophys. Acta **764,** 40–45 (1984).