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pH Dependence of the Efficiency of Binding of Iron Cations to the Donor Side of Photosystem II

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Abstract—Light-induced interaction of Fe(II) cations with the donor side of Mn-depleted photosystem II (PS II(−Mn)) results in the binding of iron cations and blocking of the high-affinity (HA_Z) Mn-binding site. The pH dependence of the blocking was measured using the diphenylcarbazide/2,6-dichlorophenolindophenol test. The curve of the pH dependence is bell-shaped with $pK_1 = 5.8$ and $pK_2 = 8.0$. The pH dependence of the O_2-evolution mediated by PS II membranes is also bell-shaped ($pK_2 = 7.6$). The pH dependence of the process of electron donation from exogenous donors in PS II(−Mn) was studied to determine the location of the alkaline pH sensitive site of the electron transport chain. The data of the study showed that the decrease in the iron cation binding efficiency at pH > 7.0 during blocking was determined by the donor side of the PS II(−Mn). Mössbauer spectroscopy revealed that incubation of PS II(−Mn) membranes in a buffer solution containing $^{57}Fe(II) + {}^{57}Fe(III)$ was accompanied by binding only Fe(III) cations. The pH dependence of the nonspecific Fe(III) cation binding is also described by the same bell-shaped curve with $pK_2 = 8.1$. The treatment of the PS II(−Mn) membranes with the histidine modifier diethylpyrocarbonate resulted in an increase in the iron binding strength at alkaline pH. It is suggested that blocking efficiency at alkaline pH is determined by competition between OH^- and histidine ligand for Fe(III). Because the high-affinity Mn-binding site contains no histidine residue, this fact can be regarded as evidence that histidine is located at another (other than high-affinity) Fe(III) binding site. In other words, this means that the blockage of the high-affinity Mn-binding site is determined by at least two iron cations. We assume that inactivation of oxygen-evolving complex and inhibition of photoactivation in the alkaline pH region are also determined by competition between OH^- and a histidine residue involved in coordination of manganese cation outside the high-affinity site.

Key words: photosystem II, oxygen-evolving complex, manganese, iron, histidine, Mössbauer spectroscopy, histidine modification

The photolysis of water in plants is accompanied by the evolution of molecular oxygen to the atmosphere. Although this reaction is of essential importance, the molecular mechanism of water oxidation remains insufficiently understood. This is mainly due to the fact that neither the structure of the manganese cluster involved in catalysis of water oxidation nor the nature of the ligands bound to manganese cations have been identified thus far. The results of X-ray diffraction analysis of photosystem II (PS II) crystals from the cyanobacteria *Thermosynechococcus vulcanus* and *Synechococcus elongatus* provided an opportunity to determine the parameters of the crystallographic cell containing the manganese cluster [1, 2].

Both oxygen atoms (in the carboxyl groups of glutamic and aspartic amino acids, phenol group of tyrosine) and nitrogen atoms (histidine) are involved in coordination of manganese cations. The presence of histidine in the coordination sphere of the manganese cations was demonstrated using ESEEM (Electron Spin-Echo Envelope Modulation) [3] and FTIR (Fourier transform IR-spectroscopy) [4]. According to the results of the X-ray diffraction analysis, the ligands are mainly located in

Abbreviations: HA_Z) high-affinity Mn-binding site; DPC) diphenylcarbazide; DCBQ) 2,6-dichloro-*p*-benzoquinone; DCPIP) 2,6-dichlorophenolindophenol; DEPC) diethylpyrocarbonate; OEC) oxygen-evolving complex; SM) silicomolybdate; PS II) photosystem II; PS II(−Mn)) manganese-depleted photosystem II; Chl) chlorophyll.

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the C-terminal domain of polypeptide D1 of the PS II reaction center [2]. Perhaps the following amino acid residues are involved in the manganese cluster binding: carboxyl group of alanine D1-A344, D1-D170, D1-E333, D1-H337, and D1-D189 (or D1-H190). However, the spatial resolution of the X-ray analysis (3.7 Å) was insufficiently high to resolve this binding.

The manganese cluster can be extracted from the oxygen-evolving complex (OEC) without inactivation of binding sites, which is evidenced by the possibility of reconstruction of the oxygen evolution activity during incubation of Mn-free PS II particles (PS II(−Mn)) in the presence of manganese and calcium cations under exposure to low-intensity light [5, 6]. Self-assembly of manganese cluster begins with oxidation of manganese cations through the high-affinity Mn-binding site (HA_Z), which is one of the sites of binding of the four-manganese cluster [7, 8]. Binding of Mn(II) cations to the high-affinity Mn-binding site is accompanied by inhibition of oxidation of exogenous donor of electrons for PS II(−Mn), diphenylcarbazide (DPC), by the secondary electron donor Y_Z [9, 10]. Some researchers used this effect to study the HA_Z Mn-binding site [11-13]. In our earlier works, we found that iron cations were also able to bind to the HA_Z site and the characteristics of the processes of binding of manganese and iron cations being comparable to each other [14, 15]. The presence of the conservative regions capable of binding of either iron or manganese cations in the C-terminal domains of the polypeptides D1 and D2 can provide a molecular basis of this effect [16]. Incubation of PS II(−Mn) membranes in the presence of cations Fe(II) under exposure to weak light (conditions of self-assembly of manganese cluster) is accompanied by binding of iron cations to the high-affinity Mn-binding site and inhibition of the process of electron donation from iron or manganese cations through the HA_Z binding site (blocking effect) [17]. The number of Fe(II) cations, whose oxidation determines the blocking effect, ranges from two to five [17]. The curve of the pH dependence of the blocking effect is bell-shaped with optimum pH at 6.0-6.5 [17]. The efficiency of binding of iron cations decreases upon increasing the pH, and causes of this phenomenon are of considerable interest, because no such decrease is observed in case of interaction of manganese cations with the HA_Z binding site (electron donation) [17]. This fact indicates that the effect of blocking is determined by at least two cation-binding sites rather than only one site. In this work, we studied the mechanism of the decrease in the efficiency of binding of iron cation to the high-affinity Mn-binding site in the alkaline pH range.

MATERIALS AND METHODS

Membrane preparations of BBY-type PS II were isolated from market spinach as described elsewhere [18]. Isolated preparations were suspended in buffer solution A containing 0.4 M sucrose, 50 mM Mes-NaOH, and 15 mM NaCl (pH 6.5) and stored in liquid nitrogen until use. The activity of the BBY-particles ranged from 400 to 500 μmol O_2/h per mg chlorophyll (Chl).

Manganese was extracted from PS II OEC by treating PS II particles (0.5 mg Chl per ml) with 0.8 M Tris-HCl buffer (pH 8.5) for 15 min at room temperature and room ambient illuminance [19].

Photochemical activity of preparations PS II and PS II(−Mn) was determined by the rate of oxygen evolution and reduction of artificial electron acceptor 2,6-dichlorophenolindophenol (DCPIP), respectively. The rate of the photoinduced oxygen evolution by PS II preparations (10 μg Chl per ml) was measured using a closed platinum Clark electrode in buffer solution A in the presence of 0.2 mM 2,6-dichloro-*p*-benzoquinone (DCBQ) (Eastman Kodak, Co., USA). The rate of electron transport in PS II and PS II(−Mn) particles (20 μg Chl per ml) was measured spectrophotometrically by the rate of DCPIP reduction (40 μM).

Buffers and solutions. Unless noted otherwise, photochemical activity (oxygen evolution or reduction of DCPIP) was measured in buffer A. Buffers Mes (pH range from 5.0 to 7.0) and Hepes (pH range from 7.5 to 8.5) were used to study the pH dependence. Silicomolybdate (SM) (silicomolybdenic acid hydrate, Aldrich, USA) was dissolved in H_2O. The resulting solution was centrifuged and concentration of SM was measured spectrophotometrically in the supernatant at 400 nm (ε_{400} = 1.07 $mM^{-1}\cdot cm^{-1}$) [20]. Solutions of $FeSO_4$ (0.5 mM), $MnCl_2$ (0.5 mM), and Fe(III) solution stabilized at neutral pH with sucrose [21] were prepared immediately before experiments. Iron solution prepared from metal powder of the ^{57}Fe isotope was used in Mössbauer spectroscopy.

Concentration of ferrous iron in solutions was measured using bathophenanthroline disulfonic acid disodium salt (Sigma, USA) (final concentration, 1 mM) [22].

Binding of Fe(II) cations to the high-affinity Mn-binding site was monitored using the DPC/DCPIP test [9-15]. According to the results of the test, binding of Fe(II) cations caused a 50% decrease in the rate of DPC oxidation at concentration of manganese and iron cations in reaction medium >2 μM. Blocking of the high-affinity Mn-binding site with iron cations was performed as follows. After membranes PS II(−Mn) (20 μg Chl per ml) had been incubated for 3 min in buffer A (pH 6.5) under exposure to a fluorescence daylight lamp (photon flux density, 15 μE/sec per m^2) in the presence of 10 μM Fe(II), the membranes were sedimented by centrifugation, and rate of DCPIP (40 μM) reduction in the presence of DPC (200 μM) was measured. The pH dependence of the blocking effect was measured in buffer solutions of corresponding composition. After that, the sample was centrifuged and assayed for activity in the

DPC/DCPIP test in buffer A (pH 6.5). The extent of the blocking effect was determined by the degree of inhibition of the rate of DPC oxidation.

Mössbauer spectra were measured at 80K using an electrodynamic spectrometer with uniformly accelerated motion of the sample (2 mg Chl per 0.3 ml sample) relative to a radiation source (^{57}Co in a Rh matrix). Mössbauer spectra were simulated using the computer program Univem (MOSTEK, Russia). Isomer shifts were measured relative to ^{57}Fe.

PS II(−Mn) particles were treated with diethylpyrocarbonate (DEPC), a histidine modifier, using the method described in [10, 23].

RESULTS AND DISCUSSION

The curve of the pH dependence of the effect of blocking of the high-affinity Mn-binding site by iron cations was studied in the initial stage of the investigation (Fig. 1). The degree of the blocking was determined by the efficiency of inhibition of electron transfer from DPC to DCPIP [9-15]. The resulting pH dependence was found to be bell-shaped with pK_1 5.8 and pK_2 8.0 (Table 1). According to the value of pK_1, the corresponding process is determined by protonation/deprotonation of the carboxyl group of aspartic and glutamic amino acids (although the pK value of the carboxyl group in the isolated amino acids is 4.2 and 3.9, respectively, the pK values of the corresponding amino acid residues in a protein molecule range from 3 to 6), whereas pK_2 in weakly alkaline region is closer to the pK of the τ-nitrogen of histidine (pK 6.1 in isolated amino acid or 5-8 in the corresponding amino acid residue in protein molecules). In this work, we studied mainly the nature of pK_2.

Table 1. Reaction medium pH corresponding to 50% inhibition of electron-transport activity of PS II and PS II(−Mn) membrane preparations and 50% inhibition of specific* or nonspecific** binding of iron cations to PS II(−Mn)

Reaction	pK_1	pK_2
O_2 evolution by PS II in the presence of electron acceptors:		
DCBQ, 200 μM	4.9	7.6
SM, 100 μM	5.4	7.5
Reduction of DCPIP by PS II(−Mn) membrane preparations in the presence of electron donors:		
DPC, 200 μM	5.4	7.6
Mn(II), 200 μM	6.3	> 8.0
Mn(II) (2 μM) + H_2O_2 (3 mM)	6.1	—
hydroxylamine, 2 mM	5.8	—
Blocking of high-affinity Mn-binding site by iron cations	5.8	8.0
Binding of Fe(III) cations by PS II(−Mn) membranes	5.9	8.1

* Binding to the high-affinity Mn-binding site of OEC (blocking).
** Binding to the membrane components of PS II(−Mn) other than the Mn-binding sites.

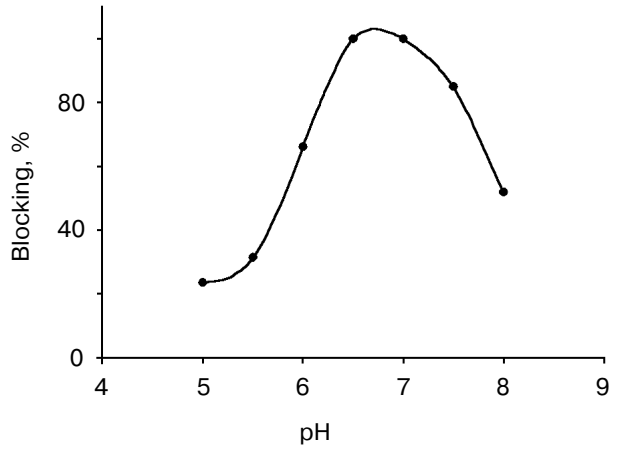

Fig. 1. pH dependence of the efficiency of blocking of the high-affinity Mn-binding site by iron cations in PS II(−Mn) membrane preparations. PS II(−Mn) membrane preparations (20 μg Chl per ml) were incubated in buffer solution of corresponding pH under exposure to light for 3 min in the presence of 10 μM Fe(II). After incubation, membranes were sedimented by centrifugation and resuspended in buffer A (pH 6.5). The fraction of the high-affinity sites (%) blocked by bound iron cations was determined using the DPC/DCPIP test, in which the rate of DCPIP reduction in the presence of DPC in buffer A (pH 6.5) mediated by untreated membranes PS II(−Mn) and such membranes treated with iron cations at pH 6.5 was taken as 0 and 100% (usually, about half-maximum rate), respectively.

With this aim, we studied the pH dependence of the O_2 evolution reaction in intact PS II preparations (Fig. 2) in the presence of various electron acceptors (DCBQ, DCPIP, and SM). DCBQ and SM are effective acceptors of electron, whereas DCPIP is a low-efficiency acceptor and the pH dependence of the process of DCPIP reduction does not allow corresponding pK value to be determined. The values pK_2 determined in the presence of DCBQ or SM are equal to each other (~7.6, see Table 1). Similar values of pK_2 (~7.5) were reported by some other researchers [24, 25]. Given the fact that SM accepts electrons either directly from Q_A^- [26] or from a site other than Q_B [20], whereas DCBQ is reduced at the Q_B-binding site [27], it is safe to suggest that decrease in the electron-transport activity at weakly alkaline pH is determined either by the donor side of the electron-transport chain or by an electron acceptor preceding Q_B^-. The possibility that histidine can be involved in the support of electron transfer on the acceptor side exists, because it was shown that

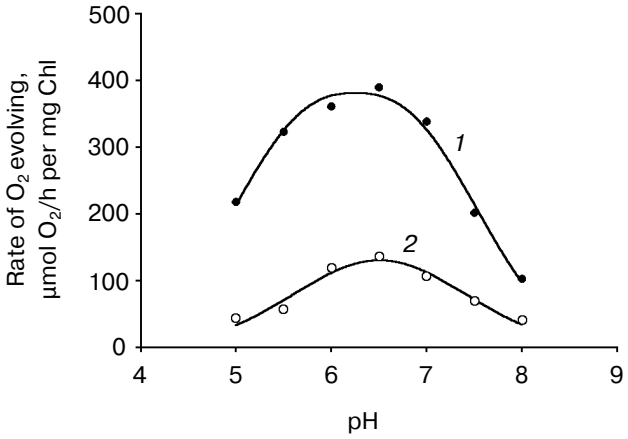

Fig. 2. pH dependence of the rate of oxygen evolution by PS II preparations in the presence of various electron acceptors: *1*) 0.2 mM DCBQ; *2*) 0.1 mM SM. The rate of oxygen evolution was measured at the corresponding pH value.

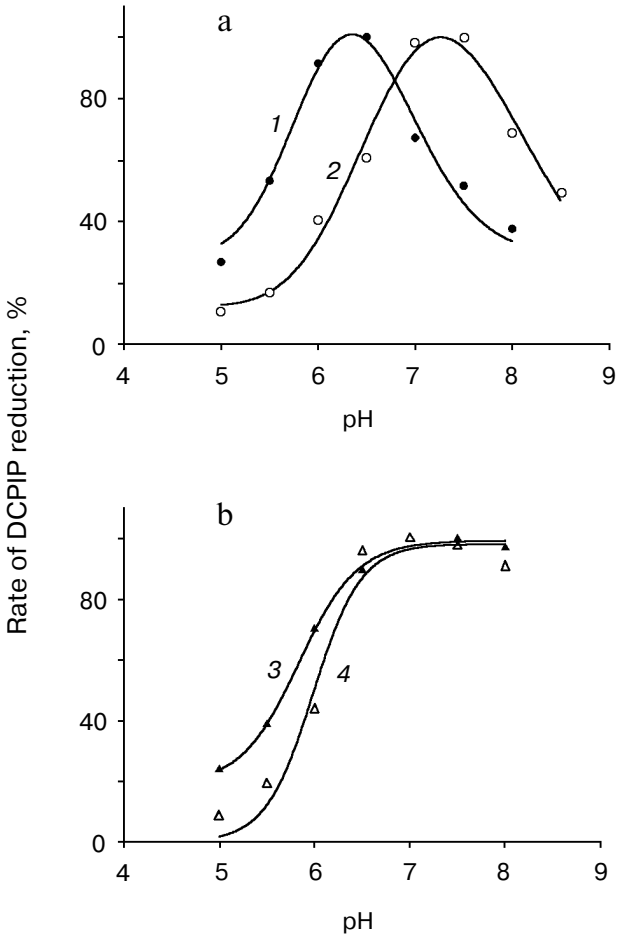

Fig. 3. pH dependence of the rate of photoinduced reduction of DCPIP in PS II(–Mn) membrane preparations (20 μg Chl per ml) in the presence of various electron donors: a) 200 μM DPC (*1*); 200 μM Mn(II) (*2*); b) 2 μM Mn(II) + 3 mM H_2O_2 (*3*); 2 mM hydroxylamine (*4*).

Q_A forms a hydrogen bond with histidine [28] (perhaps, with D2-H215 [29]).

In the intact PS II membranes the effects of pH at the donor side of the electron-transport chain (inactivation of OEC) can be associated not only with modification of manganese-binding sites but also with some other processes (e.g., release of extrinsic proteins [30] or substitution of Cl^- anions in the OEC by the hydroxyl group OH^- [31]). To exclude these effects, we studied the pH dependence of the electron transfer processes in Tris-treated PS II preparations containing no OEC (free of manganese, calcium, and the three extrinsic proteins). The rate of electron transfer was measured spectrophotometrically by the rate of reduction of the artificial electron acceptor DCPIP in the presence of various artificial electron donors. In the case of the use of DPC or Mn(II) as electron donors, the curve of the pH dependence of the electron transfer rate is bell-shaped (Fig. 3a), whereas in the case of the use of hydroxylamine or the system including Mn(II) + H_2O_2, no decrease in the efficiency of electron-transport was observed at alkaline pH (Fig. 3b). Because all these experiments were performed using the same acceptor of electrons, it is safe to conclude that the decrease in the electron-transport activity is determined by the donor rather than the acceptor side of PS II (at least at pH below 8.5). The decrease in the efficiency of DPC oxidation upon increasing the pH (pK_2 7.6) can be explained by the presence of histidine at the DPC-binding site, through which an electron is donated from DPC to $Y_Z^{\bullet}$. Such a possibility was demonstrated in [32] using a modifier of the histidine residues. No histidine residue was found in the high-affinity Mn-binding site [12, 32]. Perhaps the decrease in the rate of DCPIP reduction induced by Mn(II) cations at pH ≥ 8.0 (Fig. 3a) was due to formation of manganese hydroxide at the given pH range and concentration of manganese cations (0.2 mM). As a result of formation of manganese hydroxide, the concentration of Mn(II) decreases. Although, according to the solubility factor $4 \cdot 10^{-14}$, precipitation of $Mn(OH)_2$ at concentration 0.2 mM should begin at pH ~ 9.0 [33], the composition of the incubation medium may have a significant effect on the value of pH corresponding to the onset of precipitation of manganese hydroxide. Indeed, the rate of electron transport from Mn(II) to DCPIP rapidly decreases (within 6 min) virtually to zero upon increasing the time of preliminary (i.e., before exposure to light) incubation of Mn(II) cations in a buffer solution with pH 8.5. The conclusion that the pH dependence of the reaction of electron donation from the Mn(II) cations had no slope in the alkaline pH range was supported by the experimental data obtained using the Mn(II) + H_2O_2 donor system, in which the efficiency of electron donation was found to be pH independent at pH > 6.5 (Fig. 3b). It is well-known that electron donation in this system also occurs from the cation Mn(II) (the concentration of the cation in the system falls within sub-micromolar to

Table 2. Effect of blocking on the rate of oxidation of certain exogenous electron donors by PS II(−Mn) membrane preparations

Donor	Rate of DCPIP reduction in PS II(−Mn) membrane preparations, %			Number of donation sites
	I	II		
		cations Fe(III)	photoinhibition	
DPC, 200 μM	100	50	50	2
Mn(II) (2 μM) + H_2O_2 (3 mM)	100	7	0	1
Hydroxylamine, 2 mM	100	100	50	2

Note: Mn-binding high-affinity site is free (I) or blocked (II) by cations Fe(III) or by photoinhibition.

micromolar range) through the high-affinity Mn-binding site, whereas hydrogen peroxide is required to reduce oxidized manganese cations [12]. The concentration of Mn(II) cation in this system is too low to produce hydroxide. As a result, there is no activity decline at alkaline pH. Therefore, these data are evidence that interaction of Mn(II) cations with the HA_Z Mn-binding site is pH independent within the alkaline pH range. These results can also be regarded as evidence that neither the decline in the efficiency of binding of iron cations to the high-affinity Mn-binding site within the alkaline pH range (pK_2 8.0) (Fig. 1) nor the process of OEC inactivation within the pH range 7.5-7.6 are associated with the pH dependent rearrangement of the HA_Z Mn-binding site. The efficiency of electron donation from hydroxylamine is also pH independent within the alkaline pH range (Fig. 3b). However, it should be noted that electron donation from hydroxylamine proceeds through its own binding site rather than through the HA_Z Mn-binding site [17]. The process of electron donation in this case proceeds through the secondary electron donor $Y_Z^{\bullet}$ rather than directly to $P680^+$ [34].

Because the efficiency of electron donation through the high-affinity Mn-binding site is significantly higher than through the low-affinity site (K_m < 1 μM [9, 11, 12] and K_m ~ 200 μM [35], respectively), it is reasonable to suggest that blocking of the high-affinity Mn-binding site by iron cations causes complete inhibition of electron transport from the Mn(II) + H_2O_2 donor system because of low concentration of manganese. Indeed, blocking of the high-affinity Mn-binding site causes complete inhibition of photoinduced reduction of DCPIP when the Mn(II) + H_2O_2 system is used as electron donor (Table 2), whereas oxidation of DPC (Table 2) or Mn(II) [17] under these conditions is inhibited only by one-half. These findings can also be regarded as evidence that: 1) electron donation from the Mn(II) + H_2O_2 donor system takes place mainly through one site, and 2) neither hydrogen peroxide nor hydroxylamine [17] can reduce the Fe(III) cations capable of blocking the high-affinity Mn-binding site. It was shown in our earlier experiments that blocking of HA_Z did not prevent the hydroxylamine-mediated reduction of the secondary electron donor $Y_Z^{\bullet}$ [17]. In other words, the site of electron donation from hydroxylamine to $Y_Z^{\bullet}$ is HA_Z independent. On the other hand, it remains uncertain whether or not hydroxylamine donates electrons to $P680^+$ through another site, like in the case of donation from DPC, Mn(II), and I^- (low-affinity site directed toward an acceptor of unknown nature) [35]. To elucidate this problem, $Y_Z^{\bullet}$ was subjected to photoinactivation by exposing PS II(−Mn) preparations to saturating light, and monitoring the extent of $Y_Z^{\bullet}$ inactivation using the DPC/DCPIP test has been done [12]. As the exposure time increased, there was a decrease in the rate of DPC oxidation, which reached a 50%-photoinhibition plateau at the exposure duration of 1 min. This magnitude of the photoinhibition was evidence of complete inactivation of the site of DPC oxidation by the tyrosine Y_Z. This suggestion was also supported by the fact the Mn(II) + H_2O_2 donor system, which was capable of donating electron only through the $Y_Z^{\bullet}$ site, was unable to reduce DCPIP in the photoinhibited PS II(−Mn) preparations (Table 2). In further experiments the rate of DCPIP reduction in the presence of hydroxylamine was measured in photoinhibited PS II(−Mn) preparations. The results of these experiments shown in Table 2 indicate that although the hydroxylamine oxidation through the $Y_Z^{\bullet}$ site was inhibited, the rate of hydroxylamine oxidation was still maintained at a level equal to about 50% of the initial rate. This fact can be regarded as evidence that there are two sites of electron donation from hydroxylamine at the donor side of PS II(−Mn) membranes.

Although blocking occurs during incubation of the PS II(−Mn) membranes in the presence of Fe(II), the efficiency of this process is determined by binding of ferric rather than ferrous iron cations. This was indicated by the requirement of illumination, which induced formation of oxidants in PS II(−Mn) preparations, and by the necessity of the presence of a strong reducer (sodium dithionite) for extraction of iron cations bound to the

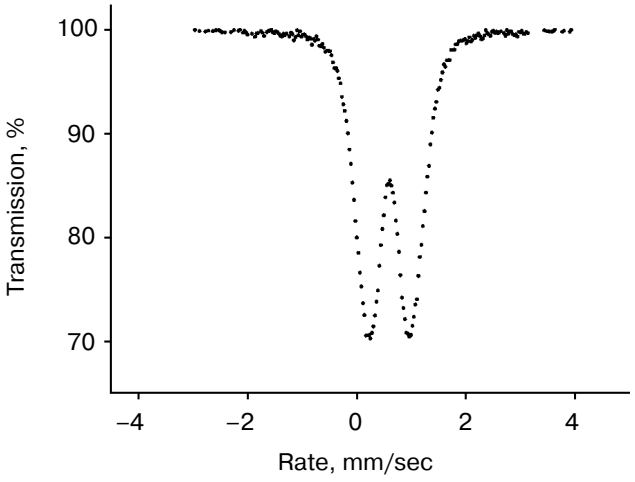

Fig. 4. Mössbauer spectra of PS II(−Mn) preparation incubated for 3 min in buffer A containing 0.14 mM ^{57}Fe. After incubation, this preparation was twice washed with buffer A to remove unbound iron. The PS II(−Mn) preparation contained 2.0 mg Chl per 0.3 ml solution. Mössbauer spectra were measured at 80K.

HA_Z site [14, 15]. This is also supported by the results of Mössbauer spectroscopy of the PS II(−Mn) membrane preparations after incubation in the light in a buffer solution containing 43% ^{57}Fe(II) and 57% ^{57}Fe(III) (Fig. 4). This spectrum contains only one component with parameters typical of ferric cation nonspecifically bound to membrane components [36] (Table 3). This preparation was free of ferrous cation, because its spectrum contained no component with corresponding parameters of quadrupole splitting and isomer shift typical of ferrous cation ($\Delta E_Q \sim 3.1$-3.3 mm/sec and $\delta \sim 1.4$ mm/sec, respectively) [36, 37]. Therefore, PS II(−Mn) membrane preparations bind specifically (through the Mn-binding site(s)) or nonspecifically (through the surface membrane components located outside the Mn-binding sites) only Fe(III). Since the amount of specifically bound iron cations should not exceed 0.5 μg/mg Chl (Table 3) (two cations Fe(III) and 250 Chl molecules per reaction center) but the amount of Fe(III) in the preparations is about 220 μg/mg Chl (Table 3), the component of the Mössbauer spectra corresponding to ferric cations mainly represents nonspecifically bound ferric cations. Although the shapes of the Mössbauer spectra of specifically and nonspecifically bound iron cations virtually coincide at 80K, the hyperfine splitting of these spectra differ from each other at near-helium temperature (5K) [38].

Because both specifically and nonspecifically bound iron cations were in the ferric state and their Mössbauer spectra have similar parameters at 80K, it is reasonable to suggest that the ligand spheres of the ferric cations both specifically and nonspecifically bound to the PS II(−Mn) membranes have similar composition. To elucidate this issue, we studied the pH dependence of the efficiency of nonspecific binding of Fe(III) to PS II(−Mn) membranes. The resulting pH dependence is shown in Fig. 5. It follows from Fig. 5 that the curve of the dependence has a descending segment at alkaline pH range with pK_2 8.1.

This raises the question: What are the causes of the decrease in the efficiency of specific (blocking, Fig. 1) and nonspecific (Fig. 5) binding of iron cations, self-assembly of the OEC catalytic center during photoactivation upon increasing the pH [5], and, perhaps, OEC inactivation at alkaline pH? As noted above, the value of pK_2 ranges within the limits of variation of pK of the pyridine nitrogen of histidine. Although inactivation of enzymes at weakly alkaline pH in some cases can be explained by deprotonation of histidine (therefore, electric charge changes, e.g., in the catalytic center), other factors are thought to play the main role in the mechanism of inhibition of the metal-containing catalytic center. This is due to the fact that the τ-nitrogen of the imidazole group of histidine is deprotonated upon increasing the pH. Therefore, the efficiency of binding of metal cations to the imidazole group of histidine should increase rather than decrease. Hence, it is an increase in the concentration of hydroxyl anions that could be the actual cause of the decrease in the efficiency of binding of cations (e.g., manganese) at high pH and, eventually, complete release of cations from the binding sites. The stability constants of complexes of iron and manganese cations with the hydroxyl anion OH^- ($\log K_1$) are equal to 7.92 (Fe(II)), 2.2 (Fe(III)), and 10.6 (Mn(II)) [39]. The stability constants of complexes of these metals with typical ligands in proteins are equal to: 1) with histidine: 5.85 (Fe(II)), 4.0 (Fe(III)), and 3.58 (Mn(II)); 2) with glutamic acid: 4.6 (Fe(II)), 12.1 (Fe(III)), and 3.3 (Mn(II)); 3) with aspartic acid: 5.8 (Fe(II)), 11.4 (Fe(III)), and 4.0 (Mn(II)) [39]. Comparative analysis of the stability constant values revealed that hydroxyl anions can begin to

Table 3. Contents of ^{57}Fe(II) and ^{57}Fe(III) in buffer solution (before and after incubation with PS II(−Mn) membrane preparations) and in PS II(−Mn) membranes (after incubation)

Object	Content of iron	
	Fe(II)	Fe(III)
Buffer A (100 ml)		
before incubation	3.42 μg/ml	4.56 μg/ml
after incubation	3.11 μg/ml	0.46 μg/ml
PS II (−Mn) membranes	0 μg/mg Chl	220 μg/mg Chl

Note: Chlorophyll content in sample, 2.0 mg. Parameters of Mössbauer spectra: isomer shift $\delta = 0.58$ mm/sec and quadrupole splitting $\Delta E_Q = 0.77$ mm/sec.

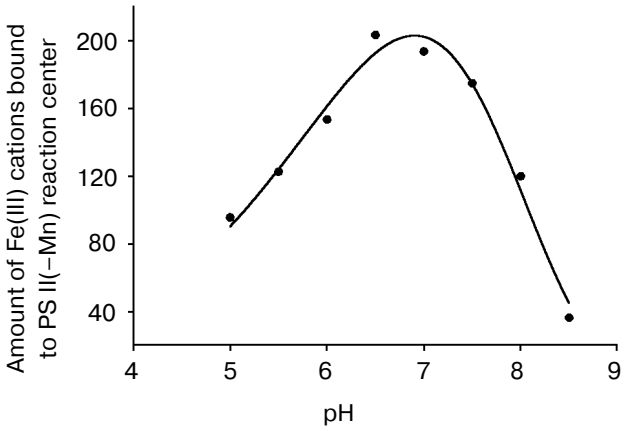

Fig. 5. pH dependence of the rate of binding of Fe(III) cations to PS II(−Mn) membrane preparations. The PS II(−Mn) membrane preparations (10 μg Chl per ml) were incubated for 1 min in buffer A containing 18 μM Fe(III). After incubation, this preparation was centrifuged, and the concentration of unbound iron was determined using bathophenanthroline (in the presence of sodium dithionite, a reducing agent).

drive histidine and carboxyl groups of amino acid residues out of the coordination sphere of Mn(II) at medium pH two to three units lower than the pK of the water molecule bound to the manganese cation (pK 10.6) [40]. It should be noted, however, that cations Mn(III) and Mn(IV) should be taken into account in actual mechanisms of binding/release of manganese cations in the binding sites, because high-valency manganese cations (2Mn(III), 2Mn(IV) [41]) form the manganese cluster in the S_1 state. On the other hand, like as Fe(III) cations, cations Mn(III) or Mn(IV) should form stronger complexes with carboxyl groups of amino acids than with histidine. Therefore, it is safe to suggest that hydroxyl anions should primarily replace histidine ligands in the coordination sphere of the manganese cluster upon increasing the pH of the reaction medium. In case of photoactivation, stronger bonds of manganese cations with the hydroxyl anion OH^- would also prevent binding of oxidized manganese cation to histidine.

The stability constant of the Fe(III) complex with the hydroxyl anion OH^- is smaller than the stability constant of the Fe(III) complex with histidine and, particularly, with carboxyl groups of amino acids. However, it should be emphasized that water molecules bound to Fe(III) are characterized by very low values of pK for the processes of formation of complexes $Fe(OH)^{2-}$, $Fe(OH)_2^-$, and $Fe(OH)_3$ (pK values are equal to 2.7, 3.6, and 3.8, respectively). The logK_3 value of these complexes is significantly larger (logK_3 ~ 10 [40]). A very low level of the coefficient of solubility of $Fe(OH)_3$ (35.06 [39]) suggests that the decrease in the efficiency of blocking of the high-affinity Mn-binding site induced by iron cations at alkaline pH is also due to intensification of competition between peptide ligands and hydroxyl ions for Fe(III) ions produced as a result of oxidation. Because the constant of stability of the Fe(III) complex with histidine is significantly smaller than constants of stability of similar complexes with carboxyl groups, hydroxyl anions, should primarily replace histidine at the binding site of iron.

It is well known that histidine is not involved in the process of binding of manganese cations at the high-affinity binding site [12, 32], but this process is mediated by the aspartic acid residue D1-D170 [42]. Therefore, it is reasonable to suggest that the histidine residue binding the second manganese (iron) cation and located at the binding site generated during photoactivation [7] or blocking [17] is a weak ligand, which is primarily replaced by the hydroxyl anion.

Special experiments were performed to test the possibility of substitution of histidine ligand by hydroxyl anion within the alkaline pH range. The effects of diethylpyrocarbonate, a histidine modifier, on the processes of binding of Fe(III) to the PS II(−Mn) membrane preparations were studied in these experiments. It was found that the following factor made it difficult to detect the effects of diethylpyrocarbonate on the processes of specific binding of iron cations (blocking). Treatment of PS II(−Mn) membranes with the histidine modifier before blocking caused an increase in the sensitivity of the membranes to light and, therefore, photoinhibition of their electron-transport chain during the process of incubation of the membrane preparations under exposure to light in the presence of iron, which was required for effective blocking (not shown). Because the values of pK_2 of the processes of blocking and nonspecific binding of Fe(III) coincide with each other (Table 1), we studied the effects of the modifier in a model of nonspecific binding of Fe(III) cations. The results of these experiments revealed that treatment of PS II(−Mn) membranes with the histidine modifier caused an insignificant (about 10%) decrease in the number of iron cations bound to the membranes. These data were not surprising, because oxygen was the main Fe(III)-binding ligand. This was evidenced by parameters of the Mцssbauer spectra of bound Fe(III) (Table 3), because the spectral parameters were typical of the coordination sphere in which oxygen atoms were dominant. In further comparative experiments we studied the resistance of bound iron cations to a chelator (*o*-phenanthroline) in membranes, in which Fe(III) interacted with both oxygen and histidine (untreated with histidine modifier), and in membranes, in which Fe(III) was bound virtually exclusively to oxygen atoms (treated with histidine modifier). The results of these experiments (Fig. 6) can be regarded as evidence that in membranes with modified histidine Fe(III) cations are extracted by the chelator at alkaline pH less effectively than in membranes with intact histidine. In other words, Fe(III) cations are more strongly bound to membranes with modified histidine than to

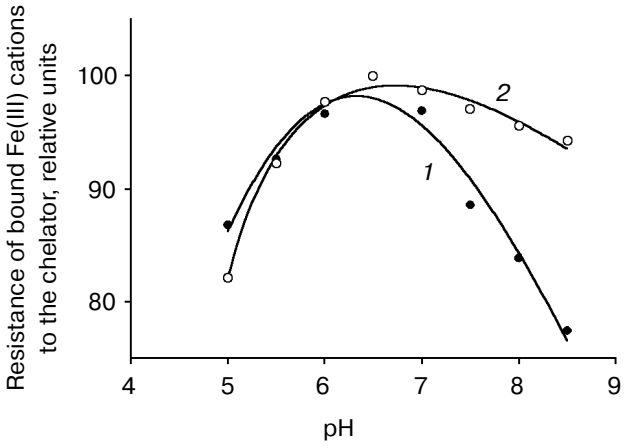

Fig. 6. pH dependence of resistance to *o*-phenanthroline of Fe(III) bound to PS II(−Mn) membranes. The PS II(−Mn) membrane preparations untreated (*1*) or treated (*2*) with histidine modifier were incubated for 1 min in buffer A (pH 6.5) containing 0.18 mM Fe(III) and then sedimented by centrifugation. After that, the membrane preparations were incubated in buffer solution of required pH containing 1 mM *o*-phenanthroline, sedimented by centrifugation, and concentration of iron in the resulting supernatant was measured spectrophotometrically in the presence of sodium dithionite.

membranes with intact histidine. Therefore, histidine is indeed a weak link that determines the sensitivity of the bound iron cations to strong ligands at alkaline pH. These results indicate that effects induced by exposure to high pH are primarily due to replacement of histidine ligand by hydroxyl anion. This histidine residue is not involved in binding of metal cation (Mn(II) or Fe(II)) to the high-affinity binding site, and more probably it is located at another (other than high-affinity) binding site, which emerges as a result of interaction of metal cations with the donor side of PS II(−Mn). Features of similarity between the processes of binding of iron and manganese cations with the donor side of the PS II(−Mn) suggest that the same mechanism is also valid in the case of binding/destabilization of manganese cluster in the oxygen-evolving complex of PS II.

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