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ELECTROSTATICS AND PROTON TRANSFER IN PHOTOSYNTHETIC WATER OXIDATION

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Abbreviations

Mn, manganese; PSII, photosystem II; P_{680}, primary donor of PSII; S_i, redox states of the catalytic center; Y_Z, tyr-161 on subunit D1.

Abstract

Photosystem II (PSII) oxidizes two water molecules to yield dioxygen plus four protons. Dioxygen is released during the last out of four sequential oxidation steps of the catalytic centre ($S_0 \Rightarrow S_1$, $S_1 \Rightarrow S_2$, $S_2 \Rightarrow S_3$, $S_3 \Rightarrow S_4 \rightarrow S_0$). The release of the chemically produced protons is blurred by transient, highly variable and electrostatically triggered proton transfer at the periphery (Bohr effect). The extent of the latter transiently amounts to more than $1H^+/e^-$ under certain conditions and this is understood in terms of electrostatics. By kinetic analyses of electron/proton transfer and electrochromism we discriminated between Bohr-effect and chemically produced protons and arrived at a distribution of the latter over the oxidation steps of 1:0:1:2. During oxidation of Y_Z its phenolic proton is not normally released into the bulk. Instead, it is shared with and confined in a hydrogen-bonded cluster. This notion is difficult to reconcile with proposed mechanisms wherein Y_Z acts as a hydrogen acceptor for bound water. Only in Mn-depleted PSII is the proton released into the bulk and this changes the rate of electron transfer between Y_Z and P_{680}^+ from normally electron- to proton-controlled. D1-His190, the proposed center of the hydrogen-bonded cluster around Y_Z, is probably farther remote from Y_Z than previously thought, because substitution of D1-Glu189, its direct neighbour, by Gln, Arg or Lys is without effect on the electron transfer from Y_Z to P_{680}^+ (in nanoseconds) and from the Mn-cluster to Y_Z^{ox}.

Introduction

Oxygenic photosynthesis of green plants and cyanobacteria uses water as the source of electrons to produce carbohydrates from carbon dioxide. Two water molecules are bound at the catalytic center at the lumenal side of photosystem II (PSII) which contains presumably four manganese atoms (Mn_4) and a redox active tyrosine residue (D1Tyr161, Y_Z). The absorption of light oxidizes the primary electron donor, a chlorophyll a monomer (P_{680}), to yield P_{680}^+. The latter oxidises Y_Z^{ox} in nanoseconds which is, in turn, reduced by $Mn_4/2H_2O$ in micro- to milliseconds. By sequential absorption of four quanta, causing one electron transfer each, the catalytic site is stepped through five increasingly oxidized states, S_0 to S_4. S_4 decays spontaneously to S_0, under release of dioxygen (see e.g. (Renger 2001) for a recent review). When a dark adapted sample is excited by a series of short laser flashes oxygen release peaks on the third flash because S_1 is the most stable state in the dark.

A structural model of PSII with 3.8 Å resolution is now available (Zouni et al. 2001b) with a boot-shaped electron density attributable to the Mn-cluster, which appears pear-shaped in another model at 3.7 Å (R.J. Shen, personal comm.). The assignment of amino acids to the electron densities is still in progress. EXAFS has revealed at least three Mn-Mn distances (e.g. 2.7, 2.8, 3.3 Å) and their minute changes as a function of the redox state (Liang et al. 2000; Robblee, Cinco, & Yachandra 2001; Dau et al. 2001; Haumann et al. 2002). Still there have remained several Mn_4-topologies which are compatible with the structural data. Despite insufficient knowledge of the structure of the Mn-centre several authors have published models for the catalytic events (for recent attempts, see Chu et al. 2001; Dau, Iuzzolino, & Dittmer 2001; Haumann & Junge 1999b; Hillier & Wydrzynski 2001; Hoganson & Babcock 1997; Hoganson & Babcock 2000; Messinger et al. 2001; Messinger 2000 ;Nugent, Rich, & Evans 2001; Renger2001; Schlodder & Witt 1999; Siegbahn & Crabtree 1999; Siegbahn 2000; Vrettos, Limburg, & Brudvig 2001). A remarkable feature of water oxidation is the

narrow window of midpoint potentials between P_{680}^{+}/ P_{680} (1.1-1.2 V) (Klimov et al. 1979; Rutherford, Mullet, & Crofts 1981); Y_Z^{ox}/Y_Z (1V) (Boussac & Etienne 1984; Boussac & Etienne 1982) and the Mn-cluster (0.9-0.95V for S_2/S_1 and S_3/S_2 (Vass & Styring 1991).

The net turnover of one full cycle, $2H_2O \rightarrow 4\ e^- + O_2 + 4H^+$, liberates four protons. They are not concertedly released with dioxygen, but distributed over the four redox transitions (reviewed in (Lavergne & Junge 1993; Haumann & Junge1999b; Haumann & Junge 1996)). It has been noted that the internal production of protons, their retention or release into the bulk, their shuffling back and forth between cofactors may provide the required 100-200mV leeway for the catalytic centre to progress between tight energetic constraints (Krishtalik 1986; Krishtalik 1989; Hoganson et al. 1995; Mulkidjanian 1999b; Tommos & Babcock 2000).

Assaying protolytic reactions within and out of the catalytic Mn-centre is complicated by the superimposition of electrostatically driven proton release into and uptake from the bulk at the periphery of PSII (Bohr effects) with the chemical proton production (by the water oxidation itself. It is furthermore complicated by the lack of *direct* indicators for internal proton rocking. In this article we review kinetic experiments aiming at a discrimination between “chemical proton production” and “electrostatic proton release/uptake” at the periphery. We describe a model for electrostatic proton/electron stoichiometries and discuss the implications for the mechanism of water oxidation.

The variable extent of proton transfer as a function of the flash number monitored by added pH-indicating dye.

Under some conditions, e.g. in thylakoids, PSII-membranes and certain PSII core particles, the extent of proton release under excitation with short laser pulses oscillates with a period of four. The extent detected under *repetitive* excitation with a ns-laser flash has been taken as one proton per single turnover of PSII. It has served to normalise the pattern of extents as function of the flash number in dark adapted and thereby mainly S_1-synchronised samples. During the first few flashes less than one or more than one proton per PSII may be released on one and the same redox transition, depending on the pH (see Fig. 1 and (Haumann & Junge 1994a; Bögershausen & Junge 1995). It is documented in Fig. 2 that under other conditions, e.g. in oxygen-evolving PSII core particles in the presence of detergent, the oscillations of proton release are lost and the release of one proton is detected on every redox transition, a pettern which is now independent of the pH (Lübbers, Haumann, & Junge 1993; Renger, Wacker, & Völker 1987; Bögershausen & Junge1995). In the same material the oscillations can be restored by the addition of glycerol as a cosolute (Haumann et al. 1997b). Despite the stoichiometric pattern of proton release being highly variable as a function of the preparation, of pH and even the solvent, the pattern of oxygen evolution is rather constant (Lübbers, Haumann, & Junge1993).

These observations have been interpreted in terms of a superposition of the chemical production of protons in the catalytic centre proper and of deprotonation/reprotonation of peripheral amino acids in response to transients in the centre, that are electrostatically (Haumann & Junge1999b; Haumann & Junge1996) or perhaps conformationally triggered (Mulkidjanian 1999a).

Kinetic properties of proton transfer at the donor side of PSII as a function of the flash number monitored by added pH-indicating dyes.

Proton transfer from the donor side of PSII to added pH-indicators is often biphasic. This is particularly evident at the third flash of Fig.1A. This particular trace merits some discussion. The rise time of the slow phase coincides with the rise time of oxygen release as detected by a time resolving (centrifugable) oxygen electrode. This holds true even in mutants of *Synechocystis* were the rise time of dioxygen release, concomitant with the one of the reduction of Y_Z^{ox}, is prolonged from 1.4 ms to 10 ms (Hundelt et al. 1998). The slowly rising phase has been attributed to chemically produced protons during the final reaction of the catalytic centre. In this way the biphasic rise has been interpreted as follows. The rapid phase represents the deprotonation of peripheral acid residues. It is electrostatically driven by the positive charge on Y_Z^{ox} and a nearby located cluster of hydrogen bonded acid/bases. This phase reverses during the electron transfer from the manganese cluster to Y_Z^{ox} which restores electroneutrality. The reversal, however, is not directly apparent because it is compensated by the synchronous appearance of the chemically produced protons. The net result is the biphasic net release as observed on giving the third flash (see Fig. 1A).

At another pH, namely 6.2, a slow uptake of protons follow the rapid release of a proton upon the third flash (Fig. 1B). How does this compare with the above interpretation? It has been interpreted as follows. At pH6.2 the extent of electrostatically triggered proton release due to earlier flashes (see e.g. the high extent at the first flash) exceeds a 1:1 stoichiometry, so that the resetting of the electrostatic situation by electron donation from water calls for the re-uptake of more protons than are chemically produced upon the third flash. One may ask whether an electrostatically triggered release can exceed a stoichiometric ratio of 1:1 of protons over electron abstracted from the catalytic centre? It can, indeed, as illustrated below.

The discrimination between “chemical” and “electrostatic” proton liberation is straightforward in the case of Fig. 1A (third flash). By kinetic and isotopic analyses we have found “chemical“ proton liberation not only upon transition $S_3 \Rightarrow S_4 \rightarrow S_0$ (Förster & Junge 1985; Haumann & Junge1994a) but also on $S_2 \Rightarrow S_3$ (Haumann et al. 1996; Hundelt, Haumann, & Junge 1997), and possibly, but for technical reasons less well defined, also on $S_0 \Rightarrow S_1$. This has led us to a pattern of the intrinsic proton production which is 1:0:1:2 over the four transitions from $S_0 \Rightarrow S_1$ to $S_3 \Rightarrow S_4 \rightarrow S_0$ (Haumann & Junge1999b). This is coincidental with the pattern which has been inferred from studies of electrochromic transients of chlorophylls in response to charge transients in the catalytic centre (Schlodder & Witt1999) (Witt 1996) (Saygin & Witt 1985b) (Saygin & Witt 1985a).

The “chemical” proton release during the transition $S_3 \Rightarrow S_4 \rightarrow S_0$ as detected upon the third flash in Fig. 1A (half-rise in 1.4ms) is kinetically distinct from its “electrostatic” precursor (some 10µs). Less obvious to inspection by the eye but well discernible by its longer rise time (some 100µs) and a greater kinetic H/D-isotope effect is the chemical release upon $S_2 \Rightarrow S_3$ (Haumann, Drevenstedt, Hundelt, & Junge1996) (Hundelt, Haumann, & Junge1997). In both cases the comparatively slow rises coincide with the rise of the electron transfer to Y_Z^{ox}.

In contrast to this behaviour we have found that the rates of the rapid phases, which are attributed to peripheral electrostatic events reveal a particular dependence on the dye concentration and on the pH that is expected for proton transfer from an immediately activated source to a sink (the indicator dye). The mentioned “immediate” activation is the electron abstraction from Y_Z by P_{680}^+ in some 30-300ns. Fig. 3 illustrates the observed behaviour with thylakoids using neutral red as amphiphilic pH-indicator (data in Fig. 3B) and with isolated PSII core particles using two hydrophilic pH-indicators. As Fig. 3D shows with thylakoids the rate rises with increasing concentration of neutral red (Haumann & Junge 1994b). Such a behaviour is indicative for a bimolecular

collision involving neutral red. It is not unexpected as neutral red, an amphiphilic dye which is adsorbed at the membrane surface, will have an effective concentration in the thylakoid lumen at least thousand-fold higher than in the bulk (Hong & Junge 1983; Junge, Schönknecht, & Förster 1986). In contrast to thalakoid membranes, the rate of proton release from solubilised PSII core particles is independent of the concentration of added hydrophilic pH-indicators. However it rises at lower pH (data in Fig. 3A) (Bögershausen & Junge1995). Such a behaviour is expected under two conditions: 1) The response is dominated by peripheral amino acids whose pK is in the range of the given pH. 2) The spontaneous protolysis of these surface groups is followed by proton uptake by the hydrophilic indicator dye. The overall rate of the sequential reaction is limited by the rate of protolysis into the bulk.

The protolysis of an acidic group, A, at the surface is induced when its pK is acid shifted, e.g. by electrostatic interaction with a positive charge in the protein. The reaction: $AH \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} A^- + H^+$ has a dissociation constant, $K = \frac{k_{off}}{k_{on}}$.

If the acid is directly in contact with bulk water, the on-rate is diffusion controlled (10^{10}-10^{11}M^{-1}s^{-1}), and the off-rate strictly pK-controlled: $k_{off} = k_{on} \cdot 10^{-pK}$ (see (Eigen 1963; Gutman & Nachliel 1995)) wherein $pK = -\log\left[H^+\right]$. If the acid group is embedded in the protein, the relation $k_{off} = k_{on} \cdot 10^{-pK}$ still holds, but as the diffusion-control of the on-reaction is lost, the *pK*-control of k_{off} remains only broadly valid (so called free-energy-relationship in elementary kinetics). Following this notion we have interpreted the pH-dependence of proton release in Fig. 3A as a pK-dependence involving a set of groups with different pKs.

In summary, the proton transfer from the donor side of PSII to a given dye can be very fast under some conditions, or by two orders of magnitude slower under other conditions. The most rapid transients of proton transfer to the respective indicator dye,

e.g. with a half-rise time of 12 µs, have been observed at a high concentration of neutral red in membranes (Haumann & Junge1994a) or at acid pH to bromocresol purple with core particles (Bögershausen & Junge1995). These rapid reactions imply a triggering at the level of oxidised Y_Z, or even P_{680}^+, so that proton release precedes the electron transfer from the Mn-cluster to Y_Z^{ox}. The slow proton release into the bulk (in core particles at alkaline pH),on the other hand, implies that the electrostatically induced release into the bulk of protons from the donor side may range into milliseconds without impairing the progress of oxygen evolution. It is noteworthy that we have not found any influence of the large variability of the extents and the rates of proton release on the rates of electron transfer between Mn and Y_Z and P_{680}^+ and on the oscillatory pattern of electrochromic absorption transients (as documented and discussed in (Lübbers, Haumann, & Junge1993)).

Super-stoichiometry of electrostatically triggered proton release in response to the deposition of a positive charge in the catalytic centre.

The above interpretation of the data implies that the electrostatic response of peripheral acid residues to the univalent oxidation of the donor side of PSII (Y_Z plus Mn-cluster) can yield a proton/electron stoichiometry greater than one, in particular in thylakoids, during transition $S_1 \Rightarrow S_2$ and at acid pH (see Fig. 1B and above). This “super-stoichiometry” is not immediately plausible, given the fact that one peripheral acid, AH, after its deprotonation to yield A^-, tends to suppress the deprotonation of a neighbouring acid, BH, and consequently the formation of the doubly deprotonated pair A^-B^-.

A Monte-Carlo treatment of electrostatically triggered proton uptake/release has been presented for the bacterial reaction centre (Beroza et al. 1991; Beroza et al. 1995). It has been based on a high-resolution structure and involves very many acid/base residues. In the case of PSII, a rigorous treatment of the electrostatics, of proton release at the periphery of PSII, and of local electrochromic transients that are ascribed to the inner

chlorophylls has to await the assignment of amino acids and the orientation of the cyclopentanone rings of the innermost chlorophyll molecules, both of which are not available in the structure at 3.8 Å resolution (Zouni et al. 2001b). Even if the amino acids were assigned, their protonation states and pKs will not be obvious without knowing their involvement in hydrogen bonding with neighbouring residues or crystallographically visible and invisible intra-protein water. It is for this reason that the following considerations are restricted to the question whether or not it is possible, in principle, to obtain a H^+/e^--super-stoichiometry by electrostatic interactions between a charge in the protein and peripheral acids. As a simple model lacking any structural detail we consider the lumenal surface of the thylakoid membrane as an infinite plane separating two homogeneous and infinitely extending phases, a conducting one, the thylakoid lumen, and a non-conducting one, the membrane core. The presence of salts in the lumen and the very rapid lateral relaxation of field inhomogeneities along the lumenal surface justifies the approximation of the lumen by a phase of "infinite" conductivity. The globular structure of the PSII with its shielding extrinsic proteins which protrude from the membrane implies a pretty curved surface between the dielectric and the conducting phase but not a flat one. The assumption of a flat geometry in the above simple model is just another approximation, which is justified as long as details on water channels and perhaps high-dielectric pockets in the peripheral proteins are not available. We assume that the "manganese cluster", M, resides 10 Å deep in the membrane and that there are only two acid groups, AH and BH, symmetrically located to M and embedded 3 Å deep in the membrane as illustrated in Fig. 4. The respective coordinates in Å-units are as follows:

[1] M: (0, 0, 10); A: (0, -6, 3); B: (0, 6, 3).

Both acids undergo reversible protonation/deprotonation with relaxation times which are shorter than typical measuring intervals used to assay the extent of the deprotonation (for kinetic features, see above). Thus they are both at equilibrium according to:

[2] $A^- + H^+ \leftrightarrow AH; \qquad B^- + H^+ \leftrightarrow BH$

with dissociation constants K_A and K_B, respectively.

The value of the dissociation constant of B depends on the charge states of M/M^+ and the respective other acid, A/A^-. As an example, if both are charged, M^+ and A^-, the pK = - log K of the acid BH deviates from the one when both partners are electroneutral, pK_B^0, as follows:

[3] $$pK_B(M^+, A^-) = pK_B^0 - \frac{e_0}{2.3k_bT}(\Phi_{MB} + \Phi_{AB})$$

wherein e_0 denotes the electric unit charge, k_b the Boltzmann, T the absolute temperature, and Φ_{MB} and Φ_{AB} the contribution to the electric potential at B as caused by M^+ and A^-. A positive potential jump which is caused by M^+ lowers pK_B and favours the deprotonation of BH, whereas the negative potential by A^- increases pK_B and tends to reduce the deprotonation.

Because the lumen is assumed to be “infinitely” conducting, the potential jumps can be calculated by the method of image charges (as presented in standard textbooks on electrostatics). The potential at the position of B, Φ_B, which is caused by a charge of magnitude $a_M\, e_0$ at the position of M is given by:

[4] $$\Phi_{MB} = \frac{a_M e_0}{4\pi\varepsilon\varepsilon_0}\left\{\begin{array}{l}\left[(x_B - x_M)^2 + (y_B - y_M)^2 + (z_B - z_M)^2\right]^{-1/2} \\ -\left[(x_B - x_M)^2 + (y_B - y_M)^2 + (z_B + z_M)^2\right]^{-1/2}\end{array}\right\}$$

wherein ε and ε_0 denote the relative and absolute dielectric permittivity. This is the well known Coulomb potential. The second term represents the contribution of the “image charge”, a fictive charge of opposite sign to the one on M^+, which is symmetrically located to M in the conducting phase. The image charge guarantees that the interface is equipotential. In the given example its coordinates are as follows: M’: (0, 0, -10). With the above coordinates of A, B, and M and their respective image charges the potentials generated by M^+ and A^- at position B as calculated by Equ. [4] are as follows:

[5] $\Phi_{MB} = +139mV$ Φ_{AB} = -32 mV

This is illustrated in Fig. 4, bottom.

They cause the following pK-shifts of B (and likewise of A):

[6] $\Delta pK_{MA} = \Delta pK_{MB} = -2.3$; $\Delta pK_{BA} = \Delta pK_{AB} = +0.53$.

Each (macro)state of M, namely M and M^+, comprises four (micro)states of the acid/base-couple A and B with *a priori* probabilities, w_1, w_2, w_3, w_4:

[7a] AH BH $w_1 = \left(1+10^{pH-pK_A}\right)^{-1} \bullet \left(1+10^{pH-pK_B}\right)^{-1}$

[7b] A^- BH $w_2 = \left(1+10^{pK_A-pH}\right)^{-1} \bullet \left(1+10^{pH-pK_B}\right)^{-1}$

[7c] AH B^- $w_3 = w_2$

[7d] A^- B^- $w_4 = \left(1+10^{pK_A-pH}\right)^{-1} \bullet \left(1+10^{pK_B-pH}\right)^{-1}$

wherein the respective figures of pK_A and pK_B differ depending on the charge state of the respective neighbours as given by Equ. [3]. The respective pK-shifts as calculated for the assumed arrangement of M, A, and B are given by Equ. [6]. If, for example, M is uncharged, pK_A denotes pK_A^0 in Equ.s [7a] and [7b], and $pK_A^0 + 0.53$ in Equ.s [7c] and [7d]. If, on the other hand, M is positively charged pK_A denotes pK_A^0-2.3 in Equ.s [7a] and [7b] and $pK_A^0 + 0.53 - 2.3$ in Equ.s [7c] and [7d].

The extent of deprotonation, ΔH^+, of the pair of interacting acids is given by the weighted contributions of the micro-states (Equ.s [7]),

[8] $\Delta H^+ = (2w_2 + 2w_4)/(w_1 + 2w_2 + w_4)$

Its magnitude varies depending on whether M is charged or uncharged. For simplicity we assume that the undisturbed pKs (M uncharged!) of both acids are equal, $pK_A^0 = pK_B^0 = pK^0$, and further that the ambient $pH = pK^0 - 1$. The extent of the deprotonation per pair of acids which is caused by the univalent up-charging of M to yield M^+ then amounts to the following:

[9] $\Delta\Delta H^+ = \left(\Delta H^+_{M^+} - \Delta H^+_M\right) = (1.738 - 0.177) = 1.561$

We found that only two acid residues at reasonable spacing to the Mn-cluster and between each other can produce a super-stoichiometry of proton release in response to the univalent up-charging of M. The extent of electrostatically driven proton release, as opposed to the one which is caused by the chemistry in the catalytic centre proper, depends on the number and the topology of peripheral amino acids relative to the centre, their original pKs, the ambient pH, and the dielectric environment of these acids as given in principle by Equ.s [3,4,7]. The simple electrostatic model presented above can, of course, be extended to incorporate the stromal bulk phase (this brings in further image charges), to account for globular protein structure and for the involvement of more acid residues. The formalism to treat such systems is standard in statistical thermodynamics. These extensions will not bear on the possibility, in principle, to obtain super-stoichiometries of proton release.

One simplifying assumption, implicit in the above considerations is, however, critical. We assume that the conversion of M to M^+ is irreversible, the appearance of the positive charge on M is not affected by the charge state of the acids. In other words we neglect the electrostatic back-pressure of the deprotonated and negatively charged peripheral acids on the redox-potentials of M and its reaction partner. This neglect appears adequate for a photochemical reaction with large driving force, but not necessarily if the driving force of the reaction $M \rightarrow M^+$ is small. Under these conditions a network approach including the equilibrium M^+/M is more appropriate.

One of us (MH) simulated the observed dramatic variation of proton release in thylakoids as function of the pH and its constancy in detergent solubilised core particles by similar reasoning as laid out for the simple model above and obtained a reasonable fit to the above presented data with only three acid groups. He also modelled the pH-independence of local electrochromic shifts. Because of the still existing freedom freedom to chose the positions and orientations coordinates of the cofactors, the dielectrically-weighted overall topology and the pKs of the interacting acid/base groups

such fits just serve illustrative purposes. That is why we refrained from presenting results. A rigorous treatment, as mentioned above, has to wait for a very much perfected structural model of PSII.

Proton rocking between Y_Z and an acid/base-cluster in response to its oxido-reduction.

Rapidly rising light-induced absorption transients have been attributed to electrochromic bandshifts of chlorophyll a accompany the oxidation of Y_Z by P_{680}^+ (Rappaport, Blanchard-Desce, & Lavergne 1994). The major portion decays with the typical time constant of the electron transfer from the Mn-cluster to Y_Z^{ox}. Rappaport and collegues also reported a smaller, more rapidly decaying component, which they attributed to a intra-protein proton transfer around Y_Z, a feature that we did not observe in our experiments despite sufficiently high time resolution (Haumann, Bögershausen, & Junge 1994; Haumann & Junge1996). The overall behaviour, rapid rise and slow partial decay of the electrochromic transient, with the latter following the reduction of Y_Z^{ox} by the Mn-cluster, point to the lack of charge neutralisation, e.g. by release into the bulk of the phenolic proton of Y_Z (Lavergne & Junge1993; Haumann & Junge1996). This view has been questioned by others (Hoganson & Babcock1997) who have interpreted one specific proton per electron stoichiometry, namely 1:1 as found under some conditions in core particles (see Fig.2C), as the unmasked release into the bulk of the proton from Y_Z^{ox} itself . The assumed electrochromic bandshifts have been re-interpreted as through-bond interactions between the tyrosine and the chlorophyll a of P_{680} (Tommos et al. 1998). Based thereupon it has been claimed that Y_Z^{ox} is reprotonated from water molecules upon each reduction (Britt 1996; Tommos & Babcock 2000). This matter has not yet been rigorously settled. There is no proton release from Y_Z^{ox} proper, at least as long as the Mn-cluster is in the S_1-state. This is documented in Fig. 1 (see first transient at pH 7.4). Here, the net extent of proton release with a half-rise time of 12µs (i.e. before any reaction with the Mn-cluster) is about 0.5 protons. If there had been the production of a chemical proton and its release from Y_Z into the bulk, a larger signal would have been observed. Admittedly, this evidence bears only on this particular redox-transition, namely $S_1 \Rightarrow S_2$.

It has been work with severely modified, i.e. Mn- and/or Ca-depleted PSII core preparations, that has provided indirect evidence for the absence of a deprotonation of Y_Z into the bulk during the other redox transitions even in the intact, i.e. oxygen evolving PSII. This line of evidence is related to the susceptibility of a neighbouring base cluster around Y_Z to allow rapid electron transfer from Y_Z to P_{680}^+. If, as in Mn-depleted material at acid pH, this cluster is saturated, the phenolic proton of the Y_Z tyrosine has to be ejected into the bulk before Y_Z can be oxidised by P_{680}^+ in a then proton-controlled reaction (Ahlbrink et al. 1998; Diner et al. 1998; Haumann & Junge 1999a; Hays et al. 1998; Hays et al. 1999; Mamedov, Sayre, & Styring 1998). Turning this argument around to oxygen evolving PSII, where the electron transfer to P_{680}^+ is very fast (30-300ns), it implies proton rocking between Y_Z and the base-cluster and its transient upcharging upon the oxidation of Y_Z. ****

The data favouring this notion, obtained with Mn-depleted PSII core particles, are given in Fig. 5. Whereas the half-rise of the electron transfer to P_{680}^+ in the intact material ranges between 30 and 300 ns, there is a biphasic rise in Mn-depleted centres. A fast phase rises in about 1µs. Its rate is almost independent of the pH (see Fig. 5A). A slower phase rises in 10-100ms and the rate decreases at lower pH. Their summed extent is constant (see Fig. 5B), if one corrects for charge pair recombination (see Fig. 5C). They are mutually inter-convertible as function of the pH, and the transition between them titrates with a pK around 7 (Fig. 5B). The rise time of the nanosecond components in fully functional, oxygen evolving core particles and of the microsecond component in Mn-depleted material are both pH-independent (between pH 5.5-7-5) (Meyer et al. 1989), nearly insensitive to H_2O/D_2O isotopic substitution (Ahlbrink et al. 1998; Haumann et al. 1997a), and they reveal a low activation energy. In contrast to the former, the rate of the slow component in Mn-depleted material decreases with decreasing pH, the kinetic H/D isotope effect is 2.5 and the activation energy is high

(0.3eV). It is obvious that the reaction between Y_Z and P_{680}^+ switches at pH7 from electron- (fast) to proton-controlled (slow) electron transfer (Ahlbrink et al..1998).

These observations have been interpreted to indicate that, in the intact system, the rapid reduction of $P680^+$ requires the presence of a receptive base cluster around Y_Z. If this cluster is protonated as at acid pH (at pH<7 in Mn-depleted centres, and possibly at pH<4.5 in intact ones), the electron transfer between Y_Z and P_{680}^+ is kinetically controlled by proton transfer (see H/D-isotope effect in (Ahlbrink et al. 1998)). Only under these conditions, the normal electrochromic transients of chlorophyll a vanished (see Fig. 9 in Ahlbrink et al. 1998), as if proton release into the bulk from the vicinity of Y_Z was then the prerequisite of the electron transfer to $P680^+$. Under these conditions we observed proton release into the bulk with a similar rate as the one of the electron transfer. These phenomena have been understood in terms of a rise of the midpoint potential of Y_Z^{ox}/Y_Z by 0.1V when the base cluster is protonated at acid pH (Ahlbrink et al. 1998). This notion is compatible with the reported difference of the midpoint potentials of Y_Z and P_{680} in Mn-depleted material, namely 0.1V at pH 6.5 (Metz et al. 1989; Mulkidjanian et al. 1996). In essence these studies have revealed that the phenolic proton of Y_Z upon oxidation can be released into and then detected in the bulk. This occurs, however, only in Mn-depleted material at acid pH. Under other conditions, and most importantly in fully functional PSII, the phenolic proton remains in the vicinity of Y_Z and this may be one construction element to increase the redox potential of Y_Z relative to the Mn-cluster (Ahlbrink et al. 1998). Models of the catalytic events wherein it is assumed that Y_Z is deprotonated into the bulk phase upon every transition, in order to function as a hydrogen acceptor for water (Tommos & Babcock 1998; Tommos & Babcock 2000), are difficult to reconcile with this notion.

It is noteworthy that the peculiar kinetic behaviour of the electron transfer from Y_Z to P_{680}^+ in Mn-depleted PSII (Fig 5A, and see Ahlbrink et al. 1998) has been almost perfectly mirrored in a synthetic ruthenium-pyridyl-tyrosine construct (see Sjödin et al.

2000, and Hammerström, in these Proceedings) where the pH-dependence of the slow phase has been interpreted as concerted proton-electron-transfer.

Two independent techniques, namely with thylakoids the electrochromic transients of intrinsic carotenoids (Junge and Witt 1968) and with PSII-liposomes an electrometric technique (Drachev et al. 1981), have been used to determine the transmembrane electrogenicity of the electron and proton transfer in PSII (Haumann et al. 1997b). The results, agreeing with each other, were as follows: taking the electrogenicity of the electron transfer from Y_Z to Q as 100%, the step fromY_Z to P_{680}^+ accounts for 15%, and from the Mn-cluster to Y_Z for about 3%. The electrogenicity of proton transfer out of the catalytic centre into the bulk (e.g. the release of chemically produced protons during $S_3 \Rightarrow S_0$) is variable, it ranges between 2 and 10%. These observations are fully in line with the above notion of the re-distribution of protons between Y_Z, its base-cluster, peripheral acids and the bulk (for data and detailed discussion, see Haumann et al. 1997). The figures for the relative electrogenicity are related to the projected positions of the cofactors along the membrane normal, through weighting by dielectric permittivity, ε, as distance times ε appear in the Coulomb energy (see Equ. [4]).

A special role of D1-His190 in the acid/base-cluster around Y_Z?

Mutant analyses of PSII have suggested that D1-His190 is one essential hydrogen bonded partner of Y_Z. One surprising and convincing result has been the rescue by soluble weak acids of the rapid electron transfer from Y_Z to $P680^+$ in a mutant where D1-His190 is replaced by neutral amino acids (Hays et al. 1998; Hays et al.1999). It has been speculated that D1-Glu189, the direct neighbour of D1-His190, is another member of the hydrogen bonded base cluster around Y_Z (Debus et al. 2000). We checked this suggestion by measuring the rates of electron transfer from the Mn-cluster to Y_Z^{ox} (in μs) and from Y_Z to $P680^+$ (in ns) and found no difference between the WT and E189Q,

E189K, and E189R (see Table (Clausen et al. 2001)). This result is surprising because one expects some effect because of different electrostatic properties of glutamic acid, glutamine, arginine and lysine. The lack of any effect of an acid, neutral or basic residue at position D1-189, then implies that it is embedded either in a strongly hydrophobic environment (all residues forcedly electro-neutral) or in high dielectric (the charge fully shielded). Otherwise, the absence of any electrostatic effect of the D1-E189-mutants could be explained by a distance between Y_Z and D1-H190, D1-E189 which is much greater than so far supposed. The advanced crystal structure of PSII (see Petra Fromme, this issue) suggests that the D1-His-190 may indeed be more than 10 Å away from the position of Y_Z.

Summary

We discriminated chemical production of protons at the donor side of PSII from electrostatic Bohr effects, as well as proton rocking between cofactors from proton release into the bulk. The results are compatible with the notion that the catalytic centre is positively up-charged during the transition $S_1 \Rightarrow S_2$ and electrostatically relaxed during $S_3 \Rightarrow S_4 \rightarrow S_0$, as inferred from previous studies on electrochromic transients. Protons are chemically produced and then released into the bulk phase at the lumenal side of PSII at least during transitions $S_2 \Rightarrow S_3$ and $S_3 \Rightarrow S_4 \rightarrow S_0$. This conforms with the notion that the oxidizing equivalent during transitions $S_2 \Rightarrow S_3$ is not stored on Mn proper but rather on some ligand (see V. Yachandra, in these Proceedings). The phenolic proton of Y_Z does not seem to be released into the bulk but rather rocking between Y_Z and a base cluster in its vicinity. The receptivity of this cluster is a prerequisite for the rapid electron transfer from Y_Z to P_{680}^+. If the cluster is pre-protonated, the electron transfer is slow and proton-controlled (see L. Hammerström, in these Proceedings, for a similar behaviour in a synthetic model system). D1-His190 the proposed candidate for the hydrogen-bonded partner of Y_Z, seems to be more remote

from Y_Z than previously thought, because electrostatically differing mutations at the neighbouring position, D1-Glu189, have little if any influence on the electron transport between the Mn-cluster, Y_Z and P_{680}. These features of proton retention and proton release are likely key elements in the remarkable tuning of the four stepped reaction cycle to one and the same energy supply.

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FIGURE CAPTIONS

FIGURE 1

Time-resolved proton release after the first four flashes of a Q-switched Ruby-laser given to unstacked, dark-adapted thylakoids from *pisum sativum* (Haumann & Junge1994a). Proton release into the lumen of thylakoids was determined from absorption transients at 548 nm. They were recorded twice, plus and minus neutral red (45µM), and in the presence of BSA as impermeant buffer of pH-transients in the external phase (Ausländer & Junge 1975) (Junge et al. 1979). Note the different time scale at the third flash, which causes mainly the oxygen evolving transition $S_3 \Rightarrow S_4 \rightarrow S_0$. The half-rise times and the extents (in parenthese) resulting from a bi-exponential fit (lines) to the data (points) upon the third flash were in Fig. 1A (pH7.4): 40µs ($0.9H^+$) and 1200µs ($0.6H^+$), and in Fig. 1B (pH6.3): 70µs ($1.0H^+$) and 5000µs ($-0.5H^+$), respectively. The extents were normalised to the extent of proton release under repetitive excitation ($1H^+$), for details, see (Haumann & Junge1994a).

FIGURE 2

The relative extent of proton release attributable to the four sequential steps of the catalytic centre of water oxidation, namely $S_0 \Rightarrow S_1$ (open triangles), $S_1 \Rightarrow S_2$ (full circles), $S_2 \Rightarrow S_3$ (full triangles), and $S_3 \Rightarrow S_4 \rightarrow S_0$ (open circles). The extent was normalised to the extent under repetitive excitation ($1H^+$). The original pattern of proton release (as in Fig. 1) was deconvoluted to yield the pattern over the steps under consideration of the S_0/S_1-distribution in the dark and of double-hits and misses. Three types of oxygen materials were used, (A) unstacked thylakoids (Haumann & Junge1994a), (B) BBY-membranes (Rappaport & Lavergne 1991), and (C) PSII core particles (Lübbers, Haumann, & Junge1993). For details, see these references.

FIGURE 3

Different mechanisms of proton transfer to an indicator dye in detergent solubilised PSII core particles (top, left) and thylakoids (bottom, left) causing different properties of the rate constant of proton release under repetitive excitation with a Q-switched Ruby-laser,

(A) as function of the concentration of neutral red in unstacked thylakoids (Haumann & Junge1994a), and (B) as function of the pH in PSII core particles and recorded by bromocresol purple (5<pH<7.5) and cresol red (7<pH<8.7) (Bögershausen & Junge1995). For details, see these references.

FIGURE 4

Model for the electrostatic interactions between a charged carrier in a protein and two acids at the periphery which cause of proton release into the bulk (Bohr effect). A photochemical reaction creates a positive charge at the position of M which is embedded 10 Å deep in the protein. The protein is viewed as a homogeneous dielectric (ε=4) facing a conducting bulk phase filled with conducting buffer. Two “peripheral” acid groups, AH and BH, which are embedded only 3 Å deep in the dielectric and 6Å remote from the normal through M are subjected to a positive potential jump of +140mV when M is charged to yield M^+. If one of them is deprotonated, A^- (see bottom part) it confers a potential of –32 mV to the other acid, BH. (For details, see text).

FIGURE 5

The rates and extents of the kinetic components of the reduction of P_{680}^+ in inactive PSII core particles as function of the pH, according to a fit with three exponentials (Ahlbrink

et al.1998). A) The rates of the two faster kinetic components. The rate of the fastest component (k_f, squares) is about pH-independent (line), whereas the rate of the slower one (k_s, circles) decreases with the pH (line).

B) The relative extents of the two kinetic components (k_f, squares; k_s, circles) from A. The extents of k_f (squares) are described by a single titration with a pK of 7 (line). C) The sum of the extents of the very slow components (k_v) as function of the pH. Open triangles: data from absorption transients at 827 nm which reflectthe oxidoreduction of P_{680}; solid triangles: data from transients at 320 nm which reflectthe oxidoreduction of Q_A. For details, see (Ahlbrink, et al.1998).

Table 1: Half times of electron transfer reactions in cells and PSII core particles from different mutants compared to wild-type. Mean values with standard deviations. For details, see {Clausen et al. 2001).

REACTION	**WT**	**E189Q**	**E189K**	**E189R**
$S_1 \Rightarrow S_2$	46 ± 5 µs n = 190	55 ± 40[b,d] µs n = 20	44 ± 7 µs n = 80	41 µs ± 7 µs n = 90
$S_2 \Rightarrow S_3$	90 µs n = 190	90 ± 50[b,d] µs n = 20	99 µs ± 7 µs n = 80	96 µs ± 7 µs n = 90
$S_3 \Rightarrow S_0$ (cores, -G)[G]	4.5 ± 0.4 ms[a,b] n = 100	4.7 ± 0.5[b,c] ms n = 80	n.d.	n.d.
$S_3 \Rightarrow S_0$ (cores, +G)[G]	1.5 ± 0.1 ms[a,b]	n.d.	n.d.	n.d.
$S_3 \Rightarrow S_0$ (cells)	1.5 ± 0.5 ms[b] n = 5	1.5 ± 0.5 ms[b] n = 5	n.d.	n.d.
Reduction of $P680^+$:				
oxygen evolving PSII	30 ± 3 ns (36%) 253 ± 21 ns (20%) > 2 µs (45%) n = 1950	38 ± 2 ns[b] (24%) 259 ± 10[b] ns(21%) > 2 µs (55%) n = 800	27 ± 0 ns (48%) 251 ± 6 ns (21%) > 2 µs (31%) n = 1800	30 ± 1 ns (41%) 304 ± 21 ns (24%) > 2 µs (35%) n = 1750
Mn-depleted PSII	780 ± 10 µs[b,c] (87%) > 2 ms (13%) n = 300	650 ± 20 µs[b,c] (96%) > 2 ms (4%) n = 150	n.d.	n.d.

(+/- G) = presence or absence of glycerol, n = number of measurements
[a]Data from (Haumann et al.1997b)
[b] error calculated from the fit routine,
[c] core particles prepared after (Hundelt et al. 1998)

FIGURE 1

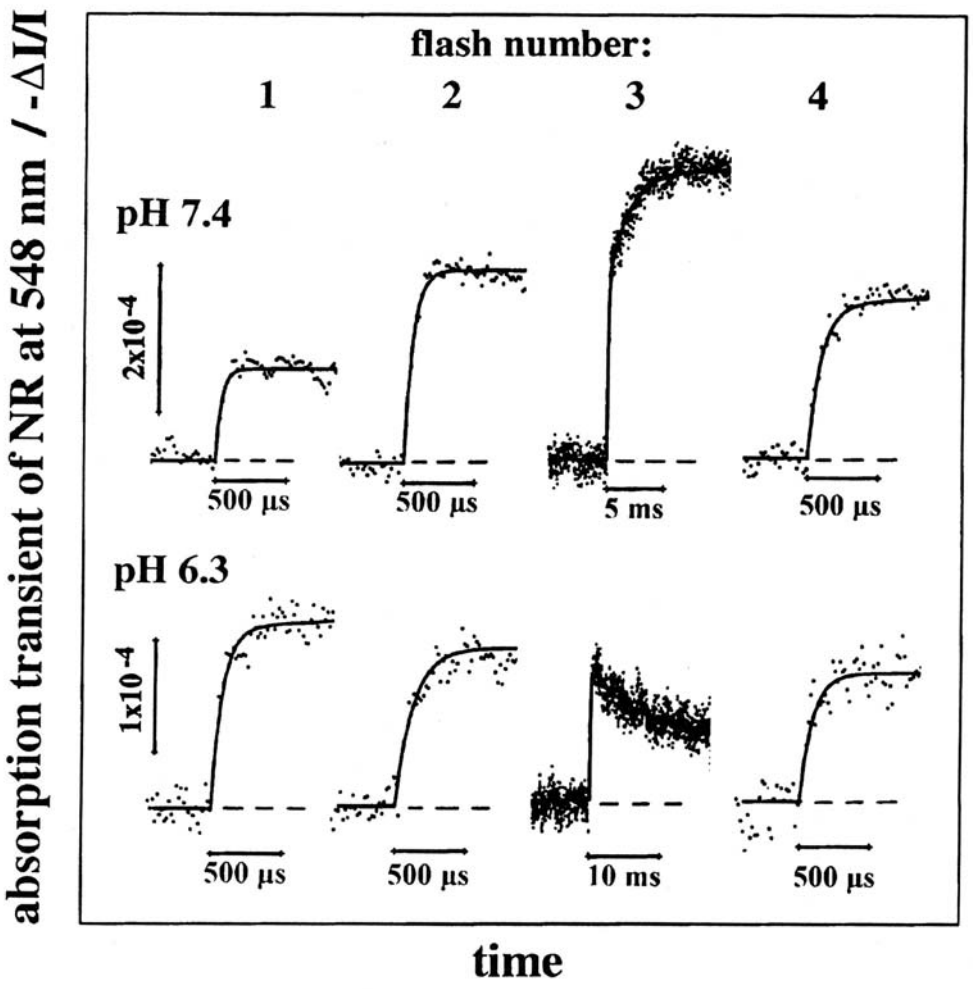

absorption transient of NR at 548 nm /-ΔI/I
flash number:
1
2
3
4
pH 7.4
2x10⁻⁴
500 µs
500 µs
5 ms
500 µs
pH 6.3
1x10⁻⁴
500 µs
500 µs
10 ms
500 µs
time

FIGURE 2

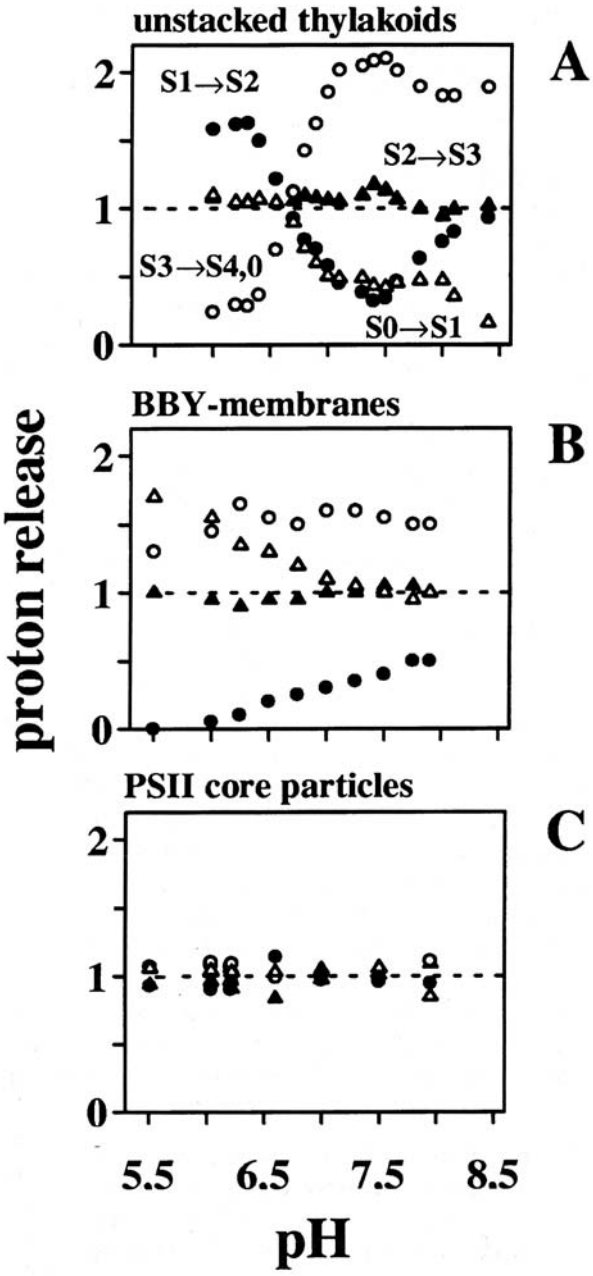

unstacked thylakoids
A
S1→S2
S2→S3
S3→S4,0
S0→S1
BBY-membranes
B
PSII core particles
C
proton release
2
1
0
5.5
6.5
7.5
8.5
pH

FIGURE 3

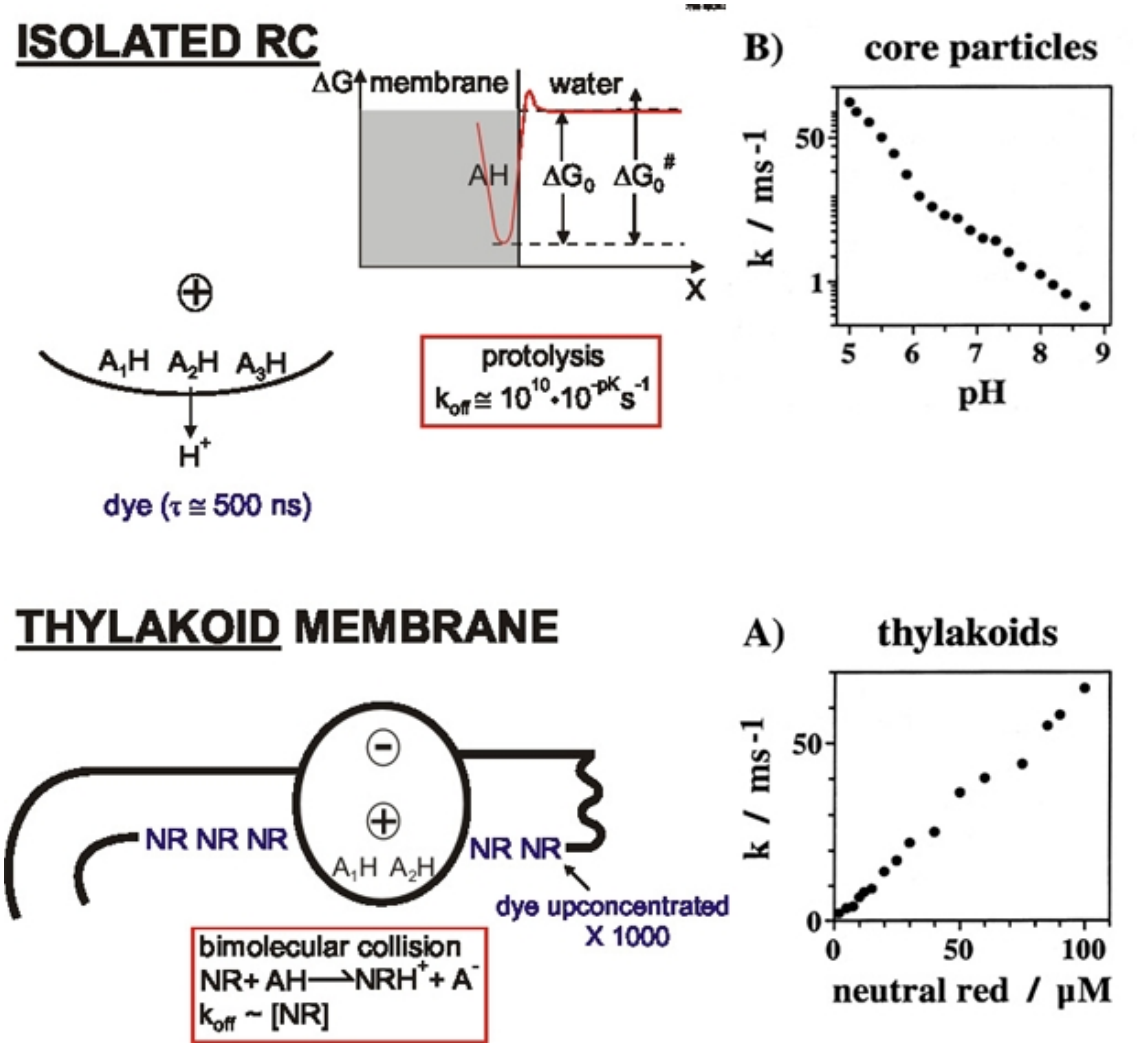

ISOLATED RC
ΔG membrane water
AH
ΔG0
ΔG0#
X
A1H A2H A3H
H+
dye (τ ≅ 500 ns)
protolysis
koff ≅ 10^10 • 10^-pK s^-1
B) core particles
k / ms-1
50
1
5 6 7 8 9
pH
THYLAKOID MEMBRANE
NR NR NR
A1H A2H
NR NR
dye upconcentrated X 1000
bimolecular collision
NR+ AH ⟶ NRH+ + A-
koff ~ [NR]
A) thylakoids
k / ms-1
50
0
0 50 100
neutral red / µM

FIGURE 4

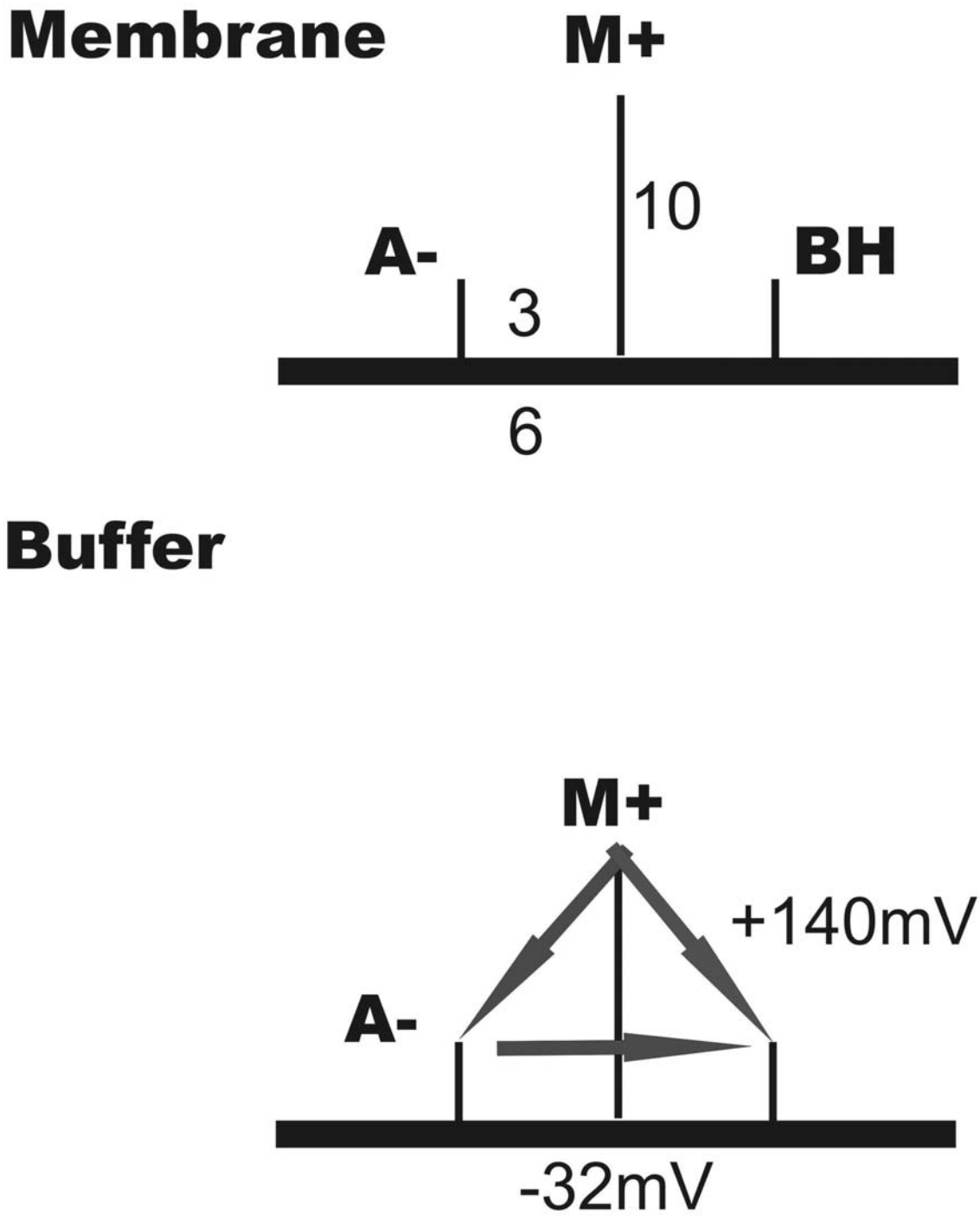
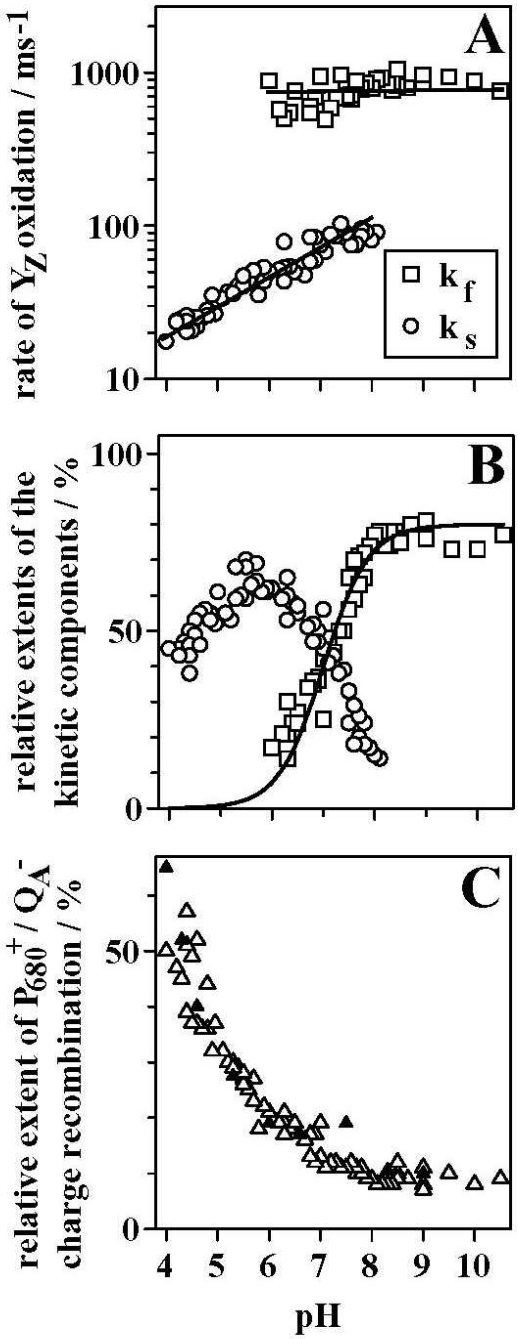


FIGURE 5


A
B
C
rate of Y_Z oxidation / ms^{-1}
1000
100
10
k_f
k_s
relative extents of the kinetic components / %
100
50
0
relative extent of P_{680}^+ / Q_A^- charge recombination / %
50
0
4
5
6
7
8
9
10
pH

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