ELECTRON TRANSFER IN THE PHOTOSYNTHETIC MEMBRANE.

Influence of pH and Surface Potential on the P-680 Reduction Kinetics

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ABSTRACT The primary electron donor P-680 of the Photosystem-II reaction center was photooxidized by a short flash given after dark adaptation of photosynthetic membranes in which oxygen evolution was inhibited. The P-680$^+$ reduction rate was measured under different conditions of pH and salt concentration by following the recovery of the absorption change at 820 nm. As previously reported for Tris-washed chloroplasts (Conjeaud, H., and P. Mathis, 1980, *Biochim. Biophys. Acta*, 590:353–359) a fast phase of P-680$^+$ reduction slows down as the bulk pH decreases. When salt concentration increases, this fast phase becomes faster for pH above 4.5–5 and slower below. A quantitative interpretation is proposed in which the P-680$^+$ reduction kinetics by the secondary electron donor Z are controlled by the local pH. This pH, at the membrane level, can be calculated using the Gouy-Chapman theory. A good fit of the results requires to assume that the surface charge density of the inside of the membrane, near the Photosystem-II reaction center, is positive at low pH values and becomes negative as the pH increases, with a local isoelectric point ~4.8. These results lead us to propose a functional scheme in which a pH-dependent proton release is coupled to the electron transfer between secondary and primary donors of Photosystem-II. The H^+/e ratio varies from 1 at low pH to 0 at high pH, with a real pK ~6.5 for the protonatable species.

INTRODUCTION

In oxygen-evolving photosynthetic organisms the electron transfer induced by the two photoreactions is coupled to a proton transfer across the membrane. At the level of the Photosystem-II (PS-II) reaction center, the first step is the photooxidation of the primary donor, P-680, which transfers an electron to a primary acceptor (probably a pheophytin *a* molecule) and then to a bound plastoquinone Q_A. In subsequent reactions P-680$^+$ is reduced, in the submicrosecond time domain (1, 2), by a first secondary donor, often named Z, which is supposed to be a quinone bound in a highly acidic environment (3–5). Z^+ gives rise to a specific ESR signal named Signal II (6).

Considering the electron transfer chain from the oxygen evolving enzyme to NADP$^+$, it appears that all electron carriers are localized in the photosynthetic membrane, or at the membrane-solution interface. However, the various approaches which are available to study these reactions under different experimental conditions require to change bulk parameters of the suspending medium. In general, these will not have simple effects at the membrane level. Some parameters of the surface membrane have been studied using various methods, such as electrophoresis (for a review see reference 7), or electron transfer to artificial charged donors or acceptors (8–11). In this work we study an intrinsic membranous electron pathway and, from its dependence upon bulk parameters, we try to obtain quantitative information on the local properties of the membrane surface around the PS-II reaction center.

The electron transfer kinetics at the level of Z have been shown to depend on the oxygen evolution ability. Inhibition of the oxygen evolving complex slows down the electron transfer from Z to P-680$^+$ (1, 12) and also the subsequent reduction of Z^+ (13). Previous studies with chloroplasts and with several subchloroplast particles enriched in PS-II have shown that, after the irreversible inhibition of oxygen evolution, the rate of electron transfer from Z to P-680$^+$ is accelerated when the pH of the suspending medium is raised from 5 to 8 (14, 15). These results have recently been confirmed by Boska et al. (16) by kinetic studies of the rise time of the ESR Signal IIf, and by Diner and De Vitry (17) who have optically shown the correlation between Z^+ formation and P-680$^+$ decay at various pH.

The influence of the pH could indicate that electron transfer at the level of Z also involves a proton (15). However, the effect is rather weak (at most a factor of 3 in the rate, per pH unit), and could not be fitted with a classical (one e^-, one H^+) reaction involving a group with one single pK. The direct involvement of a proton at the level of Z was suggested by recent experiments of Renger and Voelker (18) on inside-out chloroplast membranes in

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which oxygen evolution was inhibited by Tris treatment and of Forster et al. (19, 20) on chloroplasts. In the present work we have reinvestigated the hypothesis of a direct involvement of a proton at the level of *Z*, taking into account the pH at the membrane surface level according to the Gouy-Chapman theory. This pH was calculated on the basis of bulk pH, salt concentrations, and membrane surface charges.

MATERIALS AND METHODS

Stacks of photosynthetic membranes enriched in PS-II were prepared from market spinach leaves, by the method of Berthold et al. (21), as modified by Ford and Evans (22). These PS-II membranes were incubated with 0.8 M Tris pH 8.2 for 30 min at 4°C. After a first centrifugation (25,000 × *g*, 30 min), the pellet was then resuspended in 10 mM KCl. After another centrifugation (25,000 × *g*, 30 min) the pellet was kept on ice, in the dark.

For each experiment 100 μl of the stock (obtained by dilution of the pellet in 2–4 ml of the 10 mM KCl) was mixed with 2.5 ml of the appropriate buffer, directly in the cuvette. 0.5 mM potassium ferricyanide was added before each experiment. In some experiments 50 μM p-phenylbenzo-quinone was also added. At each pH the medium contained 10 mM of buffer and a variable amount of Tris. After addition of 10, 100, and 1,000 mM KCl or 5, 50, and 500 mM $MgCl_2$, the final pH was adjusted by small and controlled amounts of HCl or NaOH. The buffers were prepared the day before being used for spectroscopic measurements and stored at 4°C.

Flash-induced absorption changes were measured at 820 nm at room temperature (21°C) as previously described (12), except that the actinic flash was provided by a dye laser pumped by a frequency-doubled YAG laser (20 ns, broadband ~595 nm). The flash energy was attenuated so that the ΔA at 820 nm was ~80% of its saturated level. The kinetics of absorption changes were averaged in a numerical form and stored on floppy disks for computer analysis in terms of exponential components.

RESULTS

Excitation by a laser flash of dark-adapted PS-II membranes leads to an absorption increase at 820 nm due to the oxidation of P-680. The subsequent decay has been studied from pH 4 to pH 8, at every 0.5 pH unit, with several concentrations of KCl, NaCl, or $MgCl_2$. Potassium ferricyanide (0.5 mM) was added as electron acceptor and also to maintain oxidized all electron carriers, except the high potential ones. As previously observed with chloroplasts (14), the decay kinetics vary with pH. Here, with PS-II membranes, we obtained a reasonable signal-to-noise ratio with little averaging. Also we are sure that P-700$^+$ does not contribute to the absorption changes since the membranes are devoid of Photosystem-I. A few kinetic traces are presented in Fig. 1, for illustration, with a buffer at pH 7, in the presence of 10 or 1,000 mM KCl, and pH 4.0 in presence of 5 or 500 mM $MgCl_2$.

For each experiment the concentrations of the different ions in the cuvette were precisely known. They were taken into account in the analysis we will present below. To check for an eventual specific effect of the monovalent cation we have performed experiments with different concentrations of NaCl and three different pH values (data not shown). In the limits of experimental uncertainty, identical kinetics for the 820 nm absorption recovery were observed in presence of NaCl or KCl at the same concentration and pH value. As a divalent cation we chose Mg^{2+} instead of Ca^{2+} to avoid specific effects of this last cation, as shown by Yerkes and Babcock (11) and reviewed by Brand and Becker (23).

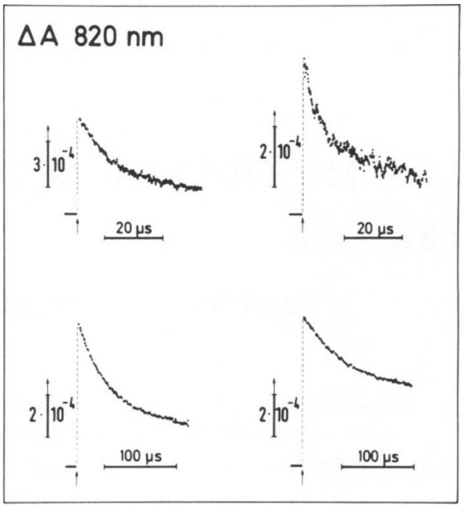

FIGURE 1 Flash-induced absorption change at 820 nm induced by a laser flash in a suspension of Tris-treated PS-II membranes. Averaged effect of four flashes. *Upper* traces: buffer at pH 7.0, without $MgCl_2$, with 10 mM KCl (*left*) or 1 M KCl (*right*). *Lower* traces: buffer at pH 4.0, without KCl, with 5 mM $MgCl_2$ (*left*) or 500 mM $MgCl_2$ (*right*). Chlorophyll concentration: 2.1×10^{-5} M.

The experimental decay kinetics were analyzed in terms of exponential components using a minimization program written by Dr. J. Thiery. At low pH (below 5.5) the experimental results can be fitted with two experimental components, with respective half-times between 15 and 45 μs, depending on pH and 125 μs (pH independent). At higher pH an additional component is needed, as previously found for chloroplasts (12): an intermediate phase of relatively small amplitude (20% to 30%) with a half-time of 22 μs, which does not seem to depend on pH and salt concentration. The major fast phase (60% to 80%) has a half-time that varies with pH and salt concentration. Our analysis and interpretation will focus essentially on the properties of that fast phase, which is attributed to the reduction of P-680$^+$ by the first secondary donor *D* or *Z* (14, 16).

Fig. 2 presents the evolution of the half-time of the fast phase of P-680$^+$ decay, observed at 820 nm, on a logarithmic scale, as a function of the inverse square root of the ionic strength. The drawn lines represent the theoretical curves predicted in the model which will be discussed below. This figure shows essentially two points (*a*) the fast phase of electron transfer to P-680$^+$ is accelerated at higher ionic strength for a pH above 4.5–5.0 units and is slowed down for lower pH. (*b*) This presentation allows a comparison of the effects observed upon addition of KCl or

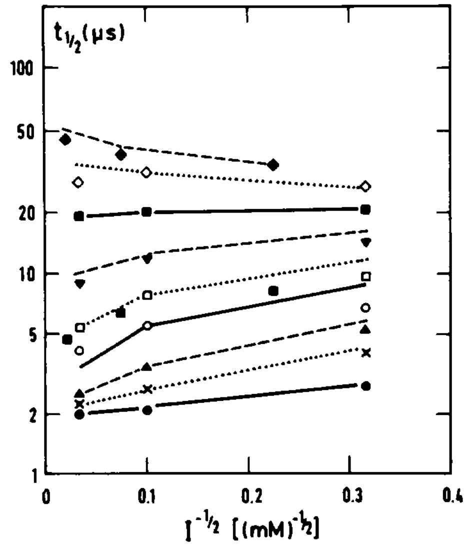

FIGURE 2 Semi-logarithmic plot of the half-time of the pH-dependent absorption change recovery at 820 nm vs. the reciprocal square root of the ionic strength. The symbols correspond to the experimental data and the drawn lines to a computation, as described in the main text. The ionic strength was changed by addition of $MgCl_2$ (pH 4.0, black lozenges; pH 6.0, crossed squares) or of KCl (all other symbols, pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0, from *top* to *bottom*).

$MgCl_2$, and suggests that their effects can be interpreted in terms of ionic strength alone.

Some results are presented in Fig. 3 as a function of the bulk pH for the three different KCl concentrations that we have used. At 10 mM KCl a small dependence of the fast phase kinetics upon the bulk pH is clearly observed (a factor of ~3 by pH unit). However, this figure also shows that the influence of pH on the kinetics of P-680$^+$ reduction is more pronounced at high salt concentration. As in Fig. 2, an inversion in the kinetic effect of the ionic strength is observable at pH 4.5–5.0, where the three curves cross each other.

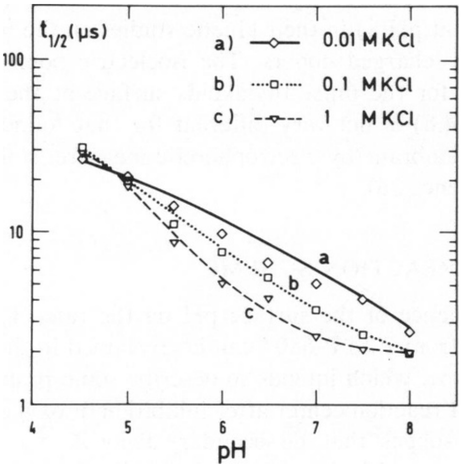

FIGURE 3 Semi-logarithmic plot of the half-time of the pH-dependent phase in the absorption recovery at 820 nm vs. bulk pH. Added salt: 0.01 M KCl (lozenges), 0.1 M KCl (squares), 1 M KCl (triangles). The drawn lines correspond to a computation, as described in the main text.

In this set of experiments the fastest kinetics we have measured, at high pH and high salt concentration, show a half-time of ~2 µs, when 1 M KCl is present in the suspending medium at pH 7.5 and 8. So there is saturation in the evolution of the fast phase at high pH (Fig. 3, curve for 1 M KCl). This effect does not seem to be artefactual since the signal amplitude observed under these conditions is not decreased as it would be if the resolution time of the apparatus were limiting.

The results we have just described correspond to the effect of a first flash, given after ~1 min of dark adaptation. Under these conditions, P-680$^+$ is essentially reduced by Z. A more complete kinetic description of the P-680$^+$ behavior should also include the recombination reaction with the reduced primary plastoquinone Q_A. In Tris-treated chloroplasts that reaction takes place after a flash given after a preflashing (12, 24). It has a half-time of 100–200 µs, essentially independent of pH (14). With the Tris-treated PS-II membranes used in this study we have measured the P-680$^+$ decay after a flash given ~10 ms after a first flash (in this material there is no rapid electron transfer away from Q_A, and we had to add 50 µM p-phenylbenzoquinone as an artificial rapid electron acceptor, in addition to ferricyanide). Under these conditions P-680$^+$ decays in a biphasic manner. A major (50–90%) slow phase has a pH-independent half-time of 125 µs, and a faster phase has the same half-time as observed after dark adaptation at the same pH. Its relative amplitude is larger at low pH, where it can make up as much as 50% of the total signal.

DISCUSSION

The present results clearly indicate that the ionic strength of the suspending medium, together with the bulk pH, control the kinetics of the electron transfer from the donor Z, presumably a highly specific protein-bound quinone, to the primary donor P-680$^+$. Both Z and P-680 are located at the inner surface of the thylakoids membrane (12). A quantitative analysis of the effect of pH on the kinetics of the electron transfer from Z to P-680$^+$ requires to know the actual pH at the level of the membrane surface. In this discussion, we will first attempt to evaluate the electrical properties of the membrane at the level of Z, using the Gouy-Chapman theory, and then we will use these data to present a more complete analysis of the kinetic properties of electron transfer in the PS-II reaction center.

SURFACE PARAMETERS

Generally the ionic strength effect on membrane surface properties can be described using the Gouy-Chapman theory (7): taking into account the surface charge density, considered as a constant, permits to calculate the difference between surface and bulk pH (pH_s and pH_b, respectively). The difference (pH_s–pH_b) is a function of the ionic strength, but is independent of the bulk pH. Our results show that, in the pH range studied, the ionic strength effect on the P-680$^+$ reduction is dependent on pH_b: no effect at pH 4.8, acceleration at higher pH, and slowing down at lower pH. Such an effect can be described if we introduce in the Gouy-Chapman theory the dependence of the surface charge density upon pH_s (25). The existence of a pH (4.8) at which the ionic strength effect changes sign (Figs. 2, 3) indicates a local isoelectric point of 4.8.

The simplest model we can suggest is the existence near the PS-II reaction center of two residues giving rise, upon ionization, to positive or negative charges at the membrane surface. The pK of these groups will be pK^+ and pK^-. In a first approximation we suppose that these two residues have the same superficial concentration (σ_m). The isoelectric point pI can be determined as $pI = \frac{1}{2}(pK^+ + pK^-)$, and the surface charge density, σ, can be written as:

$$\sigma = \frac{-\sigma_m}{1+\frac{[H_s^+]}{K^-}} + \frac{\sigma_m}{1+\frac{K^+}{[H_s^+]}}, \qquad (1)$$

where the proton concentration $[H_s^+]$ is the local one. The surface pH, pH_s, can be related to the medium pH, pH_b, by application of the Gouy-Chapman theory

$$pH_s = pH_b + \frac{F\psi_s}{2.3\,RT} \quad \text{or} \quad [H_s^+] = [H_b^+]\exp\left(-\frac{F\psi_s}{RT}\right), \qquad (2)$$

where ψ_s is the surface potential that is the solution of the well known equation (see, e.g., reference 7)

$$\sigma^2 = 2\epsilon_0\epsilon_r RT \sum_i c_i\left[\exp\left(-Z_i\frac{F\psi_s}{RT}\right) - 1\right], \qquad (3)$$

where z_i and c_i are the valency and concentration (in mol.m^{-3}) of the different ions of the suspending medium; F is the Faraday constant, R is the gas constant and T the absolute temperature (RT/F = 25.8 mV); ϵ_0 is the dielectric permittivity of the vacuum,ϵ_r the relative permittivity of the medium (we take $\epsilon_r = 30$, which is the value for the bound water as in reference 25) ($\epsilon_0\epsilon_r = 2.65 \times 10^{-10}$ $C^2J^{-1}m^{-1}$).

These three equations can be combined in only one equation including the parameter $y = \exp(F\psi_s/RT)$. We can then write

$$\left[\left(1+\frac{K^+}{[H_b^+]}y\right)^{-1} + \left(1+\frac{[H_b^+)}{K^-}\cdot\frac{1}{y}\right)^{-1}\right]^2 - 2\,\epsilon_0\epsilon_r RT\sum_i c_i\,(y^{-z_i} - 1) = 0. \qquad (4)$$

The degree of this equation is dependent upon the higher valency of the ions present in the suspending medium. This equation can be numerically solved. The physical solution y must fulfill the following conditions: (*a*) y must be positive ($\psi_s = \mathrm{Ln}\,y$), (*b*) y must be greater than unity if σ is positive and smaller than unity if σ is negative (σ and ψ_s must have the same sign), and (*c*) if the suspending medium contains only symmetrical salts, changing σ to $-\sigma$, must change ψ_s to $-\psi_s$.

The surface charge density and pH (σ and pH_s) are also functions of bulk parameters (pH_b, salt and buffer concentrations; ferricyanide has also been included) and of the following surface parameters: (*a*) the maximum surface charge density; (*b*) the pK of the two ionizable groups pK^+ and pK^-, which are linked together by the relation $pI = \frac{1}{2}(pK^+ + pK^-)$.

These three parameters have been obtained by the following methods. Between pH 7.5 and 8, at high ionic strength, the amplitude of ionic strength effect does not increase. This suggests that the maximum surface charge density has been reached at $pH_b = 7.5$, 1 M KCl. From the amplitude of the observed salt effect at high pH the maximum surface charge can be determined: $\sigma_m = 30$ mC.m^{-2} (one elementary charge per 5.3 nm^2). The isoelectric point pI is equal to the pH at which the ionic strength effect changes of sign, i.e., $pI = 4.8$. The last parameter pK^- ($pK^+ = 2\,pI - pK^-$) has been determined as the best fit in Figs. 2 and 3 (theoretical curves are drawn as continuous lines): $pK^- = 5.6$.

In consequence of these values of the surface parameters ($\sigma_m = 30$ mC.m^{-2}, $pK^+ = 4$, $pK^- = 5.6$, $pI = 4.8$) a strong dependence of the surface charge density and of the surface pH upon bulk pH and ionic strength is to be expected in our range of experimental conditions. This dependence is shown in Fig. 4 with the surface parameters previously determined. It can partially explain the relatively weak effect of bulk pH on the P-680$^+$ reduction kinetics at low ionic strength since, as shown in Fig. 4 *b*, the surface pH varies much less then pH_b in the studied pH range for 10 mM KCl.

The maximum surface charge density, which is introduced in this model, is of 30 mC.m^{-2}. This is very close to the value obtained by Yerkes and Babcock (11) (35 mC.m^{-2} at pH 8) in their kinetic studies on the reduction of Z^+ by charged donors. The isoelectric point that we measure for the inner thylakoids surface at the level of Z($pI = 4.8$) is not very different for that found for the entire membrane by electrophoretic measurements (~4.3, see reference 26).

REACTION SCHEME

The influence of the surface pH on the rate of electron transfer from Z to P-680$^+$ can be explained in the following scheme, which intends to describe some properties of the PS-II reaction center after inhibition of oxygen evolution. We suggest that the secondary donor Z, or a membranous compound in its vicinity, is partly protonated in its reduced state (with an effective pK red) and deprotonates when Z becomes oxidized (pK oxidized below 4). This model is schematically presented in Fig. 5. In the center of the scheme, the states P-680$^+$... Q_A^-, with Z protonated or

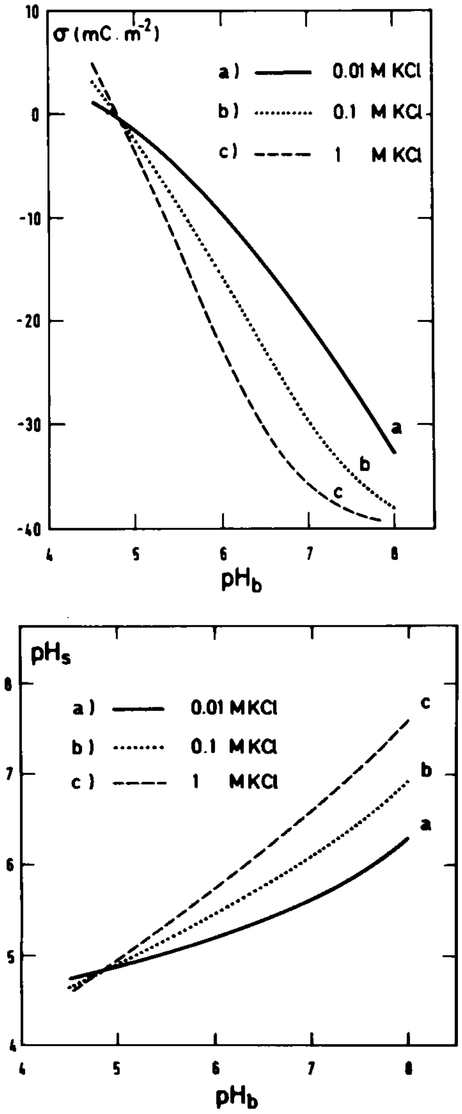

FIGURE 4 (*a*) Calculated dependence of the surface charge density upon bulk pH (pH_b), for three KCl concentrations. Same conditions as for *b*. (*b*) Calculated dependence of the membrane surface pH (pH_s) upon bulk pH (pH_b), for three KCl concentrations. The computation took into account the buffer, salt and other additions, and the following parameters: $pK^+ = 4.0$, $pK^- = 5.6$, and $\sigma_m = 30$ mC.m^{-2}.

not, are produced by the photochemical reaction. They are in equilibrium with a constant K.

From the scheme of Fig. 5, it is possible to deduce the kinetic behavior of P-680$^+$ and Z after a saturating flash following dark adaptation. Assuming that (*a*) the back-reaction rates k_i and k_r are small compared with the other reaction rates (27, 28); and (*b*) the back-reaction rates k_b' and k_b are equal, as shown by the absence of effect of pH on the recombination rate between P-680$^+$ and Q_A^-, the following kinetics are obtained:

$$P^+(t) = P_0 \exp(-k_p \cdot t), \tag{5}$$

where P_0 is the total concentration of the primary donor.

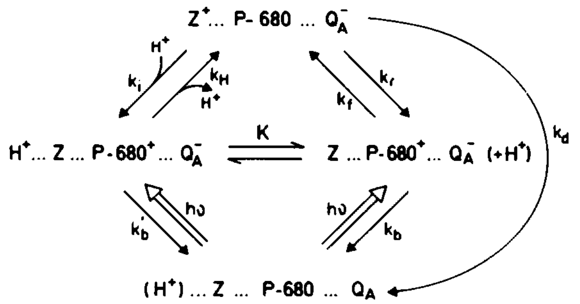

FIGURE 5 A scheme of electron transfer in PS-II membranes after inhibition of O_2 evolution by Tris-washing. We only included the states, equilibrium constants, and rate constants, which are directly relevant to the present study.

$$Z^+(t) = A(\exp(-k_z \cdot t) - \exp(-k_p \cdot t)), \tag{6}$$

where

$$k_p = k_z + k_b \tag{7}$$

$$\text{and } k_z = \frac{k_b(k_r + k_i[\mathrm{H_s^+}]) + Z \cdot k_d}{Z + k_b} \tag{8}$$

with

$$Z = \frac{k_\mathrm{H} \cdot \dfrac{[\mathrm{H_s^+}]}{K} + k_f}{1 + \dfrac{[\mathrm{H_s^+}]}{K}}. \tag{9}$$

We assume that the equilibrium $(\mathrm{H^+})..\,Z..\mathrm{P\text{-}680^+}..\,Q_A^- \rightleftharpoons Z..\mathrm{P\text{-}680^+}..\,Q_A^- + \mathrm{H^+}$ is faster than all the other reactions considered. This assumption seems reasonable since (*a*) we are concerned with the nonoxygen evolving system, in which the fastest reactions are in the microsecond range. (*b*) The equilibrium can be supposed to be independent of the redox state of the primary donor. The protons involved in this equilibrium are at the level of the membrane surface.

From Eqs. 7 and 9 it results that the reduction rate of P-680$^+$ at high pH tends toward $(k_f + k_b)$, for $pH_s >> pK$ red. This fastest reaction rate is correlated with the unprotonated state of Z (or of the membrane component in its surrounding), so that no proton release will be associated with the electron transfer at high pH. The rate of the back reaction P-680$^+$... Q_A^- → P-680... Q_A can be obtained experimentally from the slow phase of the P-680$^+$ reduction observed when a preflash has been given to the sample: (half-time = 120 μs), i.e., $k_b = 6{,}000$ s^{-1} (pH-independent). If we assume that the back reaction has the same rate when Z is reduced or oxidized, then from the value of the fastest reaction observed at high pH_s in dark adapted conditions (half-time is 2 μs), one can deduce $k_f = 35.10^4$ s^{-1}.

In the pH-dependent range ($pH_s \lesssim pK$ red), the P-680$^+$ reduction rate depends upon k_b, k_f, k_H, pK red (Eqs. 5, 7, and 9). The values of pK red and k_H have been determined as the parameters giving the best fit for the plot of the half-time of P-680$^+$ reduction vs. pH_s (Fig. 6): $k_H = 3{,}000$ s^{-1}, pK red = 6.3 (for protons at the membrane surface).

The scheme of Fig. 5 also permits to describe the kinetic evolution of Z (oxidation and reduction). From Eq. 6 it appears that the oxidized state of Z will be formed in parallel with P-680$^+$ reduction. This property has recently been established by Boska et al. (16) and Diner and De Vitry (17). The scheme also predicts that the reduction kinetics of Z^+ in the absence of exogenous donors should depend upon pH_s, in the same range as the P-680$^+$ reduction kinetics. Some experimental data on the half-time of Z^+ reduction have been obtained at different bulk pH values by Yerkes and Babcock (27), and Dekker et al. (5). From the experimental conditions they used, and the surface parameters we have found in the first part of this discussion our scheme permits to calculate the dependence of the half-time of Z^+ reduction upon the surface pH. Experimental data from these authors are presented in Fig. 6 as full or open lozenges, respectively. The theoretical curve was obtained with the values of the parameters that have been determined to account for the P-680$^+$ reduction rate, and with $k_r = 120\ s^{-1}$. We have also supposed that the rates of inverse reactions (k_i, k_d) were negligible (e.g., reference 28).

So the same set of surface parameters and rate constants can explain the pH and salt concentration dependence of the reduction rate of the primary donor (an intrinsic reaction) and of the secondary donor (by exogenous reductants [11], or by an intrinsic reaction [5, 27]), after inhibition of the oxygen evolution. These results justify in this particular case the assumption of the Gouy-Chapman theory (charges uniformly smeared over the membrane surface), which is probably not realistic.

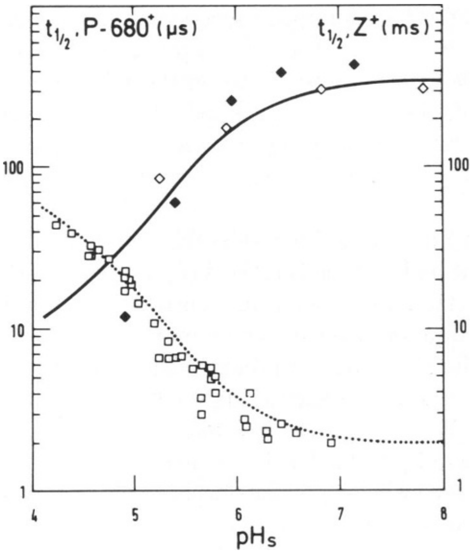

FIGURE 6 Semi-logarithmic plot of the half-time of the pH-dependent phase of absorption recovery at 820 nm (open squares; *left scale*) and of the half-time of Z^+ (open lozenges, data from reference 27; full lozenges, data from reference 5; *right scale*). The drawn lines correspond to the same half-times calculated according to the scheme of Fig. 5 (see text) with the following parameters: $pK^+ = 4.0$, $pK^- = 5.6$, and $\sigma_m = 30$ $mC.m^{-2}$.

In our model we suppose that a proton release is coupled with the electron flow from the secondary to the primary donor. The ratio H^+/e^- varies from 1 at low pH to 0 at high pH, in parallel with the relative concentration of the protonated component; Renger and Voelker (18) and Forster and Junge (20) have observed a proton release on thylakoids membranes in which oxygen evolution is inhibited. In inside-out membranes, Renger and Voelker found a H^+/e ratio of 1 for a bulk pH of 6.5, and an apparent pK of 8 (relative to the bulk pH) for reduced Z. These values should correspond, according to our analysis, to a H^+/e ratio of 1 for a pH surface of 5.6, and a real pK of 6.5 (relative to the surface pH) for the equilibrium $(H^+ \ldots Z \ldots \text{P-680}^+) \rightleftharpoons (Z \ldots \text{P-680}^+) + H^+$.

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