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Theory of proton flow along appressed thylakoid membranes under both non-stationary and stationary conditions

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Under illumination thylakoids take up protons from the suspending medium, e.g., at Photosystem II which is located in appressed portions of stacked thylakoid membranes (partitions). The rise of alkalization in the suspending medium after one single turnover of Photosystem II is rather slow (typically, half-rise time 100 ms) in stacked thylakoids and fast (2.7 ms) in unstacked ones (Polle, A. and Junge, W. (1986) Biochim. Biophys. Acta 848, 257–264). We described the transient alkalization of the suspending medium of stacked thylakoids by the theory of evaporation from a cylinder. The calculated time-course fitted the experimentally observed one with a single fit parameter, namely the 'effective' diffusion coefficient of hydroxyl anions in the narrow domain between appressed membranes. Its magnitude was 10^5-times lower than for diffusion of hydroxyl in water. This large decrease could be rationalized by the action of fixed buffers in this domain, which decreased the 'effective' diffusion coefficient (in Fick's second law), but left the 'true' diffusion coefficient (Fick's first law) unaffected. We also modeled the continuous flow of hydroxyl anions through the alkaline partitions which is required for steady ATP synthesis. For stacked thylakoids and with a diffusion coefficient as in bulk water we calculated a lateral pH drop of some 0.1 units between center and fringes of thylakoids. This provided a physical basis to understand quantitatively slightly different efficiencies of the two photosystems in ATP synthesis without necessity to invoke nebulous-localized coupling devices.

Introduction

Thylakoids in chloroplasts, disks of rod-outer segments and the invaginated plasma membrane of cones in vertebrate retinae are sheet structures with tightly appressed membranes. The narrow space between opposing membranes is loaded with proteinaceous groups and lipid headgroups which provide a buffering capacity for protons and for divalent ions. Since these membranes are ion transporting it is worthwhile to ask for the consequences of membrane appression for lateral ion flow. At the time when the calcium transmitter hypothesis of visual transduction was flourishing McLaughlin and Brown [1] tentatively attributed the induction phase of the electrophysiological response of rods to the lateral diffusion of calcium along stacked disk and towards the plasma membrane. Following theory of diffusion in media with buffering and fixed groups these authors calculated that the effective diffusion constant of calcium was lowered by up to two orders of magnitude because of calcium buffering. Viewed from todays changed concepts of visual transduction [2,3] the attribution of the induction phase to delayed diffusion of calcium, although physically attractive, may have been inappropriate from the physiological point of view.

Thylakoid membranes of chloroplasts of green plants form a sheet structure with complicated continguity. Flat disks are stacked in grana which are interconnected by more extended lamellae

(stroma membranes). It is generally agreed on that what appears as isolated disks in electron micrographic thin sections is a somehow contiguous system of sheets. A simplified representation of stacked thylakoids is illustrated in Fig. 1. One distinguishes between appressed membranes and exposed membranes. The membranes at the top and the bottom of a granum, the fringes plus the intergrana lamellae make up the second class. The gaps between appressed membranes are referred to as partitions and the inner volume is named lumen of thylakoids. With the proton pumps associated with Photosystem II (and with water oxidation) located in appressed membranes and with the ATP synthases in the exposed ones (for a recent review, see Ref. 8), continuous operation of proton pumps and ATP synthases requires lateral flow of protons both in the partitions and in the lumen. Because the partition region is alkaline the effective carrier of 'proton flow' is likely the hydroxyl anion.

Excitation of stacked thylakoids with a flash of light generates an alkalization pulse at the external side of the membrane, which reaches the suspending medium only with an unexpectedly long half-rise time (60 ms in spinach, see Ref. 4 – 100 ms for pea, see Ref. 5). We found this delay abolished (half-rise time 2.7 ms) if thylakoids were unstacked and reversibly reinstalled if they were restacked [5]. This suggested that the delay might have been caused by hindered diffusion of hydroxyl anions and of protons along the narrow and highly buffering gap between opposing membranes in the stack.

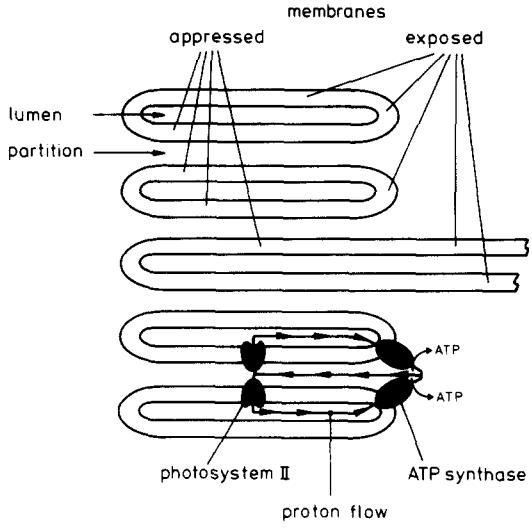

Fig. 1. Schematic representation of thylakoids in stacked configuration. The location of Photosystem II in domains with appressed membranes and of the ATPsynthase in exposed domains is indicated. Cyclic proton flow between the pumps related to Photosystem II and the synthases is marked by arrows.

In this communication we describe this diffusion problem in terms of a theory of evaporation from a solid cylinder. The calculations reproduced the experimentally observed pH transients with only one fit parameter, namely the 'effective' diffusion coefficient of hydroxyl anions in the partition domain. This had to be 10^5-times lower than the diffusion coefficient of hydroxyl in water. This, however, was the diminuation of the 'effective' diffusion coefficient which we expected based (1) on a reasonable estimate for the buffering capacity in the partition domain, and (2) on the assumption that the 'true' diffusion coefficient in the partition region was similar to the one for the hydroxyl anion in water. The latter assumption, which gained confidence by the obtained fit of the rise kinetics under pulse excitation, was used to calculate the lateral pH difference between fringes and center of thylakoids which was required to drive stationary flow of hydroxyl anions from the proton uptake sites at Photosystem II through the partitions and into the ATP synthases. This pH drop came out as a few tenth of a pH unit. Although small, it was not negligible compared with the transmembrane pH difference under continuous photophosphorylation.

Buffering capacities acting in the partition region

In line with previous work [6] the buffering capacity of a volume element is defined as follows:

$$B = -\frac{d[H_t^+]}{dpH} \qquad (1a)$$

wherein the nominator denotes the change of the total proton concentration (subscript t for total = bound and free) in this volume element. Further down we deal with an alkaline space where the hydroxyl anion is dominant for the relaxation of a pH profile. Therefore, it may be worthwhile to

recall that the ionic product of water is kept constant ($[H_f^+]\cdot[OH_f^-] = 10^{-14}$) and that the relative concentration changes of the proton and the hydroxyl anion are equal in magnitude, although opposite in sign ($d[H_f^+]/[H_f^+] = -d[OH_f^-]/[OH_f^-]$). If there is a proton sink, which makes protons disappear $d[H_t^+] = -a$, this is equivalent to a source which makes hydroxyl anions appear, $d[OH_t^-] = a$. All of this holds in aqueous environment and because of the extremely rapid neutralization reaction of water [7]. The equivalence of adding protons or hydroxyl anions to yield, a certain extent of a pH change is known to all in the field. Thus the buffering capacity may be also formulated as follows:

$$B = \frac{d[OH_t^-]}{dpH} \tag{1b}$$

And for small pH transients this is explicitly

$$B = d[OH_t^-]\cdot 2.3\cdot\frac{[OH_f^-]}{d[OH_f^-]} \tag{1c}$$

If buffering is attributable to a single species of monovalent acid at concentration c and with a dissociation constant K, the buffering capacity is

$$B = 2.3cK\cdot\frac{[H_f^+]}{([H_f^+]+K)^2} \tag{2}$$

wherein the subscript f refers to the concentration of free protons.

The total buffering capacity of the suspending medium of thylakoids as well as in the narrow partition gaps was composed from two contributions: the one by proteinacous groups at the outer side of thylakoid membranes and the one of added pH indicating dye. Other buffers (e.g., carry over from the preparation procedure) were neglected.

Cresol red at 15 μM and at pH which is equal to its pK contributes a buffering capacity of 8.6 μM/pH to a solution (see Eqn. 2). Because the partition region likely carries a slight negative net charge the contribution of cresol red to the buffering capacity of the partition region will rather be lower than its contribution to the bulk phase.

The buffering capacity of the partition region can only be indirectly inferred. Our titrations of thylakoid suspensions (prepared as in Ref. 5 and at 10 μM chlorophyll) which were washed free of soluble buffers yielded buffering capacities of between 10 and 30 μM/pH in the absence of added dye (in the presence of 10 mM NaCl, 5 mM $MgCl_2$ and at pH 7.9). (The variation resulted from different batches of starting material and from different chlorophyll concentrations in the stock suspension.) This included contributions from both sides of the thylakoid membrane, from appressed and exposed membranes and from any residual patches of the chloroplast envelope. Attributing one half of this figure to one half of the total chlorophyll molecules located in appressed membranes, we arrived at an absolute upper estimate for the specific buffering capacity in the partition region, 1 mol/mol chlorophyll per pH. For the internal surface of the thylakoid membrane we previously determined a chlorophyll-related specific buffering capacity of 70 mmol/mol chlorophyll per pH which only slightly varied with pH above neutral [6]. This contributed a buffering capacity of approx. 1 μM/pH to a suspension of thylakoids at 10 μM chlorophyll. With the volume of a partition disk we calculated the buffering capacity within the partition gap. Accepted figures for the thickness of the partition gap and the membrane area per chlroophyll molecule are the following: 5 nm (see Ref. 8) and 2.2 nm^2 [9]. Based thereupon we calculated that the buffering capacity in the partition region ranged between 20 and 200 mM/pH at slightly alkaline pH.

The above estimates showed that the buffering capacity of the indicator dye was dominant in the (otherwise unbuffered) suspending medium and negligible in the partitions.

Selectivity of a hydrophilic pH-indicating dye for events in the suspending medium of thylakoids

We considered a suspension of biological vesicles in the presence of a pH indicating dye which was distributed over several compartments numbered by the superscript i. Excitation of thylakoids with light produces pH transients in the lumen, in the partition region and in the suspending medium. This causes transient changes of absorbancy of the dye which are additive with contri-

butions from the compartments:

$$dA^i = (\varepsilon_b - \varepsilon_a) \cdot B_d^i \cdot \frac{V^i}{V^s} \cdot l \cdot dpH^i \quad (3)$$

dA^i is the change in absorbancy as reported by dye located in compartment i, $\varepsilon_b - \varepsilon_a$ is the difference of the extinction coefficients between base and acid form of the dye, B_d^i is the buffering capacity which the dye contributes to compartment i, V^i and V^s are the volumes of the compartment i and of the suspending media, l is the pathlength of the optical cell and dpH^i is the supposed very small change of the pH in the respective compartment.

Merging Eqns. 1 and 3 one obtains the ratio of absorption changes resulting from the compartment i and from the suspending medium:

$$\frac{dA^i}{dA^s} = \frac{B_d^i \cdot B_t^s \cdot V^i}{B_t^i \cdot B_d^s \cdot V^s} \quad \frac{d[OH_t^-]^i}{d[OH_t^-]^s} \quad (4)$$

$d[OH_t^-]$ is the total change of hydroxyl anion concentration (in mole) in the respective compartment and B_t is the total buffering capacity. It was assumed that the volume of the suspending medium was very large as compared with any other volume. Applying Eqn. 4 to the partition region of stacked thylakoids and to the suspending medium, one notes that $d[OH_t^-]/V$ is the same for both compartments, because the same proton uptake sites of Photosystem II caused the alkalization of the partitions and, after diffusion and with delay, of the suspending medium. The concentration of the dye was more likely to be lower in the partition region than in the bulk solution. Therefore, the ratio between the extent of absorption changes resulting from partitions (superscript p) and the one resulting from the suspending medium (superscript s) found an upper limit by the following expression:

$$\frac{dA^p}{dA^s} = \frac{B_t^s}{B_t^p} \quad (5)$$

Taking the above estimates for the buffering capacities of these compartments as approx. 20–200 mM/pH (p) and 10 μM/pH (s) into account the response ratio of hydrophilic dyes to pH transients in the bulk and in the partition ranged between $2 \cdot 10^3$ and $2 \cdot 10^4$. It followed that a hydrophilic pH indicator ignored the smaller compartments. For Cresol red and the thylakoid lumen this was previously established experimentally (Ref. 10, but see Fig. 6 in Ref. 11). (On the other hand, membrane-adsorbed indicators like neutral red are likewise sensitive to pH transients on both sides of the thylakoid membrane, since they reside at both sides of the membrane at approximately equal amounts. Only by virtue of selective buffering they were made selective, in thylakoids for the lumenal space [6,12]).

In the subsequent discussion we assumed that an alkalization pulse was generated in the narrow partition domain and that one observed the appearance of hydroxyl anions in the suspending medium via an indicator dye which was specific for this space.

Diffusion and chemical reactions with fixed groups

We discuss the following in terms of hydroxyl anions as diffusing and, for the sake of simpler wording, also as effectively buffered species (in the sense given in Eqn. 1c). The arguments, however, are fairly general.

Fick's second law relates the change rate of the local concentration of a diffusing species to the second derivative of the concentration with respect to space. The latter is proportional to the difference between the fluxes entering and leaving a thin section. If there are fixed buffering groups in that section Fick's law describes the change rate of the total concentration, which is different from the concentration of the free species. Considering hydroxyl anions Fick's second law reads as follows:

$$\frac{\partial}{\partial t}[OH_t^-] = D \cdot \Delta[OH_f^-] \quad (6)$$

wherein Δ denotes the delta differential operator, and t is the time in seconds.

That the left-hand side invokes the total concentration instead of the free as in the conventional form of Fick's law, is immediately apparent by recalling the physical meaning of this equation. Its right-hand side gives the difference of the ingoing and outgoing fluxes through the surface of an infinitesimally small volume element. The net gain

is proportional to the local transient of the total concentration.

Inserting Eqn. 1c into Eqn. 6 one obtains the differential equation for the local change of the concentration of free hydroxyl anions:

$$\frac{\partial}{\partial t}[OH_f^-] = \frac{2.3 \cdot [OH_f^-]}{B} \cdot D \cdot \Delta[OH_f^-] = D_{eff} \cdot \Delta[OH_f^-] \quad (7)$$

This implied that the concentration of free hydroxyl anions changed in time as if the 'true' diffusion constant was changed into an 'effective' diffusion constant (see also Crank, J. [13]). In media with high concentrations of fixed buffers the 'effective' diffusion constant may be very small. If we considered only the upper limit for the buffering capacity of the partition gap (200 mM/pH), we ended with a reduction of the diffusion coefficient by a factor of 10^5 at pH 8.

Fick's first law, on the other hand, states that the flux of hydroxyl anions, J, is proportional to the gradient (∇ = nabla operator) of the concentration of free anions:

$$J = -D \cdot \nabla[OH_f^-] \quad (8)$$

It is the 'true' diffusion coefficient which enters into this equation, and this is not affected by the presence of fixed buffering groups. Eqns. 7 and 8 told us that the transient dissipation of a pH profile was slowed down by fixed buffering groups, but that the steady flux induced by a given concentration gradient of hydroxyl anions was not affected by buffering groups.

The leakout of a pH-profile from the partition region into the suspending medium of thylakoids – evaporation from a solid cylinder

We approximated thylakoids as flat disks. At time zero stimulation of proton pumps generated a pH profile in the partitions, and the concentration of free hydroxyl anions was shifted by an amount $X(r, t)$ towards alkalinity. The magnitude of X was very small in comparison with the concentration of hydroxyl anions before the perturbation (a few percent). At alkaline pH in the outer medium, and in the partitions, hydroxyl anions were the major flux carrier. They leaked out from the partitions and into the suspending medium. Thus the profile was smeared out and the pH in the suspending medium raised accordingly. At a more neutral pH, two diffusing species contributed to the dissipation of the original profile. For the sake of verbal and conceptional simplicity we considered only hydroxyl leaking out.

We asked for the time-course of hydroxyl appearance in the suspending medium as viewed spectrophotometrically via a pH indicating dye. If the dye was the dominant buffer in this bulk space every hydroxyl which had crossed the cylindrical surface of a thylakoid stack was seen at equal weight, no matter how far into the bulk phase it had diffused. We asked for the amount of substance which appeared in the bulk as function of time. This problem is mathematically equivalent to evaporation from a solid cylinder [13]:

We chose the following initial condition:

$$X(r, t=0) = \begin{cases} X_0 & r \leqslant R \\ 0 & r > R \end{cases} \quad (9)$$

where R is the radius of stacked thylakoids. We assumed that the density of active Photosystems II was constant over the surface of appressed membranes and that this density ranged from the center to the boundary of the partition disk (radius $r = R$, see Fig. 1b). The boundary condition was as follows:

$$-D \cdot \left(\frac{\partial X}{\partial r}\right)_R = b \cdot X(R_-) \quad (10)$$

This equation stated that the flow of hydroxyl anions across the interface of the disk is the same at both sides of the boundary. b is a parameter which is related to the diffusion coefficient, D_b, and the thickness, d_b, of the boundary layer outside of radius R:

$$b \approx D_b / d_b \quad (11)$$

By choosing these boundary conditions we assumed that the transient, $X(r, t)$, outside of the cylindrical boundary ($r = R$) was much smaller (in fact, close to zero) than the one inside, i.e., we assumed that the pH transient in the partition domain was larger than the one in the suspending medium. This was justified if the contribution of the partition domain to the total buffering capac-

ity of the bulk medium (with dye) was small.

The solution to this cylindrical diffusion problem in terms of total substance which has leaked out into the external space has been given as function of time by Eqn. 5.49 in Ref. 13. This solution contains a parameter, L, deserving discussion:

$$L = \frac{Rb}{D^{p}_{EFF}} \tag{12}$$

where R denotes the radius of the cylinder and D^{p}_{EFF} the effective diffusion coefficient in the cylinder (here in the partitions), b is defined by the boundary conditions (Eqns. 10 and 11). As detailed above the effective diffusion coefficient in the partition region was by five orders of magnitude smaller than the one in a medium without fixed buffering groups. Moreover, the radius of thylakoid stacks is greater than the thickness of the boundary layer. This makes L very large, indeed. In the approximation of infinitely large L the analytical solution of this diffusion problem becomes:

$$\frac{M(t)}{M_{\infty}} = 1 - \sum_{n=1}^{\infty} \frac{4}{a_n^2} \exp \frac{-a_n^2 D^{p}_{EFF} t}{R^2} \tag{13}$$

In this equation $M(t)$ is the mass of substance which has diffused from the cylinder and into the surrounding space, M_{∞} is the same variable after infinite time, D^{p}_{EFF} is the effective diffusion coefficient in the cylinder (in the partitions), R is the radius of the cylinder and the a_n are roots of the Bessel function of first kind and order zero as tabulated in Table 5.2 in Ref. 13. The solution function of Eqn. 13 describes a transient which rises monotonically as a sum of exponentials. It is noteworthy that the relative contributions of these exponentials and that their respective rise times are locked with respect to each other. Therefore a fit obtained with this solution function is very different from the often ambiguous fits of transients with several exponentials at free choice of relative extents and rise times.

The first component accounts for 69% of the rise, the second for another 13% and inclusion of the first six components leaves a rest of only 6.5%. We fitted the experimenally observed rise of the pH in the suspending medium of stacked thylakoids with a calculated curve which took the first six exponentials into account. The result was plotted in Fig. 2. Except for the 100% margin of the extent the only fit parameter was D^{p}_{EFF}/R^2, the ratio between the effective diffusion coefficient in the partitions and the squared thylakoid radius. Comparison of the calculated 'dimensionless time scale' at the top of Fig. 2 with the experimental one at the bottom shows that 1 s (experimental) corresponded to 0.61 of the dimensionless time. It followed:

$$\frac{D^{p}_{EFF}}{R^2} = 0.61\ s^{-1} \tag{14}$$

Taking the typical radius of pea thylakoids as 300 nm we found that

$$D^{p}_{EFF} = 5.5 \cdot 10^{-14}\ m^2 \cdot s^{-1} \tag{15}$$

The diffusion coefficient of hydroxyl anions in water and at 25°C [14]:

$$D(OH^-) = 5.3 \cdot 10^{-9}\ m^2 \cdot s^{-1}$$

The effective diffusion coefficient of hydroxyl anions was by a factor 10^5 smaller than the one in water.

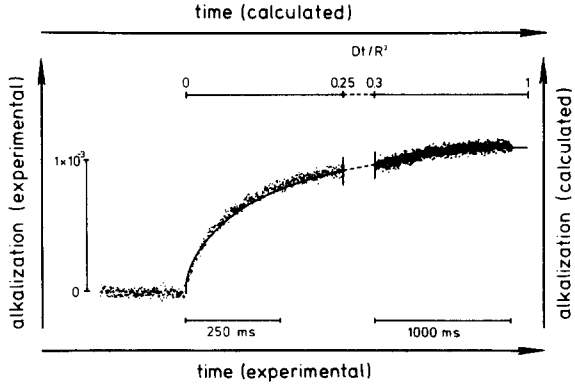

Fig. 2. Experimentally observed time-course (dots) of the flash-induced alkalization of the suspending medium of stacked thylakoids and calculated time course (line). The data were taken from Fig. 2 (upper trace) of Ref. 5. The theoretical curve was calculated according to Eqn. 13 of this message and taking the first six exponentials into account. The dimensionless time, Dt/R^2, was the only fit parameter. Its scale is given by the upper bars.

Above we calculated that buffering in the partition regions could be expected to lower the effective diffusion constant of hydroxyl anions in this domain by several orders of magnitude. The exact factor was dependent of the pH in the partition domain and of the buffering capacity. A factor of 10^5 was expected at pH 8 and for a buffering capacity of 200 mM/pH, which we considered as the upper limit. In the experiments underlying to Fig. 2 the pH of the suspending medium was 7.9 [5]. Because the partition region likely carried a residual negative net charge at the relatively low ion concentrations in the experiments (NaCl, 10 mM; $MgCl_2$, 5 mM) the pH in the partition region may have been lower than 7.9. There is no point of guessing the precise pH and the precise buffering capacity in the partition domain. However, we note that reasonable figures of both parameters brought us into the range of the observed reduction of the 'effective' diffusion coefficient of hydroxyl anions in the partitions.

Of course, we did not determine the nature of the dominant flux carrier in the pulse experiments: This could have been (a) hydroxyl anions, as assumed for the calculation, (b) protons, if their 'true' diffusion coefficient was way up above the one for hydroxyl anions and, in principle, (c) buffering proteins. Mobile proteins could speed up the relaxation of the pH profile, but they would do so via their 'true' diffusion coefficient. The true diffusion coefficient of Photosystem II, for example, was estimated to be 10^{-14} $m^2 \cdot s^{-1}$ (Ref. 20, but see also Ref. 8). This was 5-times lower than the 'effective' diffusion coefficient resulting from the fit in Fig. 2. Therefore we ignored the contribution of protein diffusion to the relaxation of an alkalization pulse.

On the pH-drop in the partition region under stationary proton flow between Photosystem II proton pumps and ATP synthases

The most interesting result from the above considerations was that the buffering capacity in the partition region could, in principle, account for the observed great delay between proton uptake at Photosystem II and the appearance of the alkalization pulse in the external medium. In turn, this implied that the 'true' diffusion coefficient in the partition region was of the same order of magnitude as that for hydroxyl anions in water. Under steady illumination both photosystems take up protons from the suspending medium and this uptake is compensated by a constant outlet of protons from the internal phase and through ATP synthases into the suspending medium. The cyclic proton path is illustrated in Fig. 1 (lower part). Deviating from the simple scheme the dominant carrier of lateral flow was likely the hydroxyl anion in the alkaline partitions and the proton in the acid lumen. It was sensible to assume that lateral flow was not driven by a lateral difference in electrical potential but by a pH difference, since a lateral voltage drop would have been compensated by other ions. In order to drive outwardly directed hydroxyl flux through the partitions a pH difference had to exist with the fringes of thylakoids more acid than the center region of the partition. At any point of the partition disk the stationary flux of hydroxyl anions is described by Fick's law (Eqn. 8). Under the assumption that the proton sinks were homogeneously distributed over the partition disk the dependence of the flux on the radius became very simple, and integration yielded the desired pH drop between fringes and center as detailed below.

The total flow of hydroxyl anions across the perimeter of a disk-shaped partition gap, I_t, was:

$$2\pi RhJ(R) = I_t \tag{16}$$

where R is the radius of the partition gap (300 nm), h its thickness (5 nm) and $J(R)$ is the flux at radius R (in $mol \cdot m^{-2} \cdot s^{-1}$). Under the assumption that Photosystems II were equally active and homogeneously distributed over appressed membranes the flux at distance r was proportional to the number of photosystems inside the given radius:

$$2\pi rhJ(r) = \left(\frac{r}{R}\right)^2 \cdot I_t \tag{17}$$

$$J(r) = \frac{r}{R} J(R) \tag{18}$$

Under consideration of Fick's law (Eqn. 8) it followed that:

$$\frac{r}{R} J(R) = -D \cdot \frac{\partial}{\partial r}[OH_r^-] \tag{19}$$

Integration and substitution of Eqn. 16 yielded:

$$[OH_f^-]_r - [OH_f^-]_0 = -\left(\frac{r}{R}\right)^2 \frac{I_t}{4\pi hD} \quad (20)$$

The geometrical features underlying to the above calculation and the parabolic radial concentration dependence of hydroxyl anions (according to Eqn. 20) were represented in Fig. 3. Eqn. 20 allows to alcculate the extent of the pH drop between fringes and center of partitions. What was the pH drop if the 'true' diffusion coefficient for hydroxyl anions in the partitions was the same as in bulk water?

The total flow, I_t, which crossed the cylindrical boundary of one partition disk could be inferred from the specific rate of ATP synthesis under linear electron flow, e.g., a relatively high rate of 360 μmol ATP/μmol chlorophyll per h. At the accepted proton/ATP-stoichiometry of 3 [15,16] this implied a proton/hydroxyl flow of 0.3 equ./chlorophyll per s. We estimated the chlorophyll content of one partition disk via the area per chlorophyll (2.2 nm^2 according to Ref. 9) and for a radius of 300 nm. Considering that both opposing membranes contributed to the total flow we arrived at $I_t = +1.3 \cdot 10^{19}$ mol · s^{-1}. The positive sign took care of the outward direction of hydroxyl flow. For the thickness of the partition region we assumed 5 nm (see Ref. 8) and for the diffusion coefficient we took the value for hydroxyl anions in bulk water, $5.3 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$ [14]. With these parameter values Eqn. 20 yielded a concentration difference of:

$$[OH_f^-]_0 - [OH_f^-]_R = 0.38\,\mu M \quad (21a)$$

In a phase where proton flow dominated and with $D(H^+) = 9.3 \cdot 10^{-9}$, the respective figure was:

$$[H_f^+]_R - [H_f^+]_0 = 0.23\,\mu M \quad (21b)$$

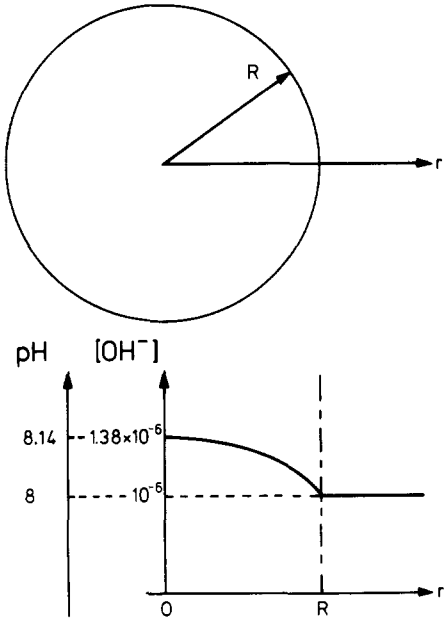

Fig. 3. Schematic representation of a partition disk as viewed from the top and calculated concentration of hydroxyl anions which supports a net lateral flux of hydroxyl/protons, which is compatible with a rate of ATP synthesis at 360 μmol ATP/μmol chlorophyll per h. For details, see text. The corresponding pH drop between the centre and the fringes of partitions is marked at the left margin.

The pH difference between the perimeter and the center of the partition domain followed from Eqn. 21 under consideration of the definition of pH. This difference depended on the pH in the partition region. Its magnitude was approximately 0.14 units if the pH was 8 and it was 0.35 units if the pH was 7.5. On the other hand we learned from Eqn. 21b that the pH difference which was necessary to drive a proton flow of same magnitude through the lumenal region (at pH < 5, under these conditions) was smaller than 0.01 units.

The foregoing showed that unhindered diffusion of hydroxyl anions through the partitions already caused lateral losses of proton-motive diring force for photophosphorylation which are small but not negligible. The losses occurring at the inner side of the thylakoid membrane, however, were shown to be negligible.

Conclusions

We showed that the delayed propagation of an alkalization pulse in thylakoid partitions can be accounted for by the known amount of fixed (or only slowly diffusing) buffers in this domain. On the other hand the 'true' diffusion coefficient of hydroxyl anions in the narrow partitions had to be the same as in bulk water. The latter is in agreement with results from elegant experiments by Gutman and Nachliel [24] who showed that the

diffusion coefficient of protons along the surface of micelles is in the same range as in bulk water. It contrasts, however, with hypotheses by Nagle and Morowitz [17] and by Haines [18] and also with the interpretation of their experiments by Teissie et al. [19] who proposed greatly enhanced diffusion of protons along lipid surfaces.

Using the bulk diffusion coefficient of OH^- for the partitions we calculated the lateral loss of the proton-motive force under steady OH^- flow through the partitions. This loss was small, but not negligible compared with the transmembrane difference of the proton-motive force under steady photophosphorylation. These losses are particularly relevant for the proton pump associated with Photosystem II, which is located in the partitions and thus remote from the ATP synthases. In fact, lower efficiency for photophosphorylation of Photosystem II was reported by Haraux and De Kouchkowsky [21,22], while no such difference between the two photosystems was found in another laboratory [23]. We showed that lateral losses of proton-motive force critically depend on the thickness of the partition gap (see Eqn. 20). It is conceivable that the seeming contradictory results reported in Refs. 22 and 23 may have been caused by different chloroplast preparations with different thickness of the partition gap.

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