Electric field–induced lateral mobility of photosystem I in the photosynthetic membrane

A study by electrophotoluminescence

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ABSTRACT Electrophoretic movement of photosystem I (PS I) along the photosynthetic membrane of hypotonically swollen thylakoid vesicles was studied by analyzing the electric field–stimulated delayed luminescence (electrophotoluminescence) emitted from PS I. The electrophoretic mobility was inferred from the differences in electrophotoluminescence (EPL) of the photosynthetic vesicles in presence and absence of trains of low amplitude (<80 V/cm) prepulses of 1 ms duration at 4 ms spacing. The average apparent electric mobility, determined from the time course of EPL increase on one hemisphere or its decrease on the other one, as function of prepulse length and intensity was of the order of $3 \cdot 10^{-5}$ $cm^2V^{-1}s^{-1}$. The assymetric distribution of the PS I reached a steady state when the diffusional, electrostatic, and elastic forces balanced the electrophoretic driving force. A lateral diffusion coefficient of $\sim 5 \cdot 10^{-9}$ cm^2s^{-1} was found for the PS I complex from the diffusional relaxation after cessation of the electric field pulse train. Experimental conditions such as concentration, temperature, and viscosity of the aqueous solution were not critical for the effect. Between 23 and 150 electron charges per moving particle were estimated from the measured electrophoretic mobility.

INTRODUCTION

The spatial organization and dynamic properties of proteins involved in energy transduction is one of the central longstanding problems in the field of bioenergetics. Of special interest are the protein complexes of the photosynthetic membrane, which carry the electron transport processes that convert the energy of oxidation into energy stored in an electrochemical proton gradient across the membrane. The vast majority of thylakoid membrane proteins is organized into five integral membrane-spanning complexes: photosystem I and II complexes (PS I and PS II correspondingly), light-harvesting complex II (LHC II), cytochrome b6-f complex, and ATP synthetase complex (1). These proteins are responsible for light harvesting, electron transport, proton gradient generation, and ATP synthesis. Both photosystem I and ATP synthetase are located in the nonappressed regions of the thylakoid membrane, whereas most of PS II and LHC II are located in the appressed regions. Hence there is a spatial segregation of PS I and PS II complexes. These two complexes are functionally linked with each other through the lateral mobility of plastoquionone pool and plastocyanin. However, under short-term environmental stresses, there is lateral redistribution of some of the complexes between the two regions. Thus, sudden increase of light intensity leads to lateral diffusion of LHC II from the appressed to nonappressed regions (2) and heat stress leads to migration of PS II into the nonappressed regions with concomitant mixing with PS I (3). The efficient conversion of light energy into an electrochemical one and its regulation by this multicomponent protein-complex system is dependent on well-orchestrated direct and indirect lateral interactions among its members. Thus, the knowledge of the diffusion coefficients of these proteins in the plane of the photosynthetic membrane is essential for understanding the dynamic aspects of the energy conversion in the photosynthetic process.

The lateral mobility of proteins and lipids in natural and model membranes was determined by different methods, in particular by fluorescence recovery after photobleaching (FRAP) (4) and electrophoresis of membrane components in the plane of the membrane (5). We used the latter approach of in situ electrophoresis to determine the electrophoretic and diffusional mobilities of PS I complex in the plane of the photosynthetic membrane. The native PS I complex corresponds to 10–12 nm protoplasmic fracture face particles observed on freeze-fracture micrographs of thylakoid membranes (6). PS I consists of a core complex of ~8 nm diameter surrounded by light-harvesting pigment proteins specifically associated with PS I. To monitor the redistribution of PS I particles during and after electrophoresis, we made use of the spatial characteristics of the electrophotoluminescence (EPL) (7–11) originating from PS I. The EPL induced by external electric fields in swollen thylakoids, formed from heat-treated chloroplasts, was shown to originate from PS I only (12). It has recently been shown that the EPL originating from PS I comes only from the

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unilamellar bleb and not from the occasional patches attached to it (13). The EPL originates from the hemisphere of the vesicles at which the induced electrical field destabilizes the photoinduced charge separation (14). The predominant effect of the electric field is at the pole, due to the angular dependence of the induced electric field (11). The study of the electrophoretic and diffusional mobilities of the PS I complex was performed on a macroscopic suspension of hypotonically swollen large thylakoid vesicles, avoiding the necessity to immobilize the vesicles for microscopic visualization as described by Poo (5). These photosynthetic membranes are devoid of extra- and intraskeletal elements and lack internalization (shedding) processes, and thus offer a model system to assess the electrophoretic and diffusional mobilities, which are unrestricted by the cytoskeletal elements.

EXPERIMENTAL PROCEDURES

Broken (class C) chloroplasts from pea (*Pisum sativum*), and tobacco (*Nicotiana tobacum*) were prepared according to Avron (15). The chloroplasts were resuspended in a medium containing 0.4 M sucrose and 10 mM Tris (pH 7.5) and stored in the same medium supplemented with 30% (vol/vol) of ethylene glycol at liquid nitrogen temperature (16). The chloroplasts (6 mg/ml chlorophyll) were heat-inactivated for 3 min at 51°C and then diluted by 1:500 with distilled water adjusted to pH 7.7 with Tris buffer. Large thylakoid vesicles (blebs) were formed by the swelling process under the hypotonic conditions. Their size distribution was determined as previously described (11).

Electrophotoluminescence measurements were performed as previously described (11). Voltage pulses were applied by a pair of parallel stainless steel electrodes with an adjustable gap. The experimental protocol consisted of preillumination with a 120-ms light pulse filtered by a 4-96 glass (Corning Glass Works, Corning, NY) (approximate wavelength band, 390–600 nm). After a darktime of 10 ms the external electric field pulse was applied and the resulting luminescence, filtered by a model RG 665 cut-off filter (Schott Glass Technologies Inc., Duryea, PA), was monitored on a fast oscilloscope (model 2430A, Tektronix, Inc., Beaverton, OR) interfaced to a compatible IBM PC computer. In all cases the amplitude of a particular EPL signal was taken at its maximum.

Before opening the illumination shutter, we applied a long electric prepulse train on the same electrodes used for the electric stimulation of luminescence. Thus, we were able to study the effect of electric prepulses on the EPL signal. To avoid electrode polarization, the electrophoretic driving force consisted of a train of 1-ms-long electrical pulses at a relatively high frequency (usually 200 pulses per second). To detect possible polarization of the electrodes, we continuously monitored the current shape and amplitude produced during the prepulse. Unless otherwise mentioned, the amplitude of the prepulses was 60 V/cm. The procedure is schematically described in Fig. 1.

In a first series of experiments, the vesicle suspension (0.8 ml) was put in a 1-cm-pathlength spectrophotometric cell, the suspension was adjusted to pH 7.7 with Tris buffer, the parallel electrodes spaced 2 mm apart were inserted, and the sample was allowed to equilibrate at 10°C for 5 min. For reference, the nonprepulsed EPL was measured. After this EPL measurement the sample was allowed to equilibrate in the dark for 60 s. Then a train of 1-ms prepulses, 4 ms apart, was applied for time periods of 1–8 s. After the prepulse, the new EPL amplitude was

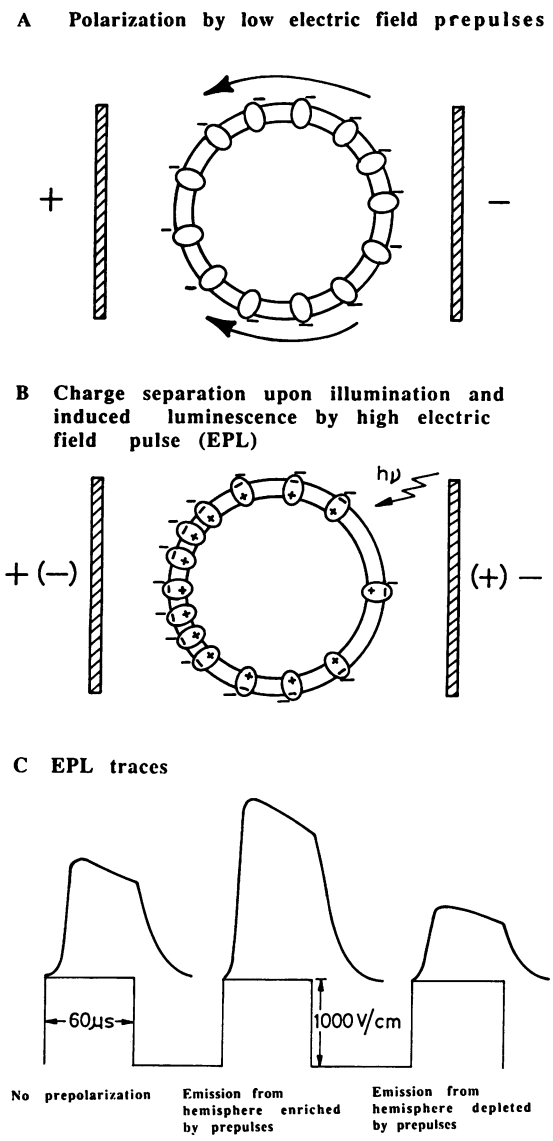

FIGURE 1 Scheme of the experimental procedure. (*A*) Electrophoretic propagation of PS I particles along the surface of the vesicle, causing accumulation on one pole and depletion on the other one. (*B*) EPL from depleted or the enriched (electrode signs in brackets) hemispheres. (*C*) EPL traces: (*a*) without prepulse, (*b*) EPL from the enriched hemisphere; (*c*) EPL from depleted hemisphere.

measured. The amplitude of the EPL signal was studied as a function of the length of the prepulse and its amplitude. After each measurement of the prepulse influence on EPL, the thylakoid vesicle sample was replaced with a fresh one.

In a second series of experiments, after a 5-s-long prepulse train, the relaxation due to the lateral diffusion was measured in low- and high-viscosity media. The high-viscosity media were obtained by performing the swelling in a 4% (wt/vol) dextrane solution (Sigma Chemical Co., St. Louis, MO, M = 500.000), which had a viscosity five times higher than that of water but did not alter the osmotic conditions.

Light scattering measurements (at right angles both to the directions

of the electric field and illumination) were carried out to study the effect of the prepulse alone on the investigated systems. This measurement was done by changing the emission cut-off filter (RG 665) to a Corning 4-96 glass when applying the electric field under constant illumination. The rate of increase of scattering upon exposure to an electric field pulse and its relaxation when the field is terminated was recorded on an oscilloscope and a recorder.

RESULTS

Fig. 2 shows the relative changes in EPL and their dependence on prepulse amplitude, duration, and polarity. The relative change in EPL is defined as the difference between the EPL in the presence of the prepulse (EPL) and in its absence (EPL_0), divided by EPL_0. For parallel polarization, namely the same polarities of the prepulse and the luminescence stimulating pulse, we observe a decrease in the EPL while the EPL increases with prepulse when in the antiparallel direction. These effects increase with the prepulse duration until saturation is approached. The saturation time decreases as the field strength increases, varying from integrated polarization time of ~0.5 s at 60 V/cm to ~0.8 s at 40 V/cm. Taking into account that PS I has to move during this time a distance of the order of 10^{-3} cm (average particle diameter of 10^{-3} cm), we obtain values of the order of $3 \cdot 10^{-5}$ $cm^2V^{-1}s^{-1}$.

The EPL signal increases with the amplitude of the prepulse up to 60 V/cm. At ~80 V/cm a maximal effect is observed after which increasing the prepulse amplitude causes decrease in the relative change of the EPL. Fig. 3 shows the relative change in EPL as a function of prepulse amplitude for prepulse duration of 2 and 3 s. The results may suggest that at high fields the electrophoretic currents induce convective surface turbulence abolishing the concentration gradients. This phenomenon seems to start around 80 V/cm. Fig. 4 shows that a fivefold increase in the aqueous viscosity does not affect the electrophoretic mobility of the photosystem. The time dependence of EPL enhancement on one pole and its lowering on the other one is the same in the presence and absence of dextran. Thus, one may conclude that the electrophoretic mobility is a function of the hydrodynamic properties of the membrane only.

Fig. 5 illustrates the relaxation of EPL after turning off the electrical field at 40 and 80 V/cm. The field free diffusional relaxation at 80 V/cm is also shown in the

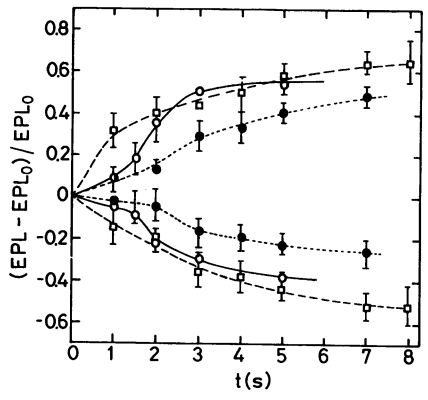

FIGURE 2 Accumulation of PS I on one pole (the relative change in EPL, $[EPL - EPL_0]/EPL_0$, obtains positive values when the EPL-stimulating pulse is in opposite direction to the prepulse) and its depletion on the other pole ($[EPL - EPL_0]/EPL_0$ obtains negative values when the EPL-stimulating pulse is in the parallel direction to the prepulse) as a function of pulse train duration time t. EPL_0 is the electric field–stimulated luminescence without prepulse train. EPL is the electric field–stimulated luminescence in the presence of the prepulse train. Prepulse amplitudes: (●) 40 V/cm, (□) 60 V/cm, and (○) 80 V/cm. Prepulse train characteristics: pulse length, 1 ms; pulse spacing, 4 ms (200 Hz).

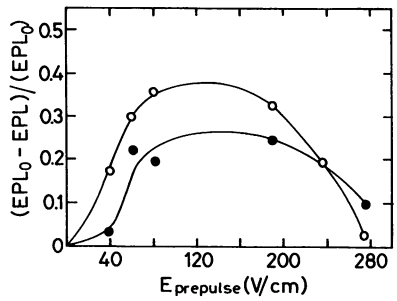

FIGURE 3 Degree of depletion of PS I from one of the poles (obtained when the EPL stimulating pulse is in the same direction as the prepulse) as a function of prepulse amplitudes. Prepulse train duration: (●) 2 s, (○) 3 s.

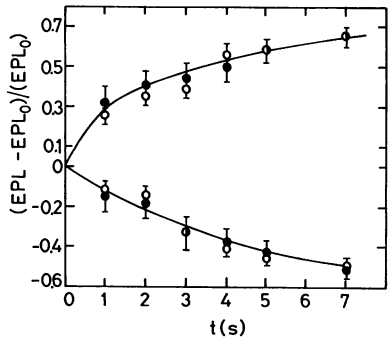

FIGURE 4 Accumulation of PS I on one pole (EPL inducing pulse and prepulse antiparallel) and its depletion on the other one (EPL inducing pulse and prepulse parallel) as a function of prepulse train duration time. Prepulse field amplitude, 80 V/cm. (○) In presence of 4% dextran; (●) in its absence.

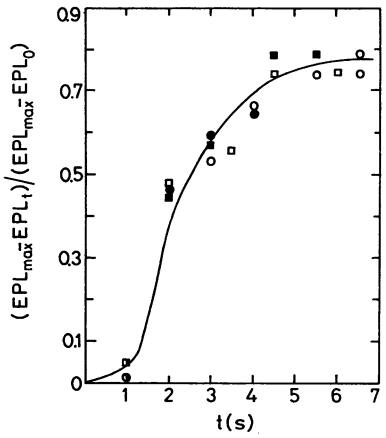

FIGURE 5 Diffusional relaxation of prepolarization. Time course of repopulation of the depleted poles by PS I. The depletion accomplished by prepulses of the following amplitudes: (○) 40 V/cm, (●) 60 V/cm, (□) 80 V/cm in the absence of dextran; (■) 80 V/cm in the presence of 4% dextran.

presence of dextran. The time dependence of the EPL after termination of the prepulse indicates that the diffusional recovery is independent of the polarization field intensity or of the viscosity of the solution. Because the effective radius of the vesicles producing most of the EPL signal is $\sim 5 \cdot 10^{-4}$ cm, the diffusion coefficient corresponding to relaxation time of 90 s, obtained from Fig. 5, is of the order of $4 \cdot 10^{-9}$ cm^2/s. The effect of the frequency of the short pulses (between 100 and 400 Hz; 9–1.5 ms spacing between the 1-ms pulses) in a prepulse train is negligible when comparing integrated net pulse durations.

The effect of vesicle concentration on the relative EPL changes is shown in Fig. 5. Its decrease in the parallel configuration of the prepulse and the stimulating pulse is independent of concentration at dilutions higher than 1/500. This EPL decrease vanishes gradually as the concentration increases (dilutions 1/200 or less). At still higher concentrations (1/100 dilution) we observe an augmentation of the EPL signal instead of a depression. At an antiparallel configuration, the measured EPL values are further increased (not shown).

We suggest that the deviations observed at high vesicle concentrations may be due to vesicle aggregation. Light scattering of unpolarized dilute vesicle suspensions (1/500 and 1/1,000 dilution) does not change with time. When 60 V/cm prepulses are applied the scattering increases by up to 5%. It decreases back when the pulse is off but to a higher value than the initial scattering. Thus a series of pulses cause a consecutive increase in scattering. This effect is more pronounced at high vesicle concentrations.

DISCUSSION

Generally, EPL is explained by an enhancement of delayed luminescence of photosystem I due to an increase of induced charge recombination rate in one hemisphere of a swollen thylakoid by the electric field after preillumination (14). Light illumination produces radial charge separation where the donor is located on the inside of the membrane and the acceptor on the outside, creating an array of dipoles in the membrane (negative outside). Stimulation of delayed luminescence occurs whenever the imposed local electric field has the same direction as the light-induced dipole. Application of an external electric field to a suspension of vesicles induces a transmembrane field with an azimuthal angular dependence. Thus, it is parallel and antiparallel to the photo-induced dipoles in the two hemispheres, destabilizing and stabilizing respectively the light-induced charge separation. As a consequence, an applied d.c. pulse of a certain polarity induces electrophotoluminescence only on the hemisphere closer to the negative electrode. The intensity of the luminescence is proportional to the concentration of the photoemitters around the pole of the hemisphere at which the externally electric field applied after illumination induces EPL. During the application of the prepulse (before illumination and the consecutive EPL-stimulating electric field) the photoemitters accumulate on the anodic side of the vesicle, which means that they are more negative than the rest of the membrane surface (17). Thus, EPL augmentation with respect to those vesicles not exposed to the prepulse is obtained when the prepulse direction is opposite to that of the EPL stimulating field and vice versa for EPL suppression.

The stimulating electric field lowers the activation energy of charge recombination and affects EPL exponentially. The local electric field E_m induced at angle θ in the spherical membrane of thickness d, for high membrane resistance compared with the solution resistivities inside and outside a vesicle of radius r, is (11)

$$E_m = 1.5\, E \frac{r}{d} \cos\theta. \quad (1)$$

The major contribution to EPL will be by the emitters on the poles of the large vesicles. In our case the radius of these vesicles was $\sim 5 \cdot 10^{-4}$ cm. Vesicles of this size should rotate very slowly, according to Stokes-Einstein relations with a rotational time constant of ~6–7 min.

Upon application of a direct electric field pulse on a suspension of vesicles containing charged surface components, electrophoretic mobility is induced. Vesicles move with respect to the solution and each charged component on the vesicular surface moves with respect to its surface environment as previously discussed (17).

Uniform electric field E in a conducting fluid is distorted near the cell and the tangential field at the cell surface E_θ producing the electrophoretic driving force is

$$E_\theta = fE \sin \theta, \tag{2}$$

where f is a numerical factor representing the field distortion (1.5 for a nonconducting sphere) and θ is the polar angle. This electrophoretic driving force propels the negatively charged PS I complex along the membrane surface until opposing forces, e.g., diffusional, electrostatic, or elastic, stop the process. The native PS I complex corresponds to the 10–12 nm in diameter particles observed on freeze-fracture micrographs of thylakoid membranes (6). It consists of a PS I complex of ~8 nm in diameter surrounded by light-harvesting pigment proteins associated with it. For the case where only back diffusion counteracts their electrophoretic mobility, the flux of these charged membrane components across a unit length of a circle at angle θ is (18–20)

$$(dN/dt)_\theta = m\Gamma_\theta f \cdot E \sin \theta - \frac{D}{r}\frac{d\Gamma_\theta}{d\theta}, \tag{3}$$

where Γ_θ is the surface concentration of the components at angle θ, m and D are the electrophoretic mobility and the diffusion coefficient of the moving components. At equilibrium $dN/dt = 0$, and

$$mfE_\theta \sin \theta \, d\theta = \frac{D}{r} d \ln \Gamma_\theta. \tag{4}$$

After integration one obtains a simple expression for the ratio of the equilibrium concentrations at the two poles,

$$(\Gamma_\pi/\Gamma_0)_{eq} = \exp\left(\frac{2fEmr}{D}\right). \tag{5}$$

Saturation of the electrophoretically driven redistribution of PS I particles seems to be reached after a prepulse of 2.5 s duration at 60 V/cm and after ~4 s at 40 V/cm. This corresponds to net pulse times of 0.5 and 0.8 s, yielding ratios of $(\Gamma_\pi/\Gamma_0)_{eq}$ which are ~5 and 2.4, respectively. The values of (mr/D) then become, according to Eq. 5, 0.011 and 0.013 V/cm, respectively. This enables us to evaluate the electrophoretic mobility m, if we can determine the diffusion coefficient D independently. D can be obtained from the diffusional relaxation as shown in Fig. 5, and it is shown in Fig. 6 to be $\sim 4 \cdot 10^{-9}$ cm^{2}s^{-1}. The value of m from Eq. 6 comes out to be $\sim 3 \cdot 10^{-7}$ cm^{2}V^{-1}s^{-1}, which is two orders of magnitude smaller than the m value estimated from the prepulse duration needed to yield saturation. Eq. 5 may, however, be incorrect if forces other than diffusional counteract the electric driving force (e.g., electrostatic and elastic forces).

The diffusion coefficient can be calculated from the diffusional relaxation by solving the diffusion equation for the initial concentration distribution determined by conditions at the end of the prepulse train. The initial surface concentration distribution is obtained by introducing the conservation of mass boundary condition.

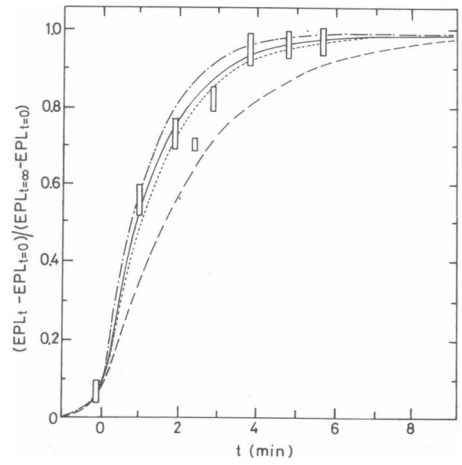

FIGURE 6 Diffusional relaxation calculated by Eq. 9 taking EPL_t to be proportional to $\Gamma_{\theta=0,t}$ (at the depleted poles of the vesicles) for the following values of D and α: (---) $D = 3 \cdot 10^{-9}$, $\alpha = 0.375$; (· · ·) $D = 5 \cdot 10^{-9}$, $\alpha = 0.8$; (——) $D = 5 \cdot 10^{-9}$, $\alpha = 0.375$; (– · – · –) $D = 6 \cdot 10^{-9}$, $\alpha = 0.375$. The rectangles encompass the experimental points from Fig. 4 after multiplying the ordinate by 1.25 to account for the incomplete recovery by setting $EPL_{t=\infty} = EPL_0$ (without prepulse).

$$\sum_0^\pi \Gamma_\theta \sin \theta \, d\theta = \frac{2}{4\pi r^2} \Gamma_{E=0}, \tag{6}$$

into the differential Eq. 4, yields for the initial conditions ($t = 0$) of the diffusional relaxation.

$$\Gamma_\theta = \Gamma_{E=0} 2\alpha \exp \alpha(1 - \cos \theta)/(\exp 2\alpha - 1), \tag{7}$$

where $\alpha = fEmr/D$.

The initial concentration distribution obtained from reference 5 for the different values of α can be obtained from Eq. 5 for different values of E. The equation for the diffusional relaxation is given by Eq. 8:

$$\frac{\partial \Gamma(\theta, t)}{\partial t} = \frac{D}{r^2 \sin \theta} \frac{\partial}{\partial \theta}\left[\sin \theta \frac{\partial \Gamma(\theta, t)}{\partial \theta}\right]. \tag{8}$$

Solving Eq. 8 according to Poo (5, 21) yields

$$\Gamma(\theta, t) = \sum_{\ell=0}^{\infty} \frac{2\alpha \exp \alpha(1 - \cos \theta)}{\exp 2\alpha - 1} \cdot K_\ell P_\ell(\cos \theta) \exp\left[-D\ell(\ell + 1)t/r^2\right], \tag{9}$$

where $P_\ell(\cos \theta)$ is the Legendre polynomial of the order ℓ and K_ℓs are constant coefficients. Solution of Eq. 9 with approximations similar to those adapted by Poo renders

an exponential dependence of $(\Gamma_{\theta-0} - \Gamma_{E-0})$ on time which is depicted for different values of D in Fig. 6. The 1-min lag period obtained experimentally is not reproduced by calculations carried out for different values of D and α. The curve representing the experimental points is shifted by 1.2 min along the time coordinate as shown in Fig. 6.

The lag period, before back diffusion starts, is just like the whole diffusion time course, practically identical for all the prepulse amplitudes. It is also independent of the viscosity of the aqueous solution. It is possible that the photosystems aggregate when compressed under the influence of the electrical field. The lag time could, in this case, be identified with disaggregation time. Because contact between particles is a necessary condition for aggregation, elastic as well as electrostatic forces are probably invoked to stop electrophoretic motion. The back diffusion coefficient of the aggregated units should be significantly lowered, and the considerations leading to Eq. 5 cannot be correct. These aggregates have to dissociate before back diffusion can start, after the removal of the electric field. As evident from Fig. 7, the recovery after the lag period corresponds to a diffusion coefficient D of $\sim 5 \cdot 10^{-9}$ cm^2s^{-1}. This value of D is just an approximation because the initial conditions, based on Eqs. 4 and 6, may not be real. This value for the lateral mobility of PS I is higher by three orders of magnitude than the previously estimated lateral diffusion of photosystem II pigment protein complex (22). From an analysis of the temperature dependence of the fluorescence changes, induced by the addition of $MgCl_2$, assumed to result from PS II particle aggregation (22), obtained estimates of $2 \cdot 10^{-12}$–$3 \cdot 10^{-11}$ cm^2s^{-1} for the lateral

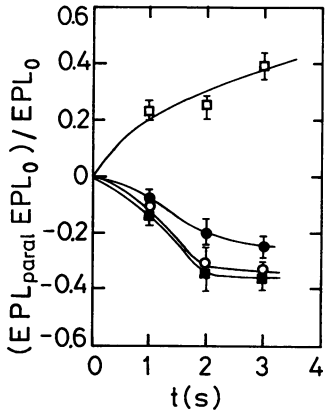

FIGURE 7 The relative change in EPL (the EPL stimulating pulse and the prepulse are parallel) as a function of the prepulse train duration time at different concentration of the vesicles. The different concentrations obtained by different dilution of the thylakoid vesicle preparation (6 mg/ml chlorophyl): (■) 1/1,000, (○) 1/500, (●) 1/200, (□) 1/100.

diffusion coefficient of PS II (in the temperature range of 10–30°C). Because the molecular dimensions of the two photosystems are of the same order to magnitude, we would expect them to possess similar lateral mobilities. The differences may arise from the different phenomena measured and the models employed in ours and others' previous work (22). It is unlikely that the difference would stem from working with swollen and unswollen thylakoids, respectively, as the membrane fluidity is not affected by swelling.

The experimental diffusional relaxation curves are nearly independent of the amplitude of the polarizing prepulse. This indicates that the initial conditions as determined by the concentration distribution on the vesicle surface are similar when saturation has been reached at polarizing field strengths between 40 and 80 V/cm or that the diffusional relaxation is only slightly dependent on m. The variation of medium viscosity by the addition of dextran did not affect the polarization rate or the diffusional relaxation pattern indicating the negligible contribution of the surrounding solvent viscosity to both processes. Furthermore, it also shows that the rotational diffusion of those vesicles contributing to the EPL signal is too slow to affect the results. Lateral diffusion by itself seems to yield only ~80% of the EPL recovery, which is in keeping with the incomplete fluorescence recovery after photobleaching (4), and with the possibility that aggregation is not completely reversible. At 80 V/cm the approach to saturation is relatively faster than at lower field strengths. When the polarizing electric field prepulses are further increased, the assymetric distribution of PS I starts decreasing. There is a possibility that the surface becomes structured or that eddy currents are induced in the surface by the electric field. The idea of field-induced eddy currents is supported by the fact that at polarization fields higher than 200 V/cm no EPL enhancement or depression is obtained (Fig. 2). The vigorous currents can stir the surface, equalizing the surface concentration over the whole surface.

There is a relation between the translational diffusion coefficient D and the electrophoretic mobility through the functional resistance or its reciprocal, namely the translational mobility. Saffman and Delbrueck (21) have shown that translational diffusion coefficient of a cylindrical molecule embedded perpendicularly in a lipid membrane can best be expressed by

$$D_T = \frac{kT}{4\pi\eta_m h}\left(\log\frac{\eta_m h}{\eta_w a} - \gamma\right) = kT \cdot b_T, \qquad (10)$$

where η_m and η_w are the viscosities of the membrane and the aqueous solution, respectively, a is the radius of the cylindrical molecule, h is height corresponding to the thickness of the membrane, and γ is Euler's constant

(0.5772). For $D_T = 5 \cdot 10^{-9}$ cm^{-2}s^{-1}, b_T is $1.25 \cdot 10^5$ g^{-1}s.

To determine the electrophoretic mobility we have to take into account also the hydrodynamic resistance of aqueous phase across the electrical double layer. At steady state the electric driving force is balanced by the hydrodynamic resistance in water and the membrane.

$$F_\theta q = \eta_w \frac{v}{\delta} 4\pi a^2 + 4\pi\eta_m h u / \left(\log \frac{\eta_m h}{\eta_w a} - \gamma\right), \qquad (11)$$

where δ is $1/\kappa$ (the thickness of the double layer), v is the velocity of the molecular surface of the moving particle with respect to the interior of the aqueous solution. u is its velocity with respect to the membrane, $u = v - v_o$ where v_o is the velocity of the vesicle.

The charge q can be replaced by the charge density $\sigma = \zeta/4\pi a^2$, or by the ζ potential. $\zeta = 4\pi\sigma\delta/\epsilon$, where ϵ is the dielectric constant.

Eq. 11 can be rewritten in terms of mobility of the molecule in the membrane $m_p = u/E_\theta$ and of the vesicle in the solution $m_v = v_o/E_\theta$.

$$q = \eta_w \frac{4\pi a^2}{\delta} m_v + m_p\left[\eta_w \frac{4\pi a^2}{\delta} + 4\pi h\eta_m / \left(\log \frac{\eta_m h}{\eta_w a} - \gamma\right)\right]. \qquad (12)$$

m_v is of the order of 10^{-4} cm^{2}V^{-1}s^{-1}, m_p estimated from the polarization time at different pulse amplitudes is $\sim 3 \cdot 10^{-5}$ cm^{2}V^{-1}s^{-1} (300 times higher in statvolts). Taking $h = 3 \cdot 10^{-7}$ cm, $\delta = 10^{-6}$ cm, $a = 5 \cdot 10^{-7}$ cm, $\eta_w = 10^{-2}$ poise, and $\eta_m = 1$ poise, then q the charge per moving particle calculated from Eq. 12 is ~ 11–$14 \cdot 10^{-9}$ esu, or ~23–30 electron charges.

This estimated number of charges is a rough approximation based on the choice of the membrane thickness and its viscosity and on the particle radius a. Taking the value of $1/b_T$ from the diffusion coefficient to be $8 \cdot 10^{-6}$ g s^{-1}, we obtain for q the value of $12 \cdot 10^{-9}$ esu or ~150 electron charges. This is an extremely large number of charges per molecular complex even if it contains a relatively large number of protein units. It is of course possible that the particles move a shorter distance, e.g., 5 μm before the counteracting forces terminate the electrophoretic movement. This would correspond only to half the mobility and only 75 electron charges per PS I complex. However, the discrepancy between the charges calculated from the estimated values of h, η_m, and a on one hand, and from the b_T derived from the diffusion coefficient on the other hand, still remains. There is a possibility that the prepulse field strength (40 V/cm or higher) induces aggregation of the charged particles forming pearl chain structures in the surface. The combined charge of these aggregates may be responsible for the high electrophoretic mobility.

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