Electroporation of the Photosynthetic Membrane: Structural Changes in Protein and Lipid-Protein Domains

Yosef Rosemberg, Michal Rotenberg, and Rafi Korenstein
Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, 69978 Tel Aviv, Israel

ABSTRACT A biological membrane undergoes a reversible permeability increase through structural changes in the lipid domain when exposed to high external electric fields. The present study shows the occurrence of electric field-induced changes in the conductance of the proton channel of the H^+-ATPase as well as electric field-induced structural changes in the lipid-protein domain of photosystem (PS) II in the photosynthetic membrane. The study was carried out by analyzing the electric field-stimulated delayed luminescence (EPL), which originates from charge recombination in the protein complexes of PS I and II of photosynthetic vesicles. We established that a small fraction of the total electric field-induced conductance change was abolished by *N,N'*-dicyclohexylcarbodiimide (DCCD), an inhibitor of the H^+-ATPase. This reversible electric field-induced conductance change has characteristics of a small channel and possesses a lifetime $\leq$ 1 ms. To detect electric field-induced changes in the lipid-protein domains of PS II, we examined the effects of phospholipase A_2 (PLA_2) on EPL. Higher values of EPL were observed from vesicles that were exposed in the presence of PLA_2 to an electroporating electric field than to a nonelectroporating electric field. The effect of the electroporating field was a long-lived one, lasting for a period $\geq$ 2 min. This effect was attributed to long-lived electric field-induced structural changes in the lipid-protein domains of PS II.

INTRODUCTION

Short exposure of cells or membrane vesicles to a high external electric field leads to a reversible transient increase in the permeability of the membrane to ions and molecules. This process, known as electroporation (for a review see Zimmermann, 1982; Tsong, 1983, 1991), has been attributed to the formation of structural defects mostly in the lipid domain of the cell membrane. The theoretical approach toward the formation of aqueous pores in the membrane (Abidor et al., 1979; Powell et al., 1986; Glaser et al., 1988), or to the induction of structural defects in it (Sugar and Neumann, 1984, Sugar et al., 1987), has been confined to electric field changes in the lipid domain. However, given that biological membranes contain integral proteins as a major component, possible electric field-induced permeability and structural changes both in protein and lipid-protein domains should also be considered. Indeed, the ability of external electric fields to affect the conformation and activity of membrane proteins is supported by numerous studies (for a review see Tsong and Astumian, 1987; Tsong, 1989). Thus, electric field-driven ATP synthesis by proton ATP synthetase (H^+-ATPase) was demonstrated in chloroplasts (Witt et al., 1976; Vinkler and Korenstein, 1982; Vinkler et al., 1982) and in submitochondrial particles (Teissie et al., 1981; Hamamoto et al., 1982). Furthermore, low electric fields were found to affect ion transport by the Na^+-K^+ ATPase in erythrocytes (Serpersu and Tsong 1983, 1984; Blank and Soo, 1990; Liu et al., 1990) and in reconstituted membrane vesicles (Rephaeli et al., 1986). In addition, electric fields were found to affect the light-driven proton pump (bacteriorhodopsin) (Shinar et al., 1977; Tsuji and Neumann, 1981; Brumfeld and Miller, 1988) and to modulate electron transfer in chloroplasts (Arnold and Azzi, 1971; Elleson and Sauer, 1976) and in photosynthetic bacterial reaction centers (Gopher et al., 1985). Moreover, it was suggested that exposure of erythrocytes to a high electric field opened a channel through the Na^+,K^+-ATPase (Teissie and Tsong, 1980). Thus, it is expected that electric field-induced conformational changes of membrane proteins may be accompanied by conductance changes occurring in the protein domain itself or taking place in the domain of the lipid-protein interface.

To monitor electric field-induced structural and conductance changes in the protein and lipid-protein domains of the photosynthetic membrane, we measured changes of electrophotoluminescence (EPL) from photosynthetic vesicles exposed to high electroporating electric fields. Electrophotoluminescence is observed when exposing preilluminated photosynthetic vesicles to external electric fields. It is explained by an enhancement of charge recombination in photosystem (PS) I and PS II by external electric fields, and its features have been described previously (Farkas et al., 1984a; Rosemberg et al. 1992).

The EPL was used, on the one hand to probe the photosynthetic apparatus and on the other as a voltage-sensitive optical probe, in exploring the mode of interaction of an external electric field with a vesicular membrane. Thus, by examining EPL, two independent EPL signals could be associated with PS I and PS II (Symons et al., 1984, 1985, 1988; Vos and van Gorkom, 1988). The study of EPL revealed some thermodynamic features of electron transport in PS I and PS II (van Gorkom et al., 1986; Vos and Van Gorkom, 1988, 1990) and established the electrophoretic and diffusional mobilities of PS I in the photosynthetic membrane (Brumfeld et al., 1989). Moreover, by using the EPL as an

Received for publication 17 September 1993 and in final form 22 June 1994.
Address reprint requests to Dr. R. Korenstein, Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, 69978 Tel-Aviv, Israel. Tel.: 972-3-6409139; Fax: 972-3-6409113.

0006-3495/94/09/1060/07 $2.00

intrinsic voltage-sensitive optical probe, the electrical properties of the photosynthetic membrane were examined (Farkas et al., 1984a), electroporation characteristics of the photosynthetic membrane were explored (Farkas et al., 1984b; Korenstein et al., 1984; Rosemberg and Korenstein, 1990b), and ionophore-mediated ion transport was studied (Farkas et al., 1982; Rosemberg and Korenstein, 1990a.

The present study employs the unique features of the EPL, which depend both on photosynthetic activity and on the conductance of the photosynthetic membrane, to elucidate structural changes both in protein and the lipid-protein domains imposed by high electric fields.

MATERIALS AND METHODS

Materials

Broken chloroplasts were prepared from spinach, pea, or tobacco according to methods described by Avron (1960). The broken chloroplasts were stored at −180°C to preserve their photosynthetic activity for a long period (Farkas and Malkin, 1979). In every set of experiments the concentrated thylakoids were thawed at room temperature. In EPL measurements involving the electric field-induced conductance change through the channel of the H^+-ATPase the thylakoids were incubated at 50°C for 3 min. This heat inactivation was performed to deplete the thylakoids from most of their PS II-related EPL activity while preserving the PS I-related activity (Symons et al., 1984, 1985). The employment of PS I-associated EPL (Fig. 1) is superior to that of PS II in measuring conductance changes, given that the PS I-associated EPL is more sensitive to an external electric field (Symons et al., 1984). After heat inactivation, the thylakoids were resuspended in a hypotonic buffered solution. The incubation in a hypotonic solution initiates a swelling process that yields spherical vesicles known as swollen thylakoids. Inasmuch as these vesicles are formed even in extreme hypotonic medium (e.g., distilled water), we have to assume the abolishment of the initial osmotic difference during and/or after the swelling process. These vesicles, composed of a single membrane with occasional patches of unswollen thylakoid fragments on it, have a size distribution of radii of 1–10 μm, with an average radius of 4 μm. The analysis of size distribution was performed by microscopic visualization. The suspension of swollen thylakoids was cooled down to 4°C after swelling for 15 min at room temperature. The concentration of the stored stock of broken thylakoids was 6–10 mg/ml, and they were diluted 1000-fold in 1 mM Tris-HCl buffer pH 8.0 (about 4×10^6 vesicles/ml). EPL associated with PS II was employed to monitor electric field-induced structural changes in the lipid-protein domain. To obtain EPL, which is associated mostly with PS II, the experiments were carried out in the presence of 150 μM methyl viologen (Sigma Chemical Co., St. Louis, MO). Methyl viologen was shown to abolish PS I-associated EPL (Symons et al., 1984, 1985). The preparation of thylakoid vesicles in this case followed the same procedure as for PS I-associated experiments, with the omission of the heat inactivation step.

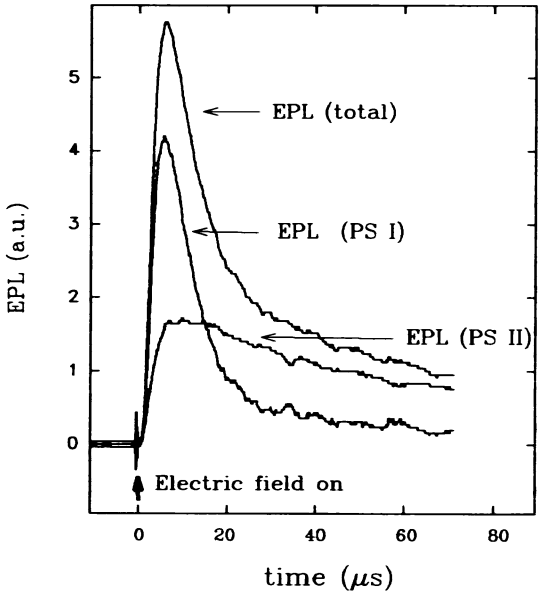

FIGURE 1 Characteristics of EPL. EPL was obtained from swollen thylakoids suspended in 1 mM Tris, pH = 8.0 and exposed to an external electric field of 1600 V/cm. The external electric field (see *arrow*) was applied 230 ms after the termination of a 120-ms preillumination. Time-dependent trace of the total EPL signal is composed of a mixture of PS I- and PS II-associated EPL. The segregation of the two components of EPL (PS I- and PS II-related EPL) was achieved by the addition of 150 μM methyl viologen, which suppresses PS I-associated EPL and leaves intact the PS II-associated EPL. A mathematic substraction of the PS II-associated EPL from the total EPL yielded the PS I-associated EPL.

Measurement of electrophotoluminescence

The experimental setup for EPL measurements was described elsewhere (Farkas et al., 1984a). The experiment was initiated by preillumination of 120 ms with a light projector. The light was filtered by a 4–96 glass filter (Corning Glass Works, Corning, NY) limiting the exciting wavelength to approximately 400–600 nm. After an appropriate dark time (230 ms in studying the conductance change through the H^+-ATPase, 50 ms in PS II activity measurements), an external electric field pulse was applied. The resultant electric field-induced luminescence was filtered by a model RG 665 cutoff filter (Schott Glass Technologies Inc., Duryea, PA) and 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 (Fig. 1). The electric field pulse was delivered by a high voltage pulse generator (model 360, Velonex, Santa Clara, CA) capable of delivering voltage pulses of 200–2500 V. The applied pulse shape, intensity, and kinetics were monitored by use of an inductive current probe. All experiments were carried out at a temperature of 5°C maintained by a thermostated Techne RB-12 bath (Techne, Princeton, NJ).

Measurement of electroporation based on EPL

Exposure of membrane vesicles to a homogeneous electric field results in the induction of a local electric field in the membrane. The induced local electric field in the membrane has been calculated for spherical vesicles by solving Laplace's equation with the appropriate boundary conditions (Farkas et al., 1984a; Ehrenberg et al., 1987). For the case where the specific conductivities of the inner and outer media, (λ_i and λ_o, respectively) are equal and where the vesicle's radius (R) is much bigger than membrane thickness d one obtains the following expression for the time-independent induced electric field (Em):

$$Em = \frac{3(R/d) \cdot \cos\Theta \cdot Eex}{2+3(R/d) \cdot (\lambda_m/\lambda_o)} \quad (1)$$

Where Eex is the externally applied electric field intensity and Θ is the angle between the applied electric field direction and the radius vector of the vesicle to a certain point on the membrane where the local electric field is induced. It was previously shown (Farkas et al., 1984a; Symons et al., 1984; Rosemberg and Korenstein, 1990a,b) that the EPL signal as a whole is not a linear function of the Eex, but it can be taken as such in the range between 1000 V/cm to 2000 V/cm (correlation coefficient >.95). Given that the EPL is a function of Em, it depends on changes of membrane conductance (λ_m) caused by the electroporation process.

Reversible electric breakdown of the photosynthetic membrane was carried out by exposing a suspension of swollen thylakoids to two consecutive high electric fields of identical strength and duration but of opposite polarity. A homebuilt device capable of delivering bipolar pulses (two DC pulses of the same voltage and duration but of opposite polarity) was described previously (Korenstein et al., 1984; Rosemberg and Korenstein, 1990b). Basically, the bipolar pulses were obtained from the high-voltage pulse generator using a homemade pulse-inverted device based on high-speed semiconductor relays. The time-spacing between the two pulses could be varied between 100 μs and 70 ms. As a result of the exposure, we observed two EPL signals where the second signal was attenuated in comparison with the first one. We have shown that the attenuation in the second EPL signal to a transient change in membrane conductance results from a reversible electric breakdown that takes place during and after the first pulse (Korenstein et al., 1984; Rosemberg and Korenstein, 1990b).

Detection of electric field-induced changes in protein and lipid-protein domains

The detection of conductance changes in the protein domain was based on monitoring changes of the PS I-associated EPL. To detect conductance changes through the proton channel of the H^+-ATPase we carried the electroporation experiments in the presence and in the absence of *N,N'*-dicyclohexylcarbodiimide (DCCD, Sigma Chemical Co.), an inhibitor of the H^+-ATPase of photosynthetic membranes. Swollen thylakoids were preincubated in the absence and presence of 20 μM DCCD, in 1 mM Tris-HCl buffer pH 8.0 for 10 min at room temperature in the dark. Under these conditions the conduction through the proton channel of the H^+-ATPase is blocked (Lill et al., 1987). After the preincubation time the vesicles were exposed to a bipolar voltage pulse of 1600 V/cm and pulse duration of 200 μs.

The measurements of electric field-induced changes in the lipid-protein domains of the photosynthetic membrane were based on monitoring changes of the PS II-associated EPL. Experiments employing phospholipase A_2 (PLA_2) were carried out by the addition of 2 μl of 4.4 mg/ml of PLA_2 from pig pancreas (Sigma Chemical Co.) to a suspension of swollen thylakoids also containing 2 mM of $CaCl_2$. When we performed electroporation in addition to exposure to PLA_2, the membrane vesicles were exposed to 10 double-voltage pulses of 1600 V/cm each possessing a duration of 200 μs. The hydrolytic activity of PLA_2 was examined by monitoring the pH decrease that accompanies the hydrolytic reaction (Gheriani-Gruszka et al., 1988).

RESULTS AND DISCUSSION

Electric field-induced conductance change through the proton channel of the H^+-ATPase

Ion channels are one type of integral proteins that are possible candidates to take part in a reversible permeability increase of a membrane upon its exposure to a high external electric field. Their choice as possible protein sites that are involved in electric field-induced conductance change is a natural one, given that they possess open and closed states for ion conduction. Detecting changes in conductance through ion channels under conditions of electroporation is difficult. This is a consequence of the fact that the conductance change through ion channels may be small because of the relatively small size of an ion channel, its ion specificity, and number of copies per cell. Thus, electric field-induced conductance changes through ion channels are expected to be relatively small compared with the large conductance changes caused by pores formed in the lipid domain during electroporation (Rosemberg and Korenstein, 1990b). Inasmuch as there are usually several types of ion channels in a biological membrane, each one contributing differently to the total conductance change, one should study the electric field-induced conductance change in the presence and the absence of a specific ion channel blocker.

The thylakoid membrane possesses an ion channel for protons, which is part of the proton-translocating ATP synthase (H^+-ATPase) known also as the CF_0-CF_1 coupling factor. The H^+-ATPase is composed of two parts: one acting as a proton channel (CF_0) and the other containing the active site(s) for ATP synthesis (CF_1). Chloroplasts have a relatively high turnover of ATP (about 400 ATP/(CF_0-CF_1.s)). This relatively high turnover requires a high conductance of protons through the CF_0 channel. DCCD is a well known inhibitor of the CF_0-CF_1 coupling factor of the thylakoid membrane. It binds covalently to a single acidic residue in the CF_0 channel under conditions of high pH leading to a blockage of the conduction through the proton channel (Sigrist-Nelson et al., 1978). Hence, we examined the behavior of the CF_0 channel in swollen thylakoids under exposure to an external electroporating electric field.

We used the PS I-related EPL of swollen thylakoids as an intrinsic voltage-sensitive optical probe to measure the changes produced in membrane conductance. Electroporation of the thylakoid membrane causes attenuation of the EPL signal (Korenstein et al., 1984; Rosemberg and Korenstein, 1990b). This attenuation was attributed to electric field-induced increase of membrane conductance (Rosemberg and Korenstein, 1990b). The increase of membrane conductance leads to a consequent decrease of the induced local electric field in the membrane and hence to a decrease of the EPL signal (Farkas et al., 1982; Rosemberg and Korenstein, 1990a,b). This reversible attenuation ($\approx$50%) decayed exponentially down to 0% from 150 μs to 0.5 s, correspondingly. This decay is a reflection of membrane conductance decrease during the resealing process of the membrane (Korenstein et al., 1984). The attenuation of the EPL signal caused by electroporation of the thylakoid membrane was significantly diminished ($p = 0.003$) by preincubating the thylakoid vesicles with DCCD (20 μM) for 10 min at room temperature (Fig. 2). Under these conditions DCCD was shown to block the proton channel specifically. The higher conductance change, observed in the absence of DCCD as compared with the one obtained in its presence, was restricted only to the time domain up to 1 ms after exposure to the electric field. At longer times (>1 ms) the electric field-induced conductance change in the presence of DCCD did not differ from the one obtained in its absence. These findings suggest that electroporation triggered an opening of the CF_0 channel, which then closed up within 1 ms.

Our detection of conductance changes is based on the response of the EPL signal to the local membrane electric field expressed in terms of a ratio between the two EPL signals elicited by a bipolar pulse. To examine the possibility that the observed effect of DCCD originates also from its possible effect on the magnitude of EPL (i.e., effect on the photosynthetic efficiency, unrelated to a conductance change), we

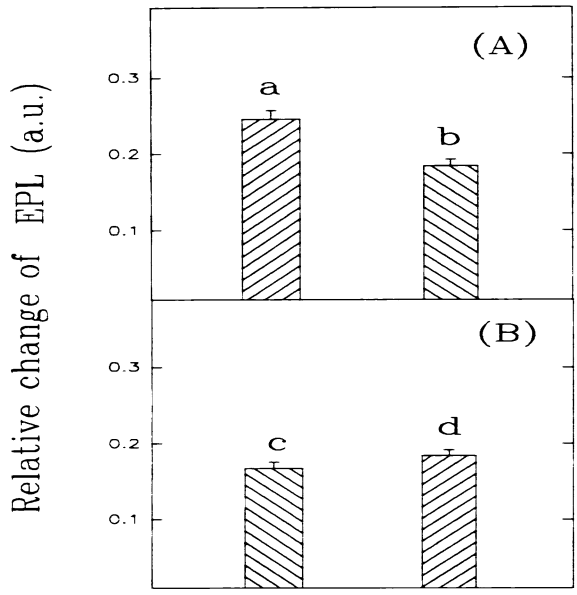

FIGURE 2 Attenuation of the EPL signal upon exposure of a suspension of swollen thylakoid vesicles to two consecutive electric fields of the same strength and duration but of opposite polarity. Difference between suspensions of swollen thylakoids that were incubated in the absence and presence of 20 μM DCCD. The results are given in terms of the relative change, which is defined as the EPL induced by the first pulse of electric field (before electroporation) minus the EPL induced by the second pulse of electric field (after electroporation) divided by the EPL of the first pulse: $EPL_{RCH} = (EPL_{\text{before electroporation}} - EPL_{\text{after electroporation}})/EPL_{\text{before electroporation}}$. A bipolar external electric field of 1600 V/cm and pulse duration of 200 μs was applied. The time between the two pulses was 600 μs. The thylakoid vesicles (~5 μg/ml chlorophyll) were suspended in 1 mM Tris buffer pH 8.0 at 5 ± 1°C. (*A*) Experiments performed in an external medium of low conductance. (*a*) Control, in the absence of DCCD; (*b*) in the presence of DCCD. (*B*) Experiments performed in an external medium of high conductance because of addition of 1 mM of TEACl. (*c*) Control, in the absence of DCCD; (*d*) in the presence of DCCD.

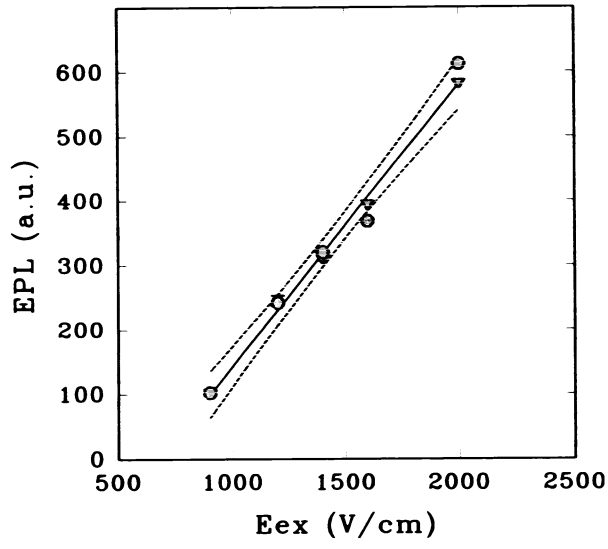

FIGURE 3 Dependence of the maximal amplitude of EPL on external electric field. The EPL signals of control suspensions of swollen thylakoids are represented by closed circles, and suspensions that were preincubated with 20 μM DCCD are represented by triangles. Whenever one of the experimental points, represented by a triangle, is absent, it reflects the complete overlap of the two experimental points. The dependence of EPL on the external electric field strength was fitted to a linear ascending function of the type $f(Eex) = A \cdot Eex + C$. The solid line represents a linear regression of control carried in the absence of DCCD (correlation of 0.99). The broken lines represent a confidence interval of 95% for the control. The experimental conditions are identical to those in Fig. 2.

examined whether the efficiency of EPL was affected by the addition of DCCD. Thus we measured the dependence of the first EPL signal (which is essentially unaffected by the electroporation process) when applying a bipolar pulse in the presence and the absence of DCCD. The EPL signal, as a whole, is not a linear function of the Eex, but it can be taken as such in the range between 1000–2000 V/cm (correlation coefficient >.95). Fig. 3 demonstrates that there is no significant change in the response of the first EPL signal to the externally applied electric field in the presence of DCCD. This finding rules out the possibility that the DCCD affects the efficiency of EPL. It also suggests that DCCD has no major effect on the lipid domain, given that electron transport was shown to be affected by alteration of the lipids in the photosynthetic membrane (Sprague, 1987). Moreover, the preincubation conditions of the thylakoids with DCCD used in our study were such that the proton transport through CF_0 was blocked by the specific reaction of DCCD with Asp^{61} to form a stable *N*-acylurea adduct. Thus it may be concluded that the effect of DCCD on electroporation is due to the blockage of the otherwise electric field-induced opening of the proton channel.

The relative change of the EPL signal caused by the electroporation of the thylakoid membrane is an explicit function of the attenuated local electric field-induced in the membrane rather than of membrane conductance change itself. However, it emerges from Eq. 1 that the local electric field depends on the ratio of λ_m/λ_o. Therefore, the relative change of the EPL depends on the ratio of λ_m/λ_o. Thus, it should be taken into consideration that an elevation of λ_o without changing λ_m leads to a decreased response of the EPL (a voltage-sensitive optical probe) to an increased ion conduction through the channels. This implies that a reduced signal/noise ratio of the optically based assay is expected upon decreasing the ratio of λ_m/λ_o. Hence, if DCCD affects λ_m, the elevation of λ_o by an impermeable ion should decrease its effect on the relative change of EPL. However, whereas if the effect of DCCD is unrelated to the change of membrane conductance, a change of λ_o should have no effect. Indeed, Fig. 2 *B* demonstrates that upon elevating λ_o by adding a large monovalent cation (tetraethylammonium, TEA^+) to the external medium, swollen thylakoids that were preincubated with DCCD show no significant difference from suspensions that were not incubated with DCCD. Similar results were found when a bivalent ion (Ca^{2+}) was used. Thus, the fact that the effect of DCCD on electroporation-dependent attenuation of the EPL signal is mediated by ions that do change λ_o but not λ_m suggests an upper limit to the electric field-induced opening of the proton channel. We may conclude that the electric field-induced opening of the channel is of a

dimension that does not allow the conduction of either TEA^+ or Ca^{2+} through it. Indeed, reconstitution experiments with CF_0 in artificial lipid bilayers have demonstrated that CF_0 loses its selectivity toward proton conduction and is permeable to monovalent cations (Wagner et al., 1989).

Electric field-induced structural changes in the lipid-protein domain of photosystem II

The possibility of detecting structural changes in the lipid-protein domain merely by monitoring electric field conductance changes does not allow assigning any fraction of this conductance change to structural changes in the lipid-protein areas. To examine the involvement of lipid-protein domains in the electroporation process, we made use of the fact that photosynthetic activity of PS II is modulated by a direct interaction with phosphatidylcholine, a minor phospholipid component of the photosynthetic membrane. Inasmuch as phosphatidylcholine is prone to hydrolysis by PLA_2, it affects the rates of electron transport through PS I and PS II (Rawyler and Siegenthaler, 1981a,b).

We examined the effect of PLA_2 on PS II-related EPL in the presence and absence of an electroporating electric field. Incubation of swollen thylakoids with PLA_2 resulted in an immediate inhibition of the PS II-associated EPL. However, when, in addition to the incubation with PLA_2, swollen thylakoids were exposed to an external electric field of amplitude and duration that induced an electroporation of the membrane, the inhibition of the PS II-dependent EPL by the PLA_2 was significantly decreased (Fig. 4, *A* and *B*). The effect of electroporation on the PLA_2-induced inhibition of the PS II-associated EPL signal was similar whether the enzyme was added ≈50 s before or after electroporation by the external electric field (cf. Fig. 4, *A* and *B*).

The activity of the photosystems is reflected by the efficiency of the forward electron transport. In contrast to forward electron transport, EPL reflects backward electron transport through the photosystems induced by an external electric field. Thus, forward electron transfer and EPL are two opposite complementary processes. Stimulation of PS I activity is expected to be associated with a decrease of backward electron flow through PS II, thereby leading to a decrease of the PS II-associated EPL. Indeed, the observed immediate inhibition of PS II-associated EPL by PLA_2 (Fig. 4, *A* and *B*) can be explained by the immediate stimulation of PS I activity upon the addition of PLA_2 (Rawyler and Siegenthaler, 1981a).

Along the same line of reasoning, the inhibition of the PS II-related forward electron transfer, caused by PLA_2-induced digestion of the inner pool of phosphatidylcholine of the thylakoid membrane, is expected to increase the backward electron flow, i.e., increase the EPL signal. PS II-related EPL from swollen thylakoids exposed to PLA_2 was enhanced after electroporation. This observation can be explained in terms of electric field-induced disorder in the phosphatidylcholine-PS II interaction domains resulting in enhanced flip-flop of the phosphatidylcholine from the inner into the outer leaflet of the photosynthetic membrane and exposing it to the action of PLA_2. This suggestion is supported by previous findings (Rawyler and Siegenthaler, 1981b), which show that the action of PLA_2 on PS II occurs only after PLA_2-induced disorder in the membrane making the inner pool of phosphatidylcholine accessible for PLA_2 digestion. Disorders of this kind that also involve an enhanced flip-flop process of the phospholipids were previously shown to occur in red blood cell membranes after electroporation (Dressler et al., 1983).

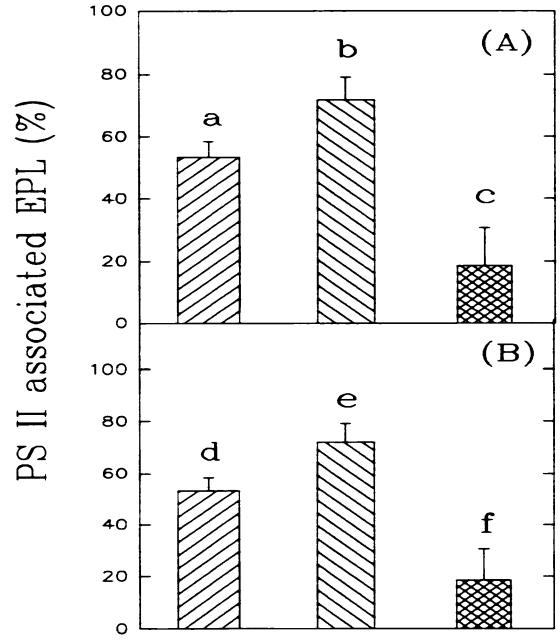

FIGURE 4 Effect of electroporation on the attenuation of PS II-related EPL signal upon exposure of a suspension of swollen thylakoid vesicles to PLA_2. Electroporated samples were exposed to 10 consecutive bipolar pulses of 1600 V/cm and duration of 200 μs at 5-s intervals. The suspensions included 2 mM of $CaCl_2$ and 150 μM methyl viologen. Other conditions as in Fig. 1. (*A*) The enzyme was added before swollen thylakoid vesicles were exposed the electric field. (*B*) The enzyme was added after the exposure to the electric field. (*a*) and (*d*) Suspensions exposed only to PLA_2; (*b*) and (*e*) suspensions exposed to PLA_2 and to electroporating electric field; (*c*) difference between (*b*) and (*a*); (*f*) is the difference between (*e*) and (*d*).

The fact that the effect of electroporation on the PLA_2-induced inhibition of the PS II-associated EPL signal was similar whether the enzyme was added before or after electroporation rules out the possibility of attributing the observed effect to the entrance of PLA_2 into thylakoid vesicles with a consecutive hydrolysis of the phospholipids located in the inner leaflet of the thylakoid membrane. This is attributed to the fact that complete resealing of the thylakoid membrane occurs 0.5 s after exposure to the external electric field (Rosemberg and Korenstein, in preparation). Moreover, it also eliminates the possibility that the effect of PLA_2 results from the direct activation of the enzyme by the external electric field. Thus, these observations suggest that electroporation leads to structural changes in the protein-lipid domains.

CONCLUSION

So far, the current view has been that a biological membrane undergoes a reversible permeability increase through structural changes in the lipid domain when exposed to high external electric fields. The presence of membrane proteins that act as ion channels in natural membranes makes them more prone to electroporation than proteins that are not ion transporters. The present study demonstrates the occurrence of electric field-induced changes in the conductance of the proton channel of the H^+-ATPase. This proton channel, in the absence of an external electric field, is selective for protons and is maintained mostly in a closed state. However, under the influence of a high induced transmembrane potential difference, the proton channel becomes conductive to small monovalent cations such as Na^+, K^+, or Cs^+ but not to large cations such as TEA^+ or bivalent cations such as Ca^{+2}. This demonstrates the limited conductive change that the protein undergoes. The conductive change reflects a conformational change of the proton channel, which decays within 1 ms. This relaxation time range is very close to the time for a single turnover of the H^+-ATPase ($\approx$2.5 ms). Thus, it may be speculated that a conformational change associated with proton conduction is a rate-limiting step of the H^+-ATPase turnover. The relatively small conduction of ions through the proton channel as compared with conduction through electropores in the lipid domain after electroporation emerges from the fact that under similar electroporation conditions we demonstrated the existence of electropores of a size that allowed the uptake of 40-kDa dextran molecules ($\approx$4.8 nm) (Rosemberg and Korenstein, 1990b). Thus, it may be concluded that although electric field-induced conductance change of ion channels with the possible loss of ion selectivity is to be expected, it contributes only marginally to the large total electric field-induced conductance change, which is confined to lipid and lipid-protein domains.

The relative conductance after electroporation in the lipid domains as compared with that occurring in the lipid-protein domains is still unknown. It may be speculated that the threshold for electroporation is different in these two membrane domains. If indeed such a difference exists, then the relative contribution of the two domains to the total conductance change will be a function of the externally applied electric field. The domain with the lower threshold for electroporation will be the major domain contributing to the conductance change.

This work was carried in partial fulfillment of a Ph.D. Thesis requirements of Y. Rosemberg.

REFERENCES

Abidor, I. G., V. B. Arakelyan, L. V. Chernomordik, Y. A. Chismadzhev, V. F. Pastushenko, and M. R. Tarasevich. 1979. Electric breakdown of bilayer lipid membranes. I. The main experimental facts and their qualitative discussion. *Bioelectrochem. Bioenerg.* 6:37–52.

Arnold, W. A., and R. Azzi. 1971. The mechanism of delayed light production by photosynthetic organisms and a new effect of electric fields on chloroplasts. *Photochem. Photobiol.* 14:233–240

Avron, M. 1960. Phosphorylation by Swiss-chard chloroplasts. *Biochim. Biophys. Acta.* 40:257–272.

Blank, M., and L. Soo. 1990. Ion activation of (Na, K)-ATPase in alternating currents. *Bioelectrochem. Bioenerg.* 24:51–62.

Brumfeld, V., and I. R. Miller. 1988. Effect of membrane potential on the conformation of bacteriorhodopsin reconstituted in lipid vesicles. *Biophys. J.* 54:747–750.

Brumfeld, V., I. R. Miller, and R. Korenstein. 1989. Electric field-induced lateral mobility of photosystem I in the photosynthetic membrane. A study by electrophotoluminescence. *Biophys. J.* 56:607–614.

Dressler, V., K. Schwister, C. W. M. Haest, and B. Deuticke. 1983. Dielectric breakdown of the erythrocyte membrane enhances transbilayer mobility of phospholipids. *Biochim. Biophys. Acta.* 732:304–307.

Elleson, J. L., and K. Sauer. 1976. The electrophotoluminescence of chloroplasts. *Photochem. Photobiol.* 23:113–123

Farkas, D. L., and S. Malkin. 1979. Cold storage of isolated class C chloroplasts. Optimal conditions for stabilization of photosynthetic activities. *Plant Physiol.* 60:449–451.

Farkas, D. L., R. Korenstein, and S. Malkin. 1982. Ionophore mediated ion transfer in a biological membrane: study by electrophotoluminescence. *In* Transport in Biomembranes. R. Antolini, F. A. Gliozzi and A. Gorio, editors. Raven Press, New York. 215–226.

Farkas, D. L., R. Korenstein, and S. Malkin. 1984a. Electrophotoluminescence and the electrical properties of the photosynthetic membrane. Initial kinetics and the charging capacitance of the membrane. *Biophys. J.* 45: 363–373.

Farkas, D. L., R. Korenstein, and S. Malkin. 1984b. Electrophotoluminescence and the electrical properties of the photosynthetic membrane. II. Electric field-induced electrical breakdown of the photosynthetic membrane and its recovery. *Biochim. Biophys. Acta.* 767:507–514.

Gheriani-Gruszka, N., S. Almog, R. L. Biltonen, and D. Lichtenberg. 1988. Hydrolysis of phosphatidylcholine in phosphatidylcholine-cholate mixtures by porcine pancreatic phospholipase A_2. *J. Biol. Chem.* 263:11808–11813.

Glaser R. W., S. L. Leikin, L. V. Chernomordik, V. F. Pastushenko, and A. I. Sokirko. 1988. Reversible electric breakdown of lipid bilayers: formation and evolution of pores. *Biochim. Biophys. Acta.* 940:275–287.

Gopher, A., Y. Blatt, M. Schonfeld, M. Y. Okamura, G. Feher, and M. Montal. 1985. The effect of an applied electric field on the charge recombination kinetics in reaction centers reconstituted in planar lipid bilayers. *Biophys. J.* 48:311–320.

Hamamoto, T., K. Ohno, and Y. Kagawa. 1982. Net adenosine triphosphate synthesis driven by external electric field in rat liver mitochondria. *J. Biochem.* 91:1759–1766.

Korenstein, R., D. L. Farkas, and S. Malkin. 1984. Reversible electric breakdown of swollen thylakoid membrane vesicles: study by electrophotoluminescence. *Bioelectrochem. Bioenerg.* 13:191–197.

Lill, H., G. Althoff, and W. Junge. 1987. Analysis of ionic channels by a flash spectrometric technique applicable to thylakoid membranes: CF_0, the proton channel of the chloroplast ATP synthase and, for comparison, gramicidin. *J. Membr. Biol.* 98:69–78.

Liu. D. S., R. D. Astumian, and T. Y. Tsong. 1990. Activation of Na^+ and K^+ pumping modes of (Na, K)-ATPase by an oscillating electric field. *J. Biol. Chem.* 265:7260–7267.

Powell, K. T., E. G. Derrick, and J. C. Weaver. 1986. A quantitative theory of reversible electrical breakdown in bilayer membranes. *Bioelectrochem. Bioenerg.* 15:243–255.

Rawyler, A., and P. A. Siegenthaler. 1981a. Regulation of photosystem I electron flow activity by phosphatidylglycerol in thylakoid membranes as revealed by phospholipase treatment. *Biochim. Biophys. Acta.* 638:30–39.

Rawyler, A., and P. A. Siegenthaler. 1981b. Transmembrane distribution of phospholipids and their involvement in electron transport, as revealed by phospholipase A_2 treatment of spinach thylakoids. *Biochim. Biophys. Acta.* 635:348–358.

Rephaeli, A., D. E. Richards, and S. J. D. Karlish. 1986. Electric potential accelerates the $E_1P(Na)$–E_2P conformational transition of (Na, K)-ATPase in reconstituted vesicles. *J. Biol. Chem.* 261:12437–12440.

Rosemberg, Y., and R. Korenstein 1990a. A novel method for measuring membrane conductance changes by a voltage sensitive optical probe. *FEBS Lett.* 263:155–158.

Rosemberg, Y., and R. Korenstein. 1990b. Electroporation of the photosynthetic membrane: a study by intrinsic and external optical probes. *Biophys. J.* 58:823–832.

Rosemberg, Y., P. Rosen, S. Malkin, and R. Korenstein. 1992. Spatial and temporal electroselection patterns in electric field stimulation of polarized luminescence from photosynthetic membrane vesicles. *Biophys. J.* 61: 1585–1594.

Serpersu, E. H., and T. Y. Tsong. 1983. Stimulation of a ouabain sensitive Rb^+ uptake in human erythrocytes with an external electric field. *J. Membr. Biol.* 74:191–201.

Serpersu, E. H., and T. Y. Tsong. 1984. Activation of electrogenic Rb^+ transport of (Na, K)-ATPase by electric field. *J. Biol. Chem.* 259:7155–7162.

Shinar, R., S. Druckmann, M. Ottolenghi, and R. Korenstein. 1977. Electric field effects in bacteriorhodopsin. *Biophys. J.* 19:1–5.

Sigrist-Nelson, K., H. Sigrist, and A. Azzi. 1978. Characterization of the dicyclohexylcarbodiimide binding protein isolated from chloroplast membranes. *Eur. J. Biochem.* 92:9–14.

Sprague, S. G. 1987. Structural and functional consequences of galactolipids on thylakoid membrane organization. *J. Bioenerg. Biomembr.* 19:691–703.

Sugar, I. P., and E. Neumann. 1984. Stochastic model for cell electric field induced membrane pores. *Biophys. Chem.* 19:211–225.

Sugar, I. P., W. Foster, and E. Neumann. 1987. Model for cell fusion: Membrane electroporation, pore coalescence and percolation. *Biophys. Chem.* 26:321–335.

Symons, M., S. Malkin, and R. Korenstein. 1984. External electric field effects on photosynthetic membrane vesicles: kinetic characterization of two electrophotoluminescence phases in hypotonically swollen chloroplasts. *Biochim. Biophys. Acta.* 767:223–230.

Symons, M., R. Korenstein, and S. Malkin. 1985. External electric field effects on photosynthetic vesicles. The relationship of the rapid and the slow phases of electrophotoluminescence in hypotonically swollen chloroplasts to PS I and PS II activity. *Biochim. Biophys. Acta.* 806:305–310.

Symons, M., S. Malkin, R. Korenstein, and D. L. Farkas. 1988. On the topological origin of the R and S phases of electric field induced luminescence in chloroplasts and blebs. *J. Photochem. Photobiol. B Biol.* 1:295–303.

Teissie, J., and T. Y. Tsong. 1980. Evidence of voltage-induced channel opening in Na/K ATPase of human erythrocyte membrane. *J. Membr. Biol.* 55:133–140.

Teissie, J., B. E. Knox, T. Y. Tsong, and J. Wehrle. 1981. Synthesis of adenosine triphosphate in respiration-inhibited submitochondrial particles induced by microsecond electric field. *Proc. Natl. Acad. Sci. USA.* 78:7473–7477.

Tsong, T. Y. 1983. Voltage modulation of membrane permeability and energy utilization in cells. *Biosci. Rep.* 3:487–505.

Tsong, T. Y., and D. Astumian. 1987. Electroconformational coupling and membrane protein function. *Prog. Biophys. Mol. Biol.* 50:1–45.

Tsong, T. Y. 1989. Deciphering the language of cells. *TIBS.* 14:89–92.

Tsong, T. Y. 1991. Electroporation of cell membranes. *Biophys. J.* 60:297–306.

Tsuji, K., and E. Neumann. 1981. Structural changes in bacteriorhodopsin induced by electric impulses. *Int. J. Biol. Macromol.* 3:231–242.

Vinkler, C., and R. Korenstein. 1982. Characterization of external electric field driven ATP synthesis in chloroplasts. *Proc. Natl. Acad. Sci. USA.* 79:3183–3187.

Vinkler, C., D. L. Farkas, and R. Korenstein. 1982. External electric field driven ATP synthesis in chloroplasts: a slow ATP synthase dependent reaction. *FEBS Lett.* 145:235–240.

Vos, M. H., and H. J. Van Gorkom. 1988. Thermodynamics of electron transport in Photosystem I studied by electric field-stimulated charge recombination. *Biochim. Biophys. Acta.* 934:293–302.

Vos, M. H., and H. J. Van Gorkom. 1990. Thermodynamical and structural information on photosynthetic systems obtained from electrophotoluminescence kinetics. *Biophys. J.*. 58:1547–1555.

Wagner, R., E. C. Apley, and W. Hanke. 1989. Single channel proton currents through reconstituted chloroplast ATP synthase CF_0-CF_1. *EMBO J.* 8:2827–2834.

Witt, H. T., E. Schlodder, and P. Graber. 1976. Membrane bound ATP synthesis generated by an external electric field. *FEBS Lett.* 69:272–276.

Zimmermann, U. 1982. Electric field-mediated fusion and related electrical phenomena. *Biochim. Biophys. Acta.* 694:227–277.