Biochimica et Biophysica Acta, 767 (1984) 507–514
Elsevier

BBA 41639

ELECTROPHOTOLUMINESCENCE AND THE ELECTRICAL PROPERTIES OF THE PHOTOSYNTHETIC MEMBRANE

II. ELECTRIC FIELD-INDUCED ELECTRICAL BREAKDOWN OF THE PHOTOSYNTHETIC MEMBRANE AND ITS RECOVERY *

DANIEL L. FARKAS, SHMUEL MALKIN and RAFI KORENSTEIN **

Departments of Biochemistry and Membrane Research, The Weizmann Institute of Science, Rehovot (Israel)

(Received May 21st, 1984)

Key words: Electrophotoluminescence; Luminescence; Thylakoid vesicle; Electric field effect; (Pea, spinach, lettuce chloroplast)

Preilluminated suspensions of swollen thylakoid vesicles ('blebs') were exposed to uni- and bipolar pairs of identical electric field pulses of variable duration, intensity and spacing. The resulting field-stimulated luminescence (electrophotoluminescence) was used as an intrinsic, voltage-sensitive optical probe to monitor electrical phenomena at the membrane level. The application of a pair of voltage pulses of opposite polarity made it possible to produce electric changes in the membrane by the first pulse and to analyse these effects by a second pulse of opposite polarity. It was found that the relative amplitudes of the two electrophotoluminescence signals depended on the intensity of the applied electric field and on the time interval (t^*) between the two pulses. When t^* varied from 0.4 to 12 ms, the second stimulated luminescence signal was at first much smaller than the first one and then increased exponentially until the two signals were equal for $t^* \geq 3$ ms. We analysed these differences between the two field-stimulated luminescence signals as a measure of the electrical breakdown of the membrane, induced during the first pulse. In this way a distinction between irreversible and reversible breakdown could be made with an estimation of the recovery kinetics of the reversible breakdown, which was found to be complete within 3 ms. Irreversible breakdown of the membrane was found to increase with lengthening the exposure time from 0.1 to 1.3 ms especially when applying high electric field of at least 2000 V/cm.

Introduction

The effect of high-intensity external electric fields on membrane conductance of cells, organelles and on artificial lipid bilayers has been considered in numerous publications [1–16 and reviews 17 and 18]. These studies demonstrate that membranes exposed to an electric field of strength exceeding a threshold value undergo electrical breakdown due to induced perforation, detected via a significant increase of membrane conductivity. Both direct and indirect methods have been used to investigate this process. The direct methods were based on electrical measurements of membrane conductance by macroscopic electrodes, in the case of black lipid bilayers, or by microelectrodes and electrofocusing Coulter counters, in the case of whole cells [1–6]. The indirect methods (of nonelectrical nature) used to detect

* For part I, see Ref. 24.

** To whom correspondence should be addressed.

Abbreviations: Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl) ethyl]glycine; EPL, electrophotoluminescence.

changes in membrane permeability relied on measuring the electric-field-induced leakage of ions or molecules of variable molecular weight from the intracellular space into the extracellular one [7–13]. The electric-field-induced increase of membrane conductance was shown to be either reversible or irreversible, depending on the experimental parameters (e.g., electric field intensity and duration). The reversible case is of more interest, both theoretically and experimentally in view of the fact that this process is fundamental in important new biotechnologies such as electric-field-induced fusion of cells [17], the use of cell ghosts for drug delivery [19] or in transformation [20]. Furthermore, a kinetic study of the interaction of natural membranes with high electric fields should help to characterize the viscoelastic properties of the studied membrane as well as to underly the basis for possible electric-field-induced changes taking place in energy-transducing membranes.

We present here an alternative optical method by which we could detect electric-field-induced conductance changes in photosynthetic membranes. This method is based on triggering of delayed luminescence from these membranes by an induced electrical field, which is therefore used as a voltage probe. This method is particularly sensitive when swollen thylakoid membrane vesicles ('blebs') are used [23]. Delayed luminescence is usually suggested to be associated with the Photosystem II complex [21], although evidence is now produced that part of the electrophotoluminescence is associated with Photosystem I (Symons, M., Korenstein, R. and Malkin, S., unpublished data). Delayed luminescence can be stimulated by orders of magnitude when exposing a suspension of blebs to an external electric field [22]. This type of triggered luminescence (coined electrophotoluminescence by Ellenson and Sauer [22]) could further be studied by the investigation of emission polarization, due to an electroselection process resulting from the interaction of the quasi-spherical vesicular membranes with an axially symmetrical external field [23]. Moreover, the kinetics as well as the amplitude of electrophotoluminescence were shown to depend on the vesicles' radii, on the conductivities of the intra and extra membranal space as well as on the membrane conductivity itself [24]. In this study we rely upon the last property, by probing with electrophotoluminescence the electric-field-induced conductance change in the photosynthetic membrane. An alternative method based on monitoring electric field induced absorption changes can also be used [35,36]. However, a serious disadvantage of the method is the need to accumulate many electric field pulses due to low level of signal/noise.

Materials and Methods

Chloroplasts from lettuce (*Lactuca sativa*), pea (*Pisum sativum*) or spinach (*Spinacea oleracea*) were prepared by a standard method [25] and stored below −100°C with complete preservation of the main photosynthetic activities [26]. Swollen thylakoid membranes were prepared by diluting the stored chloroplasts in a very low-ionic strength medium (distilled water or 5 mM Tricine, pH 7.8). The dilution was 1:100 or 1:200 yielding final chlorophyll concentrations of 20–40 μg/ml. The resulting hypotonicity leads to the formation of large spherical vesicles ('blebs') with a distribution of radii in the range of 1–12 μm with an average radius of 4–5 μm [24].

The experimental set-up for electrophotoluminescence production and detection has been described previously [23,24]. It is based on an electric field jump apparatus consisting of an optical cell containing two parallel metallic electrodes, a timing circuitry and a high-voltage pulser (Cober 606) capable of delivering high-intensity rectangular voltage pulses with very fast rise and fall times (less than 1 μs). Preillumination was accomplished by a 10 μs saturating flash. The bipolar (as well as monopolar) sequences of electric field applied to the bleb suspension were obtained from the Cober 606 high-voltage pulser, by use of a home-built pulse inverter device, based on high speed, high-voltage semiconductor relays. The only modification thus introduced in the characteristics of the original [23,24] voltage pulse – besides polarity was a slightly longer (approx. 2 μs) rise time. The spacing t^* between the two pulses (see Fig. 3) could be varied continuously, between 0.1 and 60 ms. The applied pulse shape, intensity and kinetics could independently be monitored by use of an inductive current probe. Several uni- or bipolar sequences of arbitrary spacing could be applied in succession.

Results

The emission of electrophotoluminescence includes several characteristic phases [22,24]. During an externally applied electric field pulse the electrophotoluminescence exhibits a lag period, rises to a peak value (EPL_{max}) then decays usually with a biphasic kinetics – while the field is still on. When the field is turned off the emission decays to its base level. Some of these phases can be analysed in terms of electrical events and properties. Thus for example the lag in the electrophotoluminescence onset is probably related to the charging of the membrane as capacitor [24], while the rapid decay of the electrophotoluminescence at the offset of the applied field represents the discharge of the same capacitor, probably by lateral movement of charges along the membrane surface. In general, the overall emission is simultaneously controlled by photosynthetic and electrical parameters. The photosynthetic parameters relate to the availability of precursors, which in turn depends on the time interval between the preillumination and the application of the external electric field (t_d). The electrical parameters include, among others, strength of the applied electrical field, membrane conductance, internal and external medium conductivities. By choice of appropriate fixed experimental conditions for the photosynthetic parameters one can separate the electrical effects from the photosynthetic ones, thus enabling the study of electrical processes at the membrane level through continuous monitoring of changes in the electrophotoluminescence.

It has previously been shown that stimulation of delayed luminescence occurs whenever the local electric field has an inside-outside direction (e.g., by creating a diffusion potential [27]). Light illumination produces radial charge separation where the donor is located on the inside part of the membrane and the acceptor is situated more to the outside, thus creating a continuous array of dipoles in the membrane. Application of an external electric field to a suspension of blebs induces a transmembrane field with an azimuthal angle dependence: parallel and antiparallel to the photoinduced dipoles in the two hemispheres. This in turn leads to electric-field-dependent stabilization of the light-induced charge separation in one hemisphere (closer to the positive electrode) and charge destabilization in the other hemisphere (closer to the negative electrode). As a consequence an applied d.c. pulse of a certain polarity will induce electrophotoluminescence only in one of the two hemispheres (which is closer to the negative electrode). Therefore, if a second field pulse is applied to the suspension (with no further preillumination), it will induce emission either from the same hemisphere if it is of the same polarity as the first pulse or from the opposite hemisphere if the field pulse sequence is bipolar [22,37]. Because of this, the electrophotoluminescence emission in a bipolar sequence after a single preillumination differs considerably from the one in a unipolar sequence (Fig. 1). In the case of bipolar pulses, the electric field acts practically independently on the existing (light-induced) luminescence precursors in the two hemispheres, the only condition for this being that the spacing between the pulses of opposite polarity should be short (millisecond) compared to the rotational diffusion time (t_r) of the spherical 'bleb' which can be calculated to be in the minute range [¶]. Moreover, if the dark-time elapsed from preillumination (t_d) is also much longer than the spacing (t^*) between the pulses, the amount of light-induced precursors for electrophotoluminescence in the two hemispheres will be practically the same; at the moment they are acted upon by the applied field (e.g., at $t_d = 300$ ms and $t_d + t^* = 303$ ms, respectively). In this case, if only precursors are

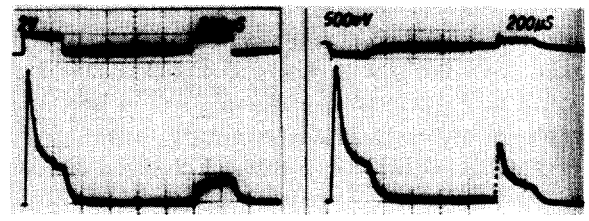

Fig. 1. Electrophotoluminescence induced by unipolar pair (left) and bipolar voltage pulses (right). Lower trace electrophotoluminescence emission; upper trace monitoring of the applied voltage pulses by a current probe. Conditions: $E = 3000$ V/cm; $t_d = 220$ ms; pH 7.8. Time scale, 200 µs/division.

[¶] Calculated by Perrin's formula ($t_r = \eta 2\pi R^3/3kT$) for $R = 5$ µm, and $\eta = 1$ cP (water); t_r is of the order of 60 s at room temperature, k is Boltzmann's constant.

determining the electrophotoluminescence amplitude, the stimulated emission should be nearly the same for each of the two pulses of opposite polarity. Thus, in principle, application of a pair of pulses of opposite polarity makes it possible to bring about electric field-induced changes in the membrane by the first pulse and to analyse these induced effects by a second pulse of opposite polarity. Such an approach was adopted in our study.

A typical experiment consisted of the following: a bipolar sequence of high-intensity d.c. electric field pulses was applied to a preilluminated suspension of blebs, and the induced electrophotoluminescence was recorded and compared for the two polarities. The independent parameters varied were: the external field intensity (E), its duration (t_E) and the spacing, t^*, between the two pulses (of opposite polarity, but otherwise identical). The intensity of the electrophotoluminescence is abbreviated by EPL_1 and EPL_2 corresponding to the consecutive pulses. Fig. 2 (left) shows EPL_1 and EPL_2 for $t^* = 600$ μs ($t_E = 110$ μs). Clearly, $EPL_2 < EPL_1$. If t^* is gradually increased (Fig. 2, right), electrophotoluminescence increases constantly. In the particular case of Fig. 2, $EPL_1 = EPL_2$ (within experimental error) for $t^* = 2.6$ ms, and stays so for t^* up to about 12 ms. Beyond this value, EPL_2 decreases again (as compared to EPL_1), but this time presumably due to the decay of the pool of precursors available for electrophotoluminescence.

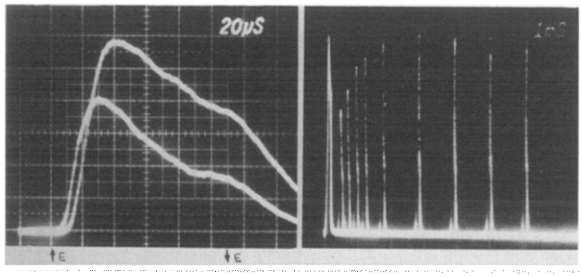

Fig. 2. Electrophotoluminescence induced by a bipolar voltage pulse. Left: EPL_1 (top); EPL_2 (bottom); $t_d = 220$ ms, $E = 2000$ V/cm; $t^* = 600$ μs, $t_E = 110$ μs. Time scale, 20 μs/division. Right: electrophotoluminescence in bipolar sequences of variable spacing t^*. Time scale, 1 ms/div. All other conditions (except t^*) as left. Each signal rises from a different experiment, with a different t^*. The EPL_2 for $t^* > 3$ ms is, on the expanded left scale (a), identical to the top trace.

We interpret $EPL_2 < EPL_1$ in a bipolar sequence as due to the electroperforation (electric breakdown) of the membrane, and the increase of the EPL_2/EPL_1 ratio with the increase in t^* as indicator of recovery following breakdown. Thus, for the experiment described, $t^* = 2.6$ ms can be considered as the (full) recovery time. If recovery leads to $EPL_1 = EPL_2$, the breakdown was reversible. If, however, the same behaviour leads to $EPL_2 = b \cdot EPL_1$ (where $b < 1$), then $(1 - b)$ will be an index of irreversible breakdown occurring in parallel with a reversible one (e.g., the largest blebs in a distribution could undergo irreversible breakdown by the action of the field, since the local electric field induced is linear with the vesicle's radius).

Another, similar experiment is presented in Fig. 3. The field intensity and duration being somewhat higher, the recovery time is slightly longer, but the general behaviour is the same. Moreover, the same recovery pattern (increase in EPL_2/EPL_1) can also be seen in the case of a unipolar sequence. This can be regarded as an additional proof that the recovery kinetics have nothing to do with the electrophotoluminescence precursors, though for quantitative assessment of breakdown the bipolar pattern is obviously more useful.

There are several characteristics of the applied electric field that do affect the recovery phenomena in blebs.

(a) *Applied field intensity.* For fast rising d.c. field pulses, reversible recovery appeared in our experiments for lower applied field intensities $E \geqslant$

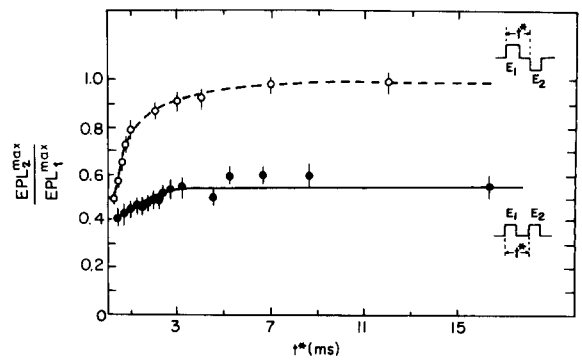

Fig. 3. EPL_2/EPL_1 ratio in a bipolar (top) and unipolar (bottom) pulse sequence as a function of inter-pulse spacing t^*. $E = 2500$ V/cm; $t_d = 300$ ms; $t_E = 300$ μs.

800 V/cm, while irreversible breakdown required at least $E = 2500$ V/cm, for the lowest field durations (approx. 10 μs) we used. These values correspond – taking into account the size distribution of 2–8 μm for the blebs – to transmembrane potential differences at the vesicle poles between 0.75 and 3.0 V (not correcting for attenuation of the actual induced electrical polarization due to the electrical breakdown of the membrane). These are of course only approximate values but they agree with existing data for crossmembrane potential difference eliciting electrical breakdown [3,10].

(b) *Applied field duration.* The longer the applied field, the more irreversible breakdown occurs. The recovery time is also lengthened, with considerable irreversible breakdown occurring for $t_E > 500$ μs even for field intensities of $E = 2000$ V/cm and below (Fig. 4). This is in line with the pulse-length dependence of the breakdown voltage in bilayers [4]. For equal peak voltages applied externally, more breakdown occurs in the bleb suspension for sinusoidal fields than for rectangular ones. This is partly related to field-duration effects (see above), but also to the lowering of the breakdown voltage by lengthening of the membrane charging time [28]. The irreversible electrical breakdown of the membrane when using extremely long d.c. pulses was verified by using a microscopic E-jump cell with interelectrode distance of 100 μm. By direct microscopic observation we could observe the irreversible breakdown (lysis) of blebs for applied d.c. field of 10 ms duration which generated crossmembrane polarization of 1.5 V.

(c) *Ageing.* Ageing of the blebs at 0°C or room temperature has the following consequences: (1) electrophotoluminescence emission declines; (2) the field-induced decay kinetics of EPL_2 vs. EPL_2 became non identical (Fig. 5). For long-ageing times (24 h), serious kinetic modifications appear, and irreversible breakdown occurs for relatively low applied-field values.

(d) *Bleb size distribution.* If the blebs are resuspended in a medium of higher osmolarity, their size distribution shifts to lower radii R. The breakdown phenomena (of both kinds) diminish – for the same applied fields – as compared to the lower osmolarity control. The explanation is clearly linked to the drop in the local electric field at the membrane, E_m by decrease in R (as E_m is proportional to R).

(e) In the presence of gramicidin (approx. 10 μM), a channel type ionophore, electrophotoluminescence emission is kinetically altered and considerably diminished. In bipolar field experiments, the t^*-dependent EPL_2/EPL_1 ratio is still observable, but at higher applied field intensities than for the control (with no gramicidin), suggesting that: (i) breakdown and recovery occur, with the effective membrane potentials diminished by

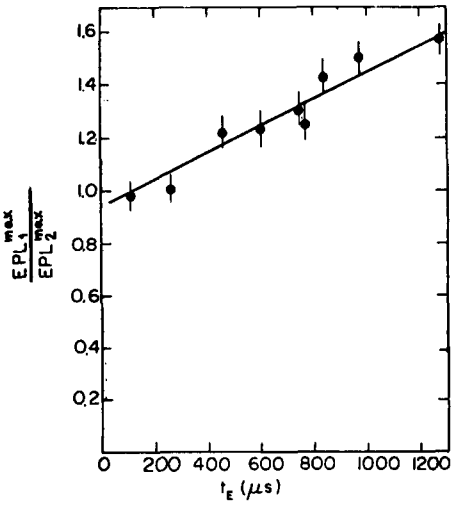

Fig. 4. EPL_1/EPL_2 ratio as function of pulse length for a bipolar sequence. Conditions: $E = 2000$ V/cm; $t_d = 300$ ms, $t^* = 6$ ms.

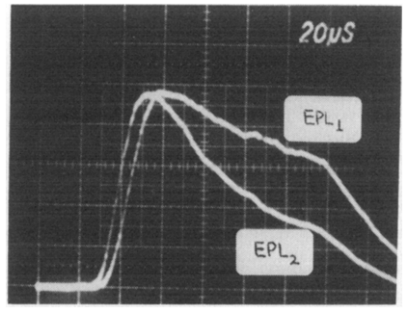

Fig. 5. Ageing effect of the photosynthetic membrane on electrophotoluminescence in a bipolar sequence. Conditions identical to Fig. 2, with $t^* = 6$ ms, except that the 'blebs' have been aged for 2 h at 0°C in the dark. Time scale, 20 μs/division.

the presence of gramicidin, and (ii) the conductivity changes in the breakdown state are much more significant than those induced by the presence of gramicidin.

Discussion

Previous studies [1–18] have demonstrated that electric fields of high intensity can cause a pronounced increase in the conductance state of membranes, usually termed electrical breakdown. The relation between the externally applied electric field intensity E and the local electric field E_m induced in the vesicular membrane, is given under steady-state conditions, by the following relation [24]:

$$E_m = \frac{\frac{3}{2}\frac{R}{d}E\cos\theta}{1+\frac{2\lambda_o+\lambda_i}{2\lambda_o\lambda_i}\lambda_m(R/d)} \quad (1)$$

where R is the radius of the vesicular membrane, d is membrane thickness, λ_m is membrane conductivity, λ_o and λ_i are the specific conductivities of the external and the internal media and θ is the angle between the external electric field and the radius vector to the plane of the membrane for any surface point (when assuming that $R \gg d$). Under our experimental conditions, where we can assume that the internal and external conductivities are equal ($\lambda_i = \lambda_o$) and that $\lambda_m \ll \lambda_o$, relation (1) written for the potential difference $\Delta\psi$ reduces to:

$$\Delta\psi = E_m d = \tfrac{3}{2}RE\cos\theta \quad (2)$$

Electrical breakdown was shown to occur (e.g., Refs. 3, 4 and 31) whenever a potential difference of the order of 1 V was established across the membrane. It can be calculated from relation (2) that under our experimental conditions $\Delta\psi$ higher than this value can be easily established across the membrane: the size distribution of the blebs formed under low-ionic condition was previously measured [24] yielding a mean radius of 5 μm; when exposing a vesicle of $R = 5$ μm to an external electric field of $E = 2$ kV/cm, a maximal $\Delta\psi$ of 1.5 V can be calculated from Eqn. 2. Therefore a dielectric breakdown is expected to occur under our experimental conditions (e.g., Fig. 2), since 1.5 V is far above the reversible breakdown threshold of membranes. Since the stimulated delayed luminescence is a monotonous function of the induced cross-membrane potential difference, any increase in membrane conductance should lead to a decrease in the induced local field as predicted by Eqn. 1.

When applying a bipolar pulse one would expect to observe equal stimulation of delayed luminescence from the two hemispheres of the 'bleb' ($EPL_1 = EPL_2$). Indeed we found this to be the case for applied electric field strengths lower than approx. 0.7 kV/cm (corresponding to a transmembrane potential difference of 525 mV for vesicles having $R = 5$ μm) and $t^* \geq 0.5$ ms. However, when the applied electric field is higher than 2 kV/cm $EPL_1 > EPL_2$ for the same interval t^*. The fact that $EPL_1 > EPL_2$ suggests that $E_1^{max} > E_2^{max}$ which means that the degree of membrane electrical polarization induced by the second pulse of the bipolar sequence is lower than that obtained by the first half. Examination of expression (1) suggests that a decrease in the local electric field E_m may be due to an electrically induced change by the first pulse in one of the four parameters, R, d, λ_o and λ_m. Let us briefly analyse these possibilities. Changes in R due to deformation of a membranous sphere field have previously [29] been considered theoretically. This should lead to changes in light scattering which were not observed under our experimental conditions. Furthermore a deformation of a spherical vesicle into an ellipsoidal one, with its long axis along the direction of the electric field should increase R, which then would lead to an increase in E_2^{max}, contrary to the experimental data. The possibility of the change in membrane thickness by an electrical field was studied experimentally by a voltage induced reflectivity relaxation of bilayer lipid membranes [30]. However, only a negligible thinning of the membrane was observed (the relative change was of the order of 10^{-4} at 100 mV). Moreover, thinning of the membrane by the electric field would lead to an increase in E_2^{max} and to concomitant increase of electrophotoluminescence which is in contrast with the observed results. Since no change in λ_o is expected when applying a field of the order of 2 kV/cm, we suggest that the

most plausible electric field induced change is in membrane conductance, λ_m. The possible mechanisms for such a process are not fully agreed upon. It is thought that the high-intensity electric field creates or enlarges aqueous pores in the membrane. This process can be highly localized (e.g., to the poles of the vesicles subjected to the field), but it may proceed by either pore 'diffusion' and resealing (for reversible breakdown) or 'accumulation' of defects, enlargement of pores, and eventually, membrane lysis (irreversible breakdown).

Concluded from this work, we think that monitoring changes in electrophotoluminescence emission elicited in a bipolar electric field sequence is a convenient alternative approach to the study of breakdown and recovery. Regarding the recovery times that have been obtained here (milliseconds for short d.c. pulses, and longer times for a.c. pulses), they are much longer than those reported for artificial membranes (approx. microseconds, see Ref. 4) or even some natural ones [31], but in agreement with those obtained for chromaffin granules [32] and erythrocytes [10–13]. However, it must be stressed that the resealing time is a function of both the amplitude and the width of the applied pulse. When comparing the resealing kinetics in Figs. 2 and 3 it is evident that a longer resealing time is obtained for higher electric field. Due to the special symmetry of our system, and the relative nature of the measurement involved (EPL_1 vs. EPL_2), it seems that the approach described here has a number of advantages (as compared to other methods): the resulting high sensitivity, high kinetics resolution and additional mechanistic handles (e.g., polarization of emission) all recommend this approach. It is possible that this method may be extended to organelles and cellular systems which lack a sensitive intrinsic optical probe by adding a suitable such one externally.

Acknowledgement

This project was supported in part by the U.S.-Israel Binational Science Foundation.

References

1 Zimmermann, V., Pilwat, G., Beckers, F. and Riemann, F. (1976) Bioelectrochem. Bioenerg. 3, 58–83
2 Zimmermann, U., Groves, M., Schnabl, H. and Pilwat, G. (1980) J. Membrane Biol. 52, 37–50
3 Benz, R., Beckers, F., Zimmermann, U. (1979) J. Membrane Biol. 48, 181–204
4 Benz, R. and Zimmermann, U. (1980) Biochim. Biophys. Acta 597, 637–642
5 Zimmermann, U. and Benz, R. (1980) J. Membrane Biol. 53, 33–43
6 Benz, R. and Zimmermann, U. (1981) Biochim. Biophys. Acta 640, 169–178
7 Sale, A.J.H. and Hamilton, W.A. (1967) Biochim. Biophys. Acta 148, 781–788
8 Sale, A.J.H. and Hamilton, W.A. (1968) Biochim. Biophys. Acta 163, 37–43
9 Neumann, E. and Rosenheck, K. (1972) J. Membrane Biol. 10, 279–290
10 Kinosita, K., Jr. and Tsong, T.Y. (1977) Biochim. Biophys. Acta 471, 227–242
11 Kinosita, K., Jr. and Tsong, T.Y. (1977) Nature 268, 438–441
12 Kinosita, K., Jr. and Tsong, T.Y. (1978) Nature 272, 258–260
13 Kinosita, K., Jr. and Tsong, T.Y. (1980) Biochim. Biophys. Acta 595, 146–150
14 Abidor, I.G., Chernomordik, L.V., Sukharev, S.I., Chizmadzhev, Yu.A. (1982) Bioelectrochem. Bioenerg. 9, 141–148
15 Chernomordik, L.V., Sukharev, S.I., Abidor, I.G., Chizmadzhev, Yu.A. (1982) Bioelectrochem. Bioenerg. 9, 149–155
16 Chernomordik, L.V., Sukharev, S.I., Abidor, I.G., Chizmadzhev, Yu.A. (1982) Stud. Biophys. 90, 221–222
17 Zimmermann, U. (1982) Biochim. Biophys. Acta 694, 227–277
18 Tsong, T.Y. (1983) Biosci. Rep. 3, 487–505
19 Zimmermann, U. (1983) in Targeted Drugs (E. Goldberg, ed.), pp. 153–200, John Wiley & Sons, New York
20 Neumann, E., Schaefer-Ridder, M., Wang, Y. and Hofschneider, P.H. (1982) EMBO J. 1, 841–845
21 Malkin, S. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 349–431, Elsevier Biomedical Press
22 Ellenson, J.L. and Sauer, K. (1976) Photochem. Photobiol. 23, 113–123
23 Farkas, D.L., Korenstein, R. and Malkin, S. (1980) FEBS Lett. 120, 236–242
24 Farkas, D.L., Korenstein, R. and Malkin, S. (1984) Biophys. J. 45, 363–373
25 Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272
26 Farkas, D.L. and Malkin, S. (1979) Plant Physiol. 64, 942–947
27 Farkas, D.L., Korenstein, R. and Malkin, S. in Transport in Biomembranes. Model Systems and Reconstitution (Antolini, R., Gliozzi, A. and Gorio, A., eds.), pp. 215–226, Raven Press, New York
28 Zimmermann, U., Scheurich, P., Pilwat, G. and Benz, R. (1981) Angew. Chem. Intern. (English edn.) 20, 325–344
29 Helfrich, W. (1974) Z. Naturforschung 29C, 182–183
30 Berestovsky, G.N., Gyulkhandanyan, M.Z., Ivkov, V.G. and Razhin, V.D. (1978) J. Membrane Biol. 43, 107–126

31 Benz, R. and Conti, F. (1981) Biochim. Biophys. Acta 645, 115–123
32 Lindner, P., Neumann, E. and Rosenheck, K. (1977) J. Membrane Biol. 32, 231–254
33 Arnold, W. and Azzi, J. (1971) Photochem. Photobiol. 14, 233–240
34 De Grooth, B.G. and Van Gorkom, H.J. (1981) Biochim. Biophys. Acta 635, 445–456
35 De Grooth, B.G., Van Gorkom, H.J. and Meiburg, R.F. (1980) Biochim. Biophys. Acta 589, 299–314
36 Schlodder, E. and Witt, H.T. (1980) FEBS Lett. 112, 105–112
37 Farkas, D.L., Korenstein, R. and Malkin, S. (1981) in Photosynthesis. I. Photophysical Processes – Membrane Energization (Akoyunoglon, G., ed.), pp. 627–636, Balaban International Science Services, Philadelphia, PA