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II. ELECTRICAL MEASUREMENTS IN THE NANOSECOND RANGE OF THE CHARGE SEPARATION FROM CHLOROPLASTS SPREAD AT A HEPTANE-WATER INTERFACE

APPLICATION OF A NOVEL CAPACITIVE ELECTRODE

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Summary

Spinach chloroplasts are spread at a heptane-water interface. Applying a novel capacitative electrode introduced in the preceding paper (Trissl, H.-W. (1980) Biochim. Biophys. Acta 595, 82—95) the changes of the interface potential induced by single laser flashes are investigated. The following results are obtained:

(1) The chloroplasts spread at the interface form a thin layer with asymmetrical orientation. The structural state of this layer is discussed.

(2) The photovoltage from the interfacial layer shows similar characteristics as the field-indicating absorption change of chloroplast suspensions, the latter reflecting the photosynthetic primary charge separation:

(a) Both can be abolished by addition of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

(b) About one half of the signals can be reactivated by addition of *N*-methylphenazonium methosulfate.

(c) Both signals saturate at low flash light intensities.

(d) Both signals can be abolished by background illumination of comparable intensities.

(e) Both signals are independent of the ionic strength.

(3) The half-rise time of the photovoltage is determined to be less than 3 ns.

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; chlorophyll a_{I}, reaction center of Photosystem I (PSI); chlorophyll a_{II}, reaction center of Photosystem II (PSII); Tricine, *N*-tris(hydroxymethyl)methylglycine.

It is suggested from these results that the photovoltage from the interfacial layer reflects the primary charge separation process in photosynthesis, i.e. the latter is accomplished also within less than 3 ns.

Introduction

The primary process of plant photosynthesis is the ejection of an electron from excited chlorophyll *a* to an electron acceptor (for review see Ref. 1). This process occurs undirectionally across the thylakoid membranes of chloroplasts from the inside to the outside of the thylakoid vesicle. The inner space becomes positive with respect to the outer space. The electric field generated by this transmembrane charge separation has been established by measurements of either electrochromic absorption changes of the accessory pigments [2] or by measurements of the photovoltage from a chloroplast suspension subjected to a light gradient [3,4] or by the microelectrode technique. [5].

The kinetics of the charge separation in chloroplast is still not time resolved. Measurements of the field-indicating absorption change have shown that the process occurs in less than 20 ns [6]. The low signal to noise ratio of optical signals in chloroplasts makes faster measurements extremely difficult.

Therefore, in this paper another method is applied for the detection of fast charge displacement: chloroplasts are spread at a heptane-water interface and the flash-induced interface potential change is measured with the capacitative electrode introduced in the preceding paper [7]. The interfacial layer which is formed by spreading the chloroplasts shall be designated in the following as 'thylakoid layer' without specifying its structure. A detailed characterization of the 'thylakoid layer' will be given in Discussion.

It will further be shown that the flash-induced interface potential change (= photovoltage) from the 'thylakoid layer' possesses five distinct properties which agree with those of the flash-induced transmembrane potential change in intact thylakoids which is caused by the primary photosynthetic charge separation. The half-rise time of the photovoltage is found to be less than 3 ns. Hence, it is suggested that the primary charge separation in photosynthesis is as fast.

Materials and Methods

Spinach chloroplasts are prepared as described elsewhere [8]. Additionally, 10 mM ascorbate are added during the grinding step. The chloroplasts are then resuspended in 0.4 M sucrose, 10 mM NaCl, 2 mM Tricine buffer (pH 7.4) and 5% Me_2SO to give a final chlorophyll concentration of about 1 mM. Samples of this preparation are frozen and stored under liquid N_2 until use.

The experimental set-up and the electrical characteristics of the capacitative electrode are described in the preceding paper [7]. A glass beaker is used and filled to one part with electrolyte and atop double-distilled *n*-heptane is layered. Into the clean interface between heptane and water (area: 25 cm^2) a droplet of the chloroplast suspension (containing 1 nmol chlorophyll) is brought by means of a thin glass capillary. The spreading reduces the interface

tension from 50 mN/m (clean interface) to about 20 mN/m (interface with spread chloroplasts). The interfacial tension is measured by the Wilhelmy plate method using a sandblasted platinum plate of 2.5 cm length coated with carbon black in order to make its surface hydrophobic.

The set-up allows illumination of the interface via a light guide from either the top or the bottom. Laser flashes (wavelength 530 nm) from a Q-switched and frequency doubled Nd-Yag-laser (Laser Associates, model 252) are delivered to that part of the interface that is capacitatively coupled. The electric signals from the 'thylakoid interface layer' are amplified with a 150 MHz pulse amplifier (Keithley, model 105) or for a lower time resolution with an electrometer amplifier (Keithley, model 602, 40 kHz) and stored in a transient recorder (Biomation, model 6500, 2 ns/point). The stored signals are then transferred to an averager (Nicolet Instrument Corporation, model 1170) in order to allow a summation when a better signal to noise ratio is wanted. Finally, the signal is displayed on a *X*-*Y* recorder.

For triggering of the transient recorder the laser beam is split and one part is used to illuminate a photodiode (Motorola, MRD 500) which directly delivers the trigger pulse to the transient recorder.

The time resolution of the total experimental set-up is determined by replacing the capacitative electrode by the photodiode (Motorola, MRD 500, rise time less than or equal to 1 ns). This measurement gives the response time of the apparatus including amplifiers and the integrated time course of the laser flash. The experimental conditions for measuring the photovoltage are chosen so that the response time of the capacitative electrode itself is less than 2 ns (see also the preceding paper [7]).

Results

When a laser flash is delivered to chloroplasts spread at the heptane-water interface a photovoltage of about 2 mV is measured with the capacitative electrode as depicted in Fig. 1a. The ordinate scale is calibrated by means of rectangular test pulses of known voltage, hence, the scale reflects the true interface potential change (see also Ref. 7). The polarity of the signal is such that the heptane phase becomes positive with respect to the aqueous phase.

In order to establish that the observed signal from the 'thylakoid layer' is due to a photosynthetic reaction DCMU is added to the aqueous subphase. DCMU is known to inhibit the primary processes of photosynthesis by binding specifically to a protein at the acceptor site of Photosystem II [10,11]. As shown in Fig. 1b the signal amplitude with DCMU is only 10—20% of that with fully working photosystems (Fig. 1a). In a further experiment Photosystem I is reactivated by addition of *N*-methylphenazonium methosulfate and ascorbate while DCMU is still present. As can be seen in Fig. 1c the photovoltage is about half of that if both photosystems are working. All three experiments have been carried out about 200 ms after a 2 s preillumination period with saturating red light. The preillumination is necessary in the experiment of Fig. 1b in order to have both reaction centers oxidized: DCMU prevents a further transfer of electrons from the reduced primary acceptor of chlorophyll a_{II} to chlorophyll a_I^+

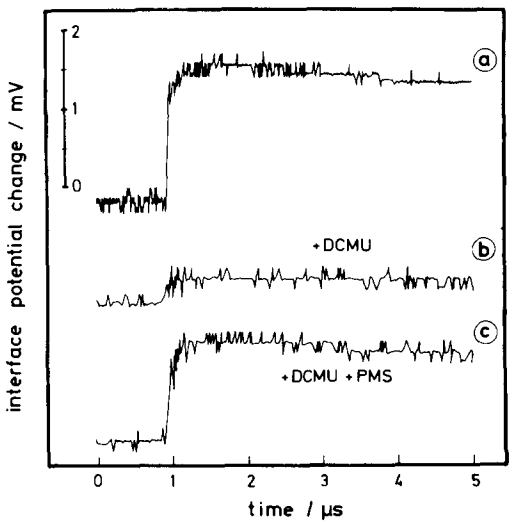


Fig. 1. Interface potential changes from chloroplasts spread at the heptane-water interface induced by single laser flashes. All signals are recorded about 200 ms after a preillumination of 2 s duration with red light (λ = 684 nm) of an intensity of $4.5 \cdot 10^{15}$ quanta/cm^2 per s. (a) Aqueous subphase: 10^{-2} M NaCl, pH 5.5. (b) Aqueous subphase: 10^{-2} M NaCl, 10^{-5} M DCMU, pH 5.5. (c) Aqueous subphase: 10^{-2} M NaCl, 10^{-5} M DCMU, 10^{-4} M *N*-methylphenazonium methosulfate, 10^{-2} M ascorbate (PMS).

while the chlorophyll a_I^+ remains in the oxidized state [12].

In the following experiments the light saturation of the generated photovoltage is investigated and compared with that of photosynthesis. Fig. 2 shows the dependence of the photoelectric signal on the flash energy. The amplitude of the photovoltage increases approximately proportional to the flash energy at low light intensities and reaches a saturation value of about 5 mV at high light intensities.

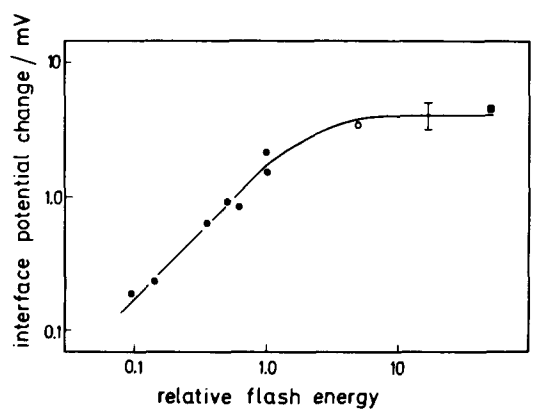


Fig. 2. Dependence of the interface potential change on the flash energy. The maximal laser flash energy is set arbitrarily to unity. Lower energies are adjusted with neutral density filters. Higher energies are obtained with a 20 µs flash lamp. •, laser flash (λ = 530 nm). ○, 20 µs flash (λ = 530 nm). ▪, 20 µs flash (white). Aqueous subphase: 10^{-2} M NaCl.

Another type of saturation experiment is depicted in Fig. 3. Shortly after layer formation a first flash is applied (Fig. 3a). Then red background light (λ = 684 nm) of an intensity of $4.5 \cdot 10^{15}$ quanta/cm^2 per s is switched on and a second flash is fired evoking only about 15% of the preceding signal (Fig. 3b). In order to establish the intactness of the layer a third flash without background illumination is applied (Fig. 3c). The smaller response to the third flash as compared to the first one corresponds with the inactivation curve shown in Fig. 4.

In a parallel experiment the saturation of the field-indicating absorption change at 515 nm of a chloroplast suspension is measured [9] as a function of the light intensity of the background illumination (λ = 684 nm). Here, a light intensity of $5.8 \cdot 10^{15}$ quanta/cm^2 per s is necessary to decrease the absorption change to 15% of its maximal value. This light intensity is similar to the one found for the layer experiment (see Discussion).

The stability of 'thylakoid layers' at two different NaCl concentrations is shown in Fig. 4. Here, the photovoltage arising from single laser flashes is plotted at different times after forming the layer. There is no difference in the signal whether the chloroplasts are spread before the capacitative electrode is

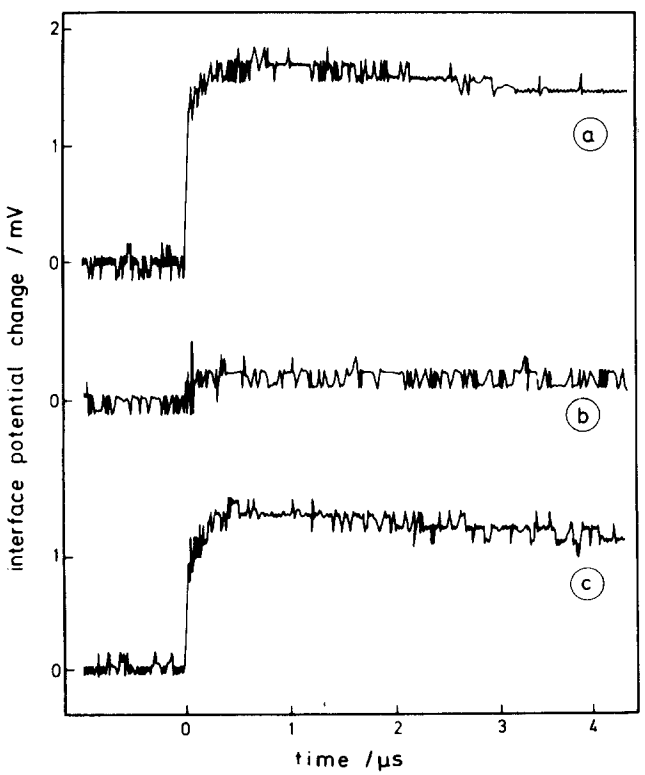


Fig. 3. Effect of red background illumination on the laser flash-induced interface potential change. Aqueous subphase: 10^{-2} M NaCl, pH 6.5. (a) First flash to the interface 1 min after formation of the layer. (b) Second flash to the interface during background illumination (λ = 684 nm, light intensity $4.5 \cdot 10^{15}$ quanta/cm^2 per s), 3 min after layer formation. (c) Third flash to the same interfacial layer, 2 min after switching off the background illumination and 6 min after the layer formation.

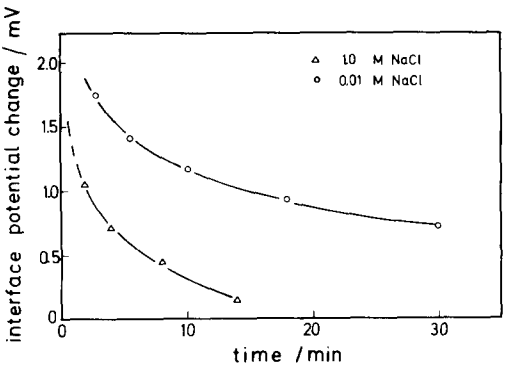


Fig. 4. Dependence of the interface potential change from 'thylakoid layers' on the time after forming the layers at two different NaCl concentrations. Single laser flashes are applied.

lowered or whether the electrode is brought to the interface and then the chloroplasts are spread (see Fig. 3 in Ref. 7). Therefore, the latter method is preferred because the time between layer formation and measurement is shorter. Obviously, the life time of a layer depends on the salt concentration. The higher it is, the shorter the life time. However, the life time does not depend on the number of flashes fired nor on the time of preillumination. Also these properties resemble those known from chloroplast suspensions and demonstrate the activity of the 'thylakoid layer'.

In order to get information about the site where the potential change is generated, the dependence of the photovoltage on the ionic strength of the aqueous phase is investigated. Fig. 5 shows that the amplitude of the photovoltage from 'thylakoid layers' does not depend on the NaCl concentration. Hence, it is likely that the charge displacement originates in a region not accessible to ions. If the generating process would result from the change of a diffuse ionic double layer the photovoltage should depend on the ionic strength

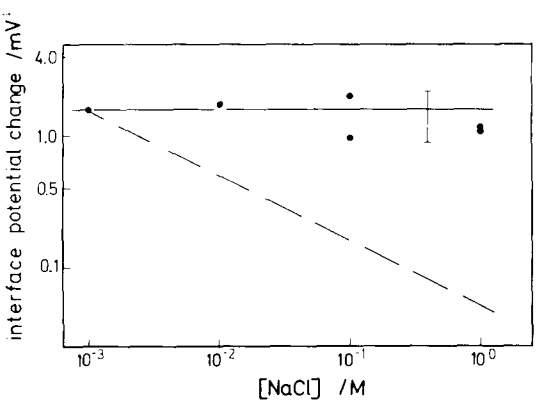


Fig. 5. Dependence of the interface potential change on the NaCl concentration in the aqueous subphase. The error bar indicates variations between individual 'thylakoid layers'. — — —, the theoretical dependence for the case that the photopotential would arise from an electrochemical double layer. The line is calculated from the Gouy-Chapman theory for a 1-1 electrolyte and for small potential changes [28] (less than or equal to 10 mV).

as described by the Gouy-Chapman theory. This hypothetical dependence is indicated by the dashed line in Fig. 5 (for discussion see Ref. 28).

All these results indicate that the observed photovoltage from chloroplasts spread at the heptane-water interface behaves, with respect to the examined properties, similar to the flash-induced transmembrane potential change in intact thylakoids.

It may be asked whether the interface potential change arises from a layer of closed thylakoid vesicles or from a layer of disrupted thylakoid membranes. From other experiments it is known that photoelectric signals can be measured in suspensions of chloroplasts subjected to a light gradient [3,4]. In this case the photovoltage is explained by the fact that the membrane side directed to the light source absorbs more light than the rear side. Consequently, the polarity of the measured potential depends on the direction of the incident light. However, it is demonstrated in Fig. 6 that the polarity in the layer experiment is independent of whether the flash is sent from the top or from the bottom to the 'thylakoid layer'.

Fig. 7 shows a quantitative spreading experiment. The interface tension of a clean *n*-heptane-water interface is measured to be 50 mN/m in agreement with literature data. Addition of increasing amounts of chloroplasts to the interface (area: 25 cm^2) decreases the interface tension to a limiting value of (20 mN/m. The half-maximal effect is observed upon addition of an amount of chloroplasts corresponding to $0.5 \cdot 10^{-9}$ mol chlorophyll. A comparison of the calculated thylakoid membrane area corresponding to this amount of chlorophyl makes a layer of thylakoid vesicles at the interface unlikely but

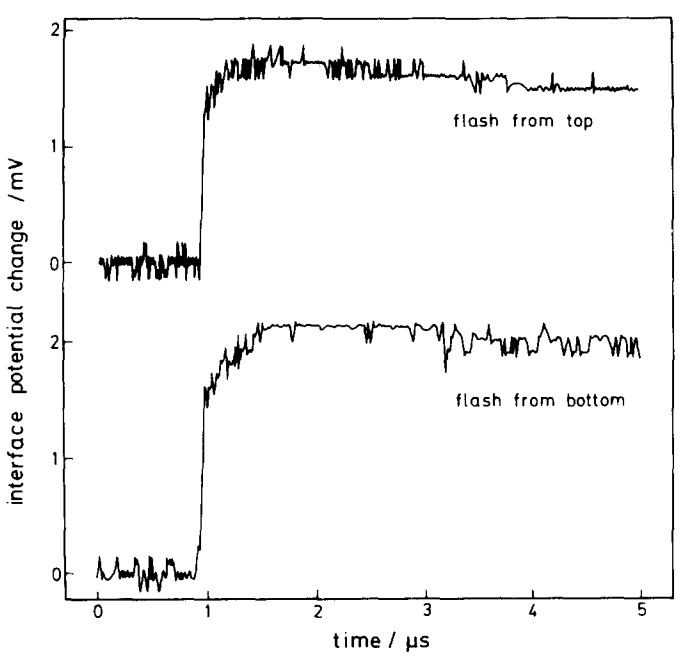
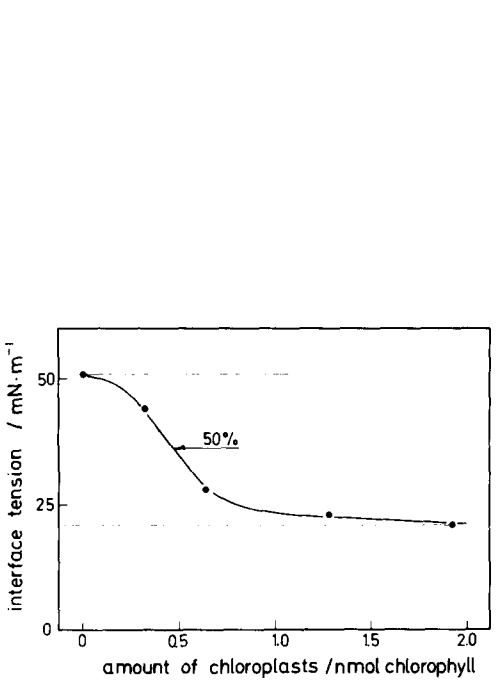


Fig. 6. Interface potential changes from a 'thylakoid layer' flashed from the top and from the bottom. Aqueous subphase: 10^{-2} M NaCl, pH 6.5.



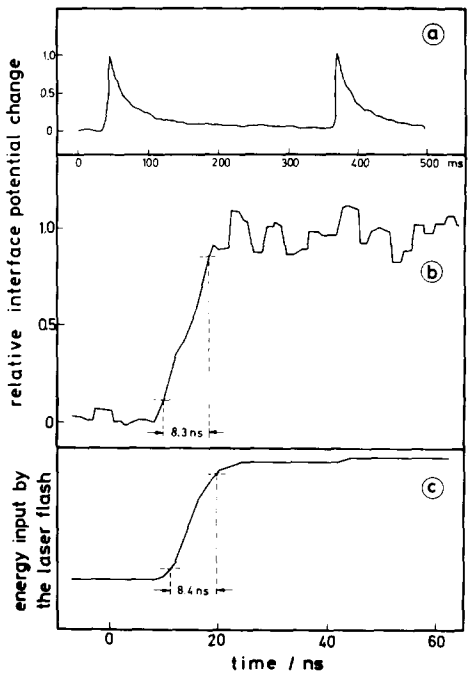


Fig. 7. Interface tension as a function of the amount of chloroplast added to a clean *n*-heptane/1 M NaCl interface of an area of 25 cm^2 as measured by the Wilhelmy plate method.

Fig. 8. (a) Complete time course of the relative interface potential change from a 'thylakoid layer' under repetitive measuring conditions. Laser flash frequency: 3 Hz; 16 accumulated sweeps; electrometer amplifier; aqueous subphase: 10^{-1} M NaCl, pH 6.5. (b) Time course of the rising phase of the relative interface potential change from a 'thylakoid layer' with the highest time resolution of the apparatus (pulse amplifier). The auxiliary dashed lines show how the 10—90% values are read. Aqueous subphase: 1 M NaCl, pH 6.5. (c) Response time of the apparatus including the integrated time course of the laser flash as measured with a fast photodiode.

rather supports a kind of monomolecular architecture (see Discussion).

In Fig. 8a the complete time course of the photovoltage from a 'thylakoid layer' is shown. The decay time of the photovoltage is in the order of 50 ms and depends only little on the ionic strength. (The decay is not caused by the capacitative coupling [7].)

The rising phase of the signal is shown in Fig. 8b under the condition of highest time resolution of the apparatus. Analysis of the trace yields a 10—90% rise time of τ_t = 8.3 ns. For comparison, Fig. 8c shows the response time of the apparatus including the integrated time course of the laser flash (see Materials and Methods). A 10—90% rise time of τ_a = 8.4 ns is found for the response of the apparatus.

The mean values of ten such single-shot experiments with different 'thylakoid layers' are found to be for both $\overline{\tau}_t$ and $\overline{\tau}_a$ 9 ns with a standard deviation of ±1 ns. From these data the rise time of the photoreaction $\overline{\tau}_{ph}$ can be calculated [6] by:

$$\overline{\tau}_{ph}^2 = \overline{\tau}_t^2 - \overline{\tau}_a^2$$

The upper limit of τ_{ph} is estimated assuming the upper limit of τ_t ($\overline{\tau}_t$ = 10 ns)

and assuming the lower limit of τ_a ($\overline{\tau}_a$ = 8 ns):

$\overline{\tau}_{ph} \leqslant 6$ ns

Therefore, the half-rise time of the photoreaction is

$\overline{\tau}_{ph,0.5} \leqslant 3$ ns

Also Photosystem I vesicles (prepared as described in Ref. 13; PS I/PS II approx. 12) and 'inside-out' thylakoids [14,15] have been spread at the heptane-water interface. In both cases similar signals are observed, especially the same polarities.

Discussion

It is well documented from in vitro experiments that under suitable conditions excited chlorophyll, carotenoids and other dyes are able to generate photoelectric effects at interfaces of lipid bilayer membranes [16—21]. However, these effects are not the same as the primary charge separation process in photosynthesis which takes place in highly specialized pigment-protein complexes (reaction centers) asymmetrically embedded in the thylakoid membranes. Therefore, in the following the properties of the observed photoelectric signals from 'thylakoid layers' are compared with the characteristic properties known from the photosynthetic charge separation occurring in the intact thylakoid membranes of chloroplasts.

An unspecific photochemistry of two of the chloroplast pigments can be excluded with the following control experiments: Monolayers from pure lutein and pure chlorophyll *a* are prepared at the heptane-water interface and flashed under identical conditions as the 'thylakoid layers'. The lutein layer is completely unresponsive whereas the chlorophyll layer displays a negative signal of about 0.5 mV. In contrast to the 'thylakoid layers' the photovoltage from the chlorophyll layer is unaffected by background illumination (same background light as in Fig. 3b). The negative polarity of the signal and its insensitivity to background illumination indicates that the response of the chlorophyll layer is basically different from the response of a 'thylakoid layer'. Since chlorophyll is present in a high concentration in the thylakoid membrane one might have suspected that the chlorophyll could be molecularly dissolved into the interface during the spreading process. In this case a negative component of the photovoltage from the 'thylakoid layers' would be expected. Such a negative signal should be visible, especially when the response from photosynthetic processes is suppressed, i.e. in Figs. 1b and 3b. Since this is not the case this result is a first indication that the pigment-protein complexes of photosynthesis are not significantly disassembled at the interface.

Five different experiments show that the properties of the interface potential change from 'thylakoid layers' resemble those of the light-induced transmembrane potential change in intact thylakoids.

1. The electron transport inhibitor DCMU abolishes the photovoltage. DCMU is known to inhibit the electron transport by binding to a protein at the acceptor site of Photosystem II [10,11]. Reactivation of Photosystem I by addition of *N*-methylphenazonium methosulfate in the presence of DCMU

leads again to a photoelectric signal which is about half of that when both photosystems are working (Fig. 1). This finding is in accordance with corresponding measurements of the electrochromic absorption change in chloroplast suspensions which indicate that both photosystems contribute to about one half to the transmembrane potential [1,22].

2. In the thylakoid membrane each reaction center is surrounded by about 200 antenna pigments which channel absorbed light quanta to the reaction centers. These antenna systems characteristic for plant photosynthesis supply the reaction centers very efficiently with light quanta so that the photoreaction is saturated at relatively low light intensities. In order to find out to what degree the antenna system is functioning in the 'thylakoid layer' the light intensity of red background light to reach 85% saturation of the photoreaction was compared with the corresponding value for chloroplast suspensions. As quoted in Results the red background light intensity necessary to saturate 85% of the electrochromic absorption change at 515 nm is $5.8 \cdot 10^{15}$ quanta/cm^2 per s. This value has to be corrected for the absorption within the cuvette (transmission at 684 nm: $T = 35\%$), since only those thylakoids being located at the light entrance side of the cuvette are exposed to that intensity. Thylakoids at the rear side see only 35% of that intensity. For simplicity we compare the background light intensity in the middle of the cuvette, $3.4 \cdot 10^{15}$ quanta/cm^2 per s, with the $4.5 \cdot 10^{15}$ quanta/cm^2 per s found for the 'thylakoid layer'. Both values almost coincide and one has to conclude that the functional organization of the antenna pigment system and the connection between the reaction centers are not significantly altered in the 'thylakoid layer'.

3. The curve in Fig. 2 shows that the laser flash is not saturating, but with a 20 μs flash lamp enough energy can be provided to reach saturation and routinely photovoltages of about 5 mV are observed (from some layers even 10 mV have been measured). This saturation behavior can be utilized again to compare the 'thylakoid layer' with the chloroplast suspension: to induce the half-maximal electrochromic absorption change in a chloroplast suspension 80% of that flash energy is sufficient which is necessary to induce the half-maximal photovoltage in the 'thylakoid layer'. If one accounts for the absorption within the cuvette (transmission at 531 nm: $T = 61\%$) and again compares the flash energy in the middle of the cuvette as described above, then this value becomes 65%. This points out again the similarity between the organization of the antenna pigment system in the 'thylakoid layer' and in the chloroplast suspension. It should be mentioned that a measurement of the electrochromic absorption change with a 'thylakoid layer' is not feasible at present because the amount of chloroplasts in the 'thylakoid layer' is almost 200 times smaller than necessary for the detection of an optical absorption change.

4. It can be seen in Fig. 4 that the photovoltage decreases with time. The effect is more pronounced at high salt concentrations. In contrast, the photovoltage from a pure chlorophyll layer is stable for more than 1 h (data not shown). We interpret this result by the well-known effect that proteins may unfold more or less rapidly at interfaces with a concomitant loss in activity (for review see Ref. 23). Accordingly, the generating mechanism of the photovoltage from the 'thylakoid layers' seems to be located within a pigment-protein complex. For comparison the inactivation of chloroplasts suspended in

1 M NaCl is investigated as measured by the amplitude of the electrochromic absorption change. This inactivation is comparable to that shown in Fig. 4, however, the stability is about ten times higher.

5. As seen in Fig. 5 the amplitude of the photovoltage immediately after the spreading process is independent of the ion concentration in the aqueous subphase within the experimental error. Hence, the generating process seems to occur at a site which is not reached by ions. If, for example, the photovoltage would be created by a charge separation between an electron donor bound at the interface and an acceptor in the aqueous phase, then such a photovoltage should decrease with increasing ion concentration according to the Gouy-Chapman theory of the electrochemical double layer. This hypothetical dependence is depicted in Fig. 5 by the dashed line. Obviously, a generating mechanism based on such a process can be excluded. This property is also found for chloroplasts: the electrochromic absorption change in chloroplast suspensions indicates under single-turnover conditions that the transmembrane charge separation is independent of the ion concentration [1,2]. (In continuous light, however, the electrochromic absorption change indicates the superposition of the transmembrane charge separation and the change of the surface potential. The latter does depend on the ion concentration [1].)

With respect to these criteria the 'thylakoid layer' at the heptane-water interface shows properties very similar to those known for the primary photosynthetic process in intact chloroplasts. Therefore, we suggest the interpretation that the photovoltage arising from the 'thylakoid layer' reflects the primary charge separation in photosynthesis.

In the preceding paper [7] the electrical properties of the capacitative electrode have been determined by feeding external voltage pulses into the measuring device. However, the question had been remained open whether this procedure is electrically equivalent to voltage pulses which are generated at the interface. This particular study with a photosynthetic interfacial layer offers a possibility to test this assumption.

Measurements of the electrochromic bandshift of chloroplast suspensions have shown that the potential change across the thylakoid membrane generated by a short laser flash has a half-rise time $\leqslant$20 ns, whereas the half-life time of the decay is between 10 and 100 ms [2,6]. Hence, in the microsecond range the potential change due to the photosynthetic charge separation is a step function. Such a step function should also be expected for the 'thylakoid layer'. Therefore, the photosynthetic charge separation may be used as an 'interface test pulse'. As seen in Figs. 1, 3, 6 and 8b the measured photovoltage is indeed a step function. This result demonstrates first the high pulse fidelity of the capacitative electrode and second it shows that the application of external test pulses is an appropriate method to control the transmission characteristics of the capacitative electrode. Furthermore, it is noteworthy to mention that the results can be understood and interpreted without the necessity of using the concept of the 'chemical capacitance' introduced by Hong and Mauzerall [24].

Based on the two experiments depicted in Figs. 2 and 6 one can exclude that the observed photovoltage originates from a layer of thylakoid vesicles being asymmetrically charged due to the different light absorption in the upper and lower sides of a vesicle (non-saturating flashes) [3,4]: first, the polarity of the

photovoltage from the layer is independent of the incident light direction and second, the photovoltage saturates at high-flash energies in contrast to a decrease of the photovoltage from chloroplast suspensions subjected to saturating flashes [3].

An argument that the chloroplasts may form a sort of monolayer at the interface can be derived from the results shown in Fig. 7. The amount of chloroplasts necessary to cause a half-maximal decrease of the interface tension corresponds to $0.5 \cdot 10^{-9}$ mol chlorophyll. It is estimated that twice the amount of chloroplasts is sufficient to yield a densely packed layer over the experimental area of 25 cm^2.

The total thylakoid membrane area present in that amount of chloroplasts can be calculated separately from literature data. Assuming a membrane area of $4 \cdot 10^7$ $Å^2$ for one thylakoid [25] and assuming $1.1 \cdot 10^5$—$1.8 \cdot 10^5$ chlorophyll molecules per thylakoid [25—27] then the membranes corresponding to 10^{-9} mol chlorophyll would cover 13—22 cm^2. If the thylakoid membrane would be a bimolecular membrane and if it is transformed at the interface into a monolayer, then an area of 26—44 cm^2 would result. If, however, a thylakoid vesicle layer would be present at the interface an area of only 7—11 cm^2 could be covered.

A comparison of these calculated areas with the experimentally given heptane-water interface (25 cm^2) makes it most likely that the spreading process leads to a rupture of the thylakoid vesicles forming a thylakoid interfacial layer with a structure which is between a bilayer and a monolayer. This interpretation is in accordance with recent studies of Pattus et al. [29] reporting the rupture of liposomes at the clean air-water interface.

Further evidence for the rupture of thylakoids at the interface is provided by the observation that spreading of chloroplasts, Photosystem I vesicles and 'inside-out' chloroplasts lead to interfacial layers displaying the same polarity of the photovoltage (heptane phase positive). One has to assume therefore, that the orientation of the reaction centers is determined by their amphiphilic properties alone and not by the type and the sidedness of the different vesicles spread. As concluded from the polarity of the photovoltage the electron donor sites of both photosystems behave as more hydrophobic than the acceptor sites when they are brought to the interface. It seems that the clean heptane-water interface breaks the thylakoid vesicles and the membrane components (proteins, for example the reaction centers, and lipids) orient individually according to their amphiphilic properties.

In chloroplasts the transmembrane potential difference generated by a single-turnover flash is about 50 mV according to different methods [1]. If such intact membranes would be perfectly oriented at the interface, this potential difference should be measurable. However, the routinely measured photovoltage in saturating flashes is about 5 mV although up to 10 mV have been observed occasionally. This discrepancy of a factor 5—10 can be explained by a dilution effect caused by the heptane penetrating the membrane which leads additionally to a concomitant loss of the degree of orientation of the reaction centers.

In Fig. 8a the decay of the photovoltage from the 'thylakoid layer' is shown. The half-life time of the decay is in the order of 50 ms. As discussed above the

fast rise of the signal most likely reflects the primary charge separation in the reaction centers. However, based on the present results it cannot be decided whether the decay represents a recombination of the separated charges or a potential equilibration caused by an ion flux through the interface phase (similar as in intact thylakoids).

Fig. 8b shows the time course of the photovoltage with the highest time resolution of the apparatus. From such experiments the upper limit of the half-rise time of the photoreaction is calculated to be less than 3 ns. We conclude for the reasons discussed above that the primary process of charge separation in plant photosynthesis is also faster than 3 ns. Since this charge separation is thought to be realized by the photooxidation of the reaction center chlorophylls [1] the oxidation of both, chlorophyll a_I and chlorophyll a_{II}, must be as fast.

In our present experiments the duration of the laser flash (Fig. 8c) limits the time resolution whereas the capacitative electrode would allow for measurements of much faster events. Experiments are in preparation to increase the time resolution by one order of magnitude.

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