Biochimica et Biophysica Acta, 767 (1984) 223–230
Elsevier

BBA 41603

EXTERNAL ELECTRIC FIELD EFFECTS ON PHOTOSYNTHETIC MEMBRANE VESICLES

KINETIC CHARACTERIZATION OF TWO ELECTROPHOTOLUMINESCENCE PHASES IN HYPOTONICALLY SWOLLEN CHLOROPLASTS

MARC SYMONS [a,b], SHMUEL MALKIN [b] and RAFI KORENSTEIN [a]

Departments of [a] Membrane Research and [b] Biochemistry, The Weizmann Institute of Science, Rehovot 76100 (Israel)

(Received May 11th, 1984)

Key words: Electrophotoluminescence; Membrane vesicle; Thylakoid membrane; Delayed luminescence; Electric field effect; (Lettuce, spinach chloroplast)

Strong externally applied electrical field pulses are known to stimulate delayed luminescence from preilluminated blebs (hypotonically swollen vesicles originating from thylakoid membranes of broken chloroplasts) by up to 3 orders of magnitude. This phenomenon is known as electrophotoluminescence. Previous analysis showed the kinetics of the electrophotoluminescence to be biphasic, displaying a rapid (R) phase which decays towards a slower one (S) (Ellenson, J.L. and Sauer, K. (1976) Photochem. Photobiol. 23, 113–123). We demonstrate that these two components represent different processes. At low pH, a good kinetic separation is obtained between the two phases, which become distinct, with the S phase manifesting also an initial rise period. Under these conditions, it is possible to estimate separately the approximate rise times of the two phases. It is shown that the R and S components have a different dependence on the pH and on the time between the actinic flash and onset of the field. The field dependence is also different, with the S phase requiring a lower threshold field than R. From these observations, it is concluded that the R and S luminescence components are formed by different precursors. The difference in behaviour of the two phases during formation of the bleb indicates that the precursors of the R and S phases belong to different parts of the bleb. We suggest that R precursors are located in the wall of the swollen thylakoid and S precursors in the membrane formations which are attached to this wall.

Introduction

Delayed luminescence from photosynthetic membranes presumably results from a reversal of the light-induced charge separation [1–3]. It is very sensitive to the prevailing conditions of the membrane [4]. For instance, in blebs (i.e., swollen vesicles originating from the chloroplasts under hypotonic conditions) externally applied electrical fields can stimulate the luminescence up to 3 orders of magnitude [5]. This phenomenon, called electrophotoluminiescence [5], was first reported by Arnold and Azzi [6] and studied extensively by Ellenson and Sauer [5]. Through its dependence on the experimental conditions, including photosynthetic-related parameters (Photosystem II activity, preillumination, presence of inhibitors and membrane permeability) and parameters of the suspension medium (osmolarity, viscosity, electrical conductivity, pH and temperature), analysis of electrophotoluminescence promises to be a valuable

Abbreviations: DCMU, 3-(3-4-dichlorophenyl)-1,1-dimethylurea; PS II, Photosystem II.

tool for monitoring structural and functional parameters of the photosynthetic membrane [7,8,19,20]. However, the present state of understanding of this phenomenon is still unsatisfactory in terms of specifying a detailed mechanism [7–9], except for a general belief that it involves recombination of charges formed by the obligatory preillumination [1].

Previous analysis of the kinetics of electrophotoluminescence for chloroplasts suspended in distilled water showed that upon onset of the external electrical field, the luminescence rises rapidly (within 50 μs) to a maximum (R phase) and subsequently decays with multiphasic kinetics, rapid (typically within 400 μs) and slow (in the ms time range). The slow S phase was rather arbitrarily defined as the extent of the stimulated luminescence at some time during the slow decay [5,9]. The rise of the R phase appears to be determined by the charging capacitance of the membrane with a requirement for a threshold value of the stimulating electric field [9]. The purpose of this research is to characterize the R and S components and to find the conditions under which they can be studied separately. Furthermore, we demonstrate that the two phases belong to different precursors and argue that these precursors are located in different parts of the bleb.

A preliminary account of part of this work has been presented at the VIth International Congress of Photosynthesis.

Materials and Methods

Class C chloroplasts from lettuce and spinach were prepared according to Avron [10]. The isolation medium routinely comprised 0.4 M sucrose/10 mM NaCl/5 mM $MgCl_2$/20 μM ascorbic acid/30 mM Tris (pH 7.5). The washing and storage medium comprised 0.4 M sucrose/10 mM Tris (pH 7.5). The chloroplasts were freshly used or stored at liquid nitrogen temperature. In the latter case, the storage medium was supplemented by 30% ethylene glycol [11]. The results were independent of the way of storage. In some cases, 10 mM NaCl and 5 mM $MgCl_2$ were added to the washing and storage media (see Discussion). Upon dilution in various hypotonic media (typically by a factor of 500), blebs [8,13,14] were formed. The blebs were viewed and characterized by a phase-contrast microscope equipped with an image-intensified video camera, as described previously [12]. They had a spectrum of diameters ranging up to 20 μm. In distilled water, the average diameter of the blebs was between 3 and 11 μm, depending on the preparation. In a buffered suspension (10 mM Tris), the average size was about 25% smaller, and virtually pH-independent. However, in these conditions, a substantial amount of the chloroplasts did not seem to bleb, amounting to about 30 and 20% at pH 6 and 9, respectively. This did not influence the results to any significant extent, since electrophotoluminescence from chloroplasts suspended in an isotonic medium is about an order of magnitude smaller. In distilled water, virtually all the chloroplasts appeared to form blebs. Full bleb formation, as monitored by electric-field-stimulated luminescence, could take up to an hour or more, depending on the preparation and pH (see Results), the slower-formed blebs remaining stable for several hours at room temperature. We routinely prepared the blebs by incubating the broken chloroplasts during the appropriate time in the respective media at room temperature. The suspension was then transferred to ice until measured. Special care was taken not to break the extremely fragile bleb membrane formations: shaking, handling with microsyringes or even the slight pressure of a microscopic cover glass was sufficient to destroy part of the material. We observed substantial variations in the ratio of the extent of the R and S luminescence components and in the separation between their rise times depending on the preparation, in otherwise similar conditions, however. The time needed to complete bleb formation was also strongly variable. No systematic difference was observed between material from different origin (i.e, lettuce or spinach). Bleb formation was faster however with fresh chloroplasts than with those stored in liquid nitrogen. Moreover, in the latter case, at low pH, S was more predominant. Final chlorophyll concentration varied between 5 and 20 μg/ml. Specific conductivity was measured with a conductometer (Radiometer, Copenhagen).

The experimental set-up was essentially as described by Farkas et al. [15]. The sample holder was provided by a pair of stainless-steel electrodes

with a variable gap. A typical experiment started by preillumination with either a 10 μs flash or with continuous illumination of variable duration, both filtered by a Corning 4-96 filter limiting the wavelength band to approx. 400–600 nm. After a variable dark time, t_d, an external electrical field pulse was applied. The minimum t_d value was limited by the shutter system: 8 ms after a flash and 21 ms after continuous illumination. The electric field pulses were delivered by a high-voltage pulser (Cober 606) capable of delivering rectangular direct current pulses of up to 2500 V with a duration t_E between 20 and 5000 μs. For the double-pulse experiments, a trigger system was used eliciting two pulses of the same polarity with a variable time between them. All experiments were carried out at room temperature.

Results

The kinetics of the electric-field-induced luminescence obtained from chloroplasts suspended in Tris buffer at different pH are shown in Fig. 1. At pH 4.5, the emission intensity has two maxima (Fig. 1A), indicating the existence of two components, which we will term R and S, respectively. These components can be characterized by their respective rise times t^R and t^S, defined here as the time needed to reach the respective maxima from the onset of the field. At higher pH, the slow phase may be visible as a shoulder or may be completely buried under the fast phase (Fig. 1B and C). Previously, the electric-field-stimulated luminescence was only observed under conditions showing a similar pattern as in Fig. 1C. R was also defined as the maximum extent of the fast-rising component, but what then has been defined as S phase represents evidently only the tailing edge of the slow component [5,9]. This is corroborated by the fact that under conditions where the maximum of the S phase is usually not resolved from the R phase, e.g., at pH 9, as depicted in Fig. 1C, S is distinctly visible during the initial stages of bleb formation. This is demonstrated in Fig. 2, showing kinetics of stimulated luminescence at different times during the swelling process of the thylakoids at pH 9. It is seen that during the first 0.5 min, the S phase is observed alone and only afterwards the R phase develops. Subsequently, as bleb formation progresses, the S phase becomes hidden under the R phase.

Another parameter which differentiates the two phases is the polarization of the emission: it was demonstrated previously that the R phase was polarized (perpendicular to the field) whereas the S phase was not [15]. This is actually what is observed at low pH (Fig. 3), where there is a clear separation between the two phases. The polarization extent decays in time and tends already to zero when the S phase is still growing.

Fig. 4 shows the electric field dependencies of the peak values of the R and S phases. Corre-

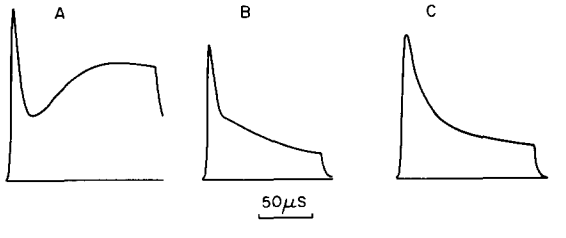

Fig. 1. Kinetics of electric-field-induced luminescence of lettuce blebs suspended in various media. Applied field strength is 1.6 $kV \cdot cm^{-1}$. t_d after flash is 10 ms. Medium contains 10 mM Tris. A, pH 4.5, B, pH 7, C, pH 8. Bleb formation time is 20 min, 1 h and 2 h, respectively.

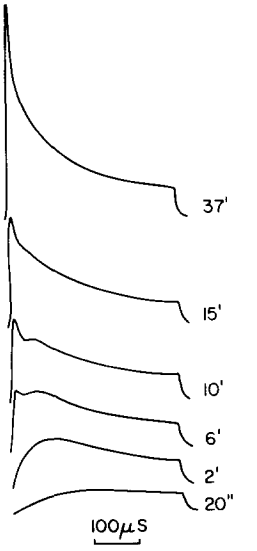

Fig. 2. Kinetics of electric-field-induced luminescence at various times during formation of blebs from lettuce chloroplasts. Suspension medium is 10 mM Tris (pH 9). Other conditions as in Fig. 1.

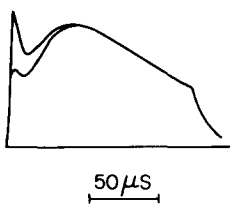

Fig. 3. Polarization of electric-field-induced luminescence. Suspension medium is 10 mM Tris (pH 9). t_d after flash is 10 ms. Applied field strength is 2 kV·cm^{-1}. Upper and lower traces are polarized ⊥ and ||, respectively.

sponding kinetics of the stimulated luminescence at three different external field strengths are depicted in Fig. 5. Although there is an overlap of the two phases, for the present purpose the maximum extents of R and S are a sufficiently accurate measure of the relative size of the two components. As seen in Fig. 4, the field dependencies of both R and S follow a similar trend. There are significant differences however, which are mostly

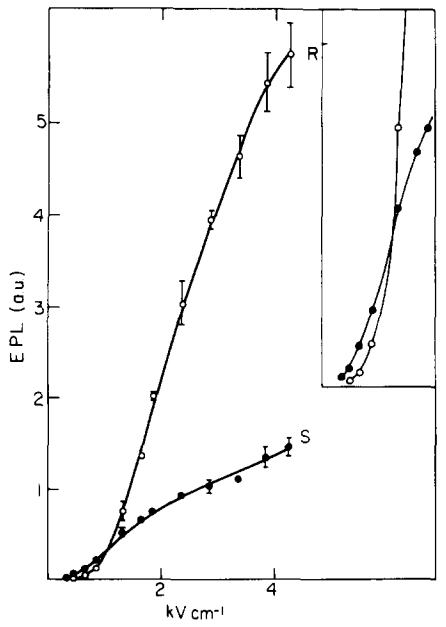

Fig. 4. External electric field dependence of R and S components of electric-field-induced luminescence. t_d after flash is 10 ms. ○——○, R component; ●——●, S component. Points are an average of 3 or 4 measurements. Error bars mark standard deviations. For each experiment, another sample was taken. Suspension medium is 8 mM Tris (pH 4.5). Bleb formation time, 15 min. Inset, expansion of the low external field region. EPL, electrophotoluminescence.

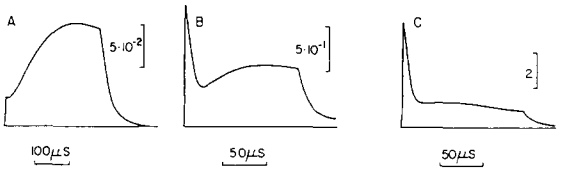

Fig. 5. Kinetics of electric-field-induced luminescence at various external electrical field strengths. (A) $E = 0.9$ kV·cm^{-1}; (B) $E = 1.2$ kV·cm^{-1}; (C) $E = 4$ kV·m^{-1}. Other conditions as in Fig. 4.

marked at lower fields. Part of these differences can be accounted for by a difference in threshold field (the minimal field strength below which no stimulation of the luminescence occurs). The effect of this threshold is evidently more pronounced at low external electrical fields. Farkas et al. [9] suggested the existence of a threshold in order to explain the field dependence as well as the initial kinetics of the field-induced luminescence. They experimentally determined the value of the (intramembrane) threshold field for the R phase to be about 240 mV [9]. In Fig. 4, one can thus see that there is a difference between R and S components with respect to the threshold field and that the threshold for S is lower than for R. This is substantiated by the fact that the two curves cross each other. Due to increased overlap between the two components, it is much more difficult to observe this phenomenon above pH 5 or 6. Another difference between the two phases is that, for field strengths smaller than 1.5 kV · m^{-1}, the field dependence of R is significantly stronger than that of S. The field dependence of both R and S show a tendency to saturate at higher fields. This is substantiated by the presence of an inflection point which is located at about the same field (2 kV · cm^{-1}) for the two components. The trend towards saturation could be brought about by electroperforation of the membrane [8,16]. This electrically induced breakdown limits the membrane potential generated by the external field by causing a drastic increase in the conductance of the membrane [17].

The R and S differ also in their dependence on the dark time between preillumination and the electrical pulse. Increasing the dark time from 10 ms to 1 s at pH 4.5 and for an applied field of 2.8 kV · cm^{-1} causes R to decrease by a factor of 120,

whereas S decreases by less than a factor of 20. Similar differences between R and S were observed at higher pH or in distilled water.

The degree to which the two phases can be separated is evidently determined by the difference in their respective rise times t^R and t^S. The rise times of both R and S become shorter with increasing field strength. They are also affected in a similar way by increasing the dark time from 10 ms to 1 s; both t^R and t^S increase by about 30% in this case. The pH affects t^R and t^S in an opposite way, however. The t^R increases from pH 4.5 to 9, which can be quantitatively explained by the lower conductivity of the medium at high pH (cf. Ref. 9). The t^S decreases markedly in the same pH range, a phenomenon which at this moment we cannot explain.

The inset of Fig. 6 shows the kinetic of the field-stimulated luminescence in a double-pulse experiment for two different preillumination times. In these experiments, the blebs are exposed to a sequence of two pulses of identical polarity, instead of a single rectangular one. The blebs, here, are formed in distilled water. It can be seen that in the second pulse only the S phase remains: all the precursors creating the R component seem to have been depleted. In this way, for some conditions, we can study the two phases separately. A detailed analysis of these double-pulse experiments will be covered in a subsequent paper. Suffice it to say here that these experiments clearly illustrate that the precursors of R and S are different. The complete preillumination time dependence of the two compounds is presented in Fig. 6. It can be seen that the illumination time required for half saturation of S is more than 5-times longer than for R and this holds for all the intensities of actinic illumination tested. In the presence of DCMU, this effect is less marked but still significant (about a factor of 2). Furthermore, when preillumination is provided by a short light flash, the S/R ratio is twice as large in the presence than in the absence of DCMU.

As already demonstrated in Fig. 2, it is possible to follow the process of bleb formation by monitoring the electric field luminescence at different times after dilution of the chloroplast in the suspension medium. The shortest time lapse possible between the initiation of bleb formation and the first measurement was about 20 s. Fig. 7 shows how both phases develop at pH 4.5. The S phase seems to be fully developed within 20 s and decays before R reaches its maximum, which takes about 7 min. Afterwards, R and S decay at a similar pace. The difference in the formation of the two components indicates that, at least to some extent, they arise independently of each other. Similar discrepancies between the development of R and S

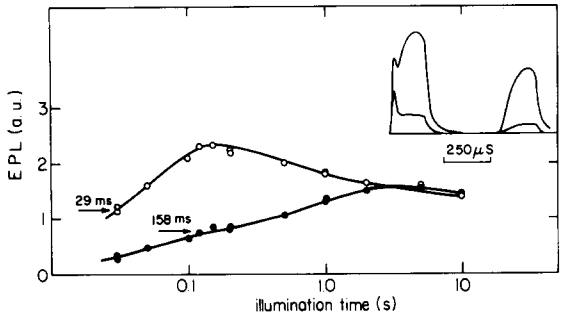

Fig. 6. Preillumination time dependence of R and S components of electric field luminescence. t_d = 21 ms. External field 1.6 kV·cm^{-1}. Illumination intensity is 0.6 nE·cm^{-2}, which is about 1% of saturating light intensity. Suspension medium, distilled water. Bleb formation time is 1 h. Inset, kinetics of the luminescence stimulated by two unipolar field pulses corresponding to (1) 30 ms and (2) 5 s illumination time. EPL, electrophotoluminescence.

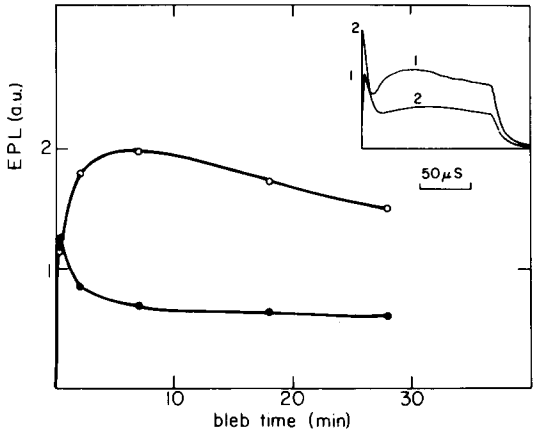

Fig. 7. Rise and decay of R and S components of electric-field-induced luminescence during formation of bleb from lettuce chloroplasts at pH 4.5. Suspension medium, 8 mM Tris. t_d after flash is 10 ms. Applied field strength is 2 kV·cm^{-1}. ○——○, R phase; ●——●, S phase. Inset, kinetics of electrophotoluminescence (EPL) at (1) 20 s and (2) 7 min.

have been observed at higher pH, but here the swelling process is substantially slower: at pH 6, full formation of S takes about 2 min. R is only half formed during this time, taking more than 1 h to reach saturation. At pH 9, half formation of S and R takes about 1 and 30 min, respectively (see Fig. 2). Here, R was maximally developed after 3 h and decayed slowly thereafter. Bleb formation at 0 °C takes about 5-times as long. There is a strong variation of the kinetics of the swelling process depending on the preparation, however.

Discussion

In this work, the R and S phases of the electric-field-induced luminescence reported previously [5,7,9] were further characterized. We show that the S phase is a separate component from R and is probably always present, even when only a single emission component is apparent at first sight. It is the difference between the respective rise times of R and S, t^R and t^S, which will determine whether S will be differentiated as a separate phase. In those cases where t^S is close to t^R (e.g., at high pH; see Fig. 1), both R and S are mingled and show up as a single component. This conclusion is important to the study of electric-field-induced luminescence in general, since both phases depend differently on a variety of parameters such as external field strength, pH, dark time, and show different properties of the emission polarization. Therefore, in those cases in which there is no clear separation between the two components, the effect of electrical as well as photosynthetic-related parameters cannot be unambiguously studied.

The kinetic separation of the two components depends on several experiemtnal parameters. The predominant factor is the pH of the final suspension medium: it is shown in Fig. 1 that R and S are separated optimally at low pH (between pH 4.5 and 5). Below pH 4, however, the overall intensity of the luminescence is very much lower. Both t^R and t^S are similarly influenced by the external electrically field strength and the dark time, which implies that the latter parameters are less critical for the separation of the R and S components. These parameters do however drastically alter the ratio between R and S, which also influences the cross-contamination between the two phases. This can be observed in Fig. 5, where at the lower and upper values of the external field, the extents of R and S, are defined less precisely.

The data in this report provide evidence that the R and S components of the electric-field-induced luminescence are created by different precursors. This is particularly substantiated by the marked difference in their dependency on photosynthetic parameters such as the preillumination and dark time, as well as by the effect of DCMU. Concerning the difference in dark time dependency of the two phases, it could be alternatively argued that the induced variation in the R/S ratio (e.g., by a factor of 6 when t_d varied between 10 ms and 1 s) is caused by the decay of the light-induced transmembrane potential, which adds itself to the external field [9]. This argument must be rejected, however. A typical potential difference set up by the light flash is about 50 mV [18]. Given a membrane thickness of 5 nm, this corresponds to 10^5 V · cm^{-1} in the bleb membrane. The maximum value of the intramembrane field E_m created by an externally applied field E is given $E_m = 1.5(R/d)E$, where R is the bleb radius and d the thickness of the bleb membrane [9]. An external field of 2.8 kV · cm^{-1} across a bleb with a radius of 1.5 m amounts then to $1.26 \cdot 10^6$ V · cm^{-1}. The contribution of the actinic-induced potential to the electrical field in the photosynthetic membrane is much lower and thus negligible. Therefore, the light-induced transmembrane potential should not influence the R/S ratio to any significant extent.

As shown in Fig. 3, a remarkable difference between R and S is that R is polarized and S is not. The small apparent residual polarization of the S phase is probably due to contamination by R, as no such polarization was observed at low field strengths, where S is predominant (Fig. 5A). This also renders support for the R and S components to originate from different precursors. The polarization has been explained by electroselection of membrane regions which have a close to perpendicular orientation with respect to the external field direction and the fact that the emitting transition dipoles are oriented in the plane of the membrane [8,15]. Thus, it could be that the pigment environment of the system which gives rise to S is different and is much less membrane-oriented. This point should be investigated in more detail. It also

has been observed previously that the luminescence polarization decreases with time during the application of the external field [8,15]. De Grooth and Van Gorkom [8] explained the phenomenon describing a mechanism whereby the precursors are first depleted at the top (pole) of the bleb (with respect to the electric field direction) so that the overall emission during field application appears to migrate from the pole to the equator of the bleb, thus decreasing the degree of polarization. From the present work, it appears that the substantial contribution to the polarization decay is caused by the fact that the field-induced luminescence contains indistinguishable R and S components of which the R decays much faster. Thus, the relative extent of the unpolarized S increases with time.

The two luminescence phases show also marked differences in their field dependency, as can be seen in Fig. 4. For instance, the apparent threshold field for S seems to be lower than the one for R. This difference in apparent threshold field could be explained if one assumes that the two luminescence phases reside in different populations of blebs, i.e., if S belongs to bigger blebs the effective field would be larger and an apparent lower threshold would be observed. Such a possibility is not consistent however with the observations of irreversible electrical breakdown, occuring at high electric fields, which are expected to destroy the largest blebs first. On the contrary, it was found that R and S are inhibited to a similar extent by such high fields (data not shown). Similarly, both R and S diminish equally upon shaking the bleb suspension or very mild sonication (1 or 2 s in a sonication bath). Furthermore, from inspection of the blebs by phase contrast microscopy, no indication for two distinct bleb polulations was obtained. Considering the large stimulation of delayed luminescence (an order of magnitude larger than in chloroplasts) to produce the components of electrophotoluminescence by the external electric field pulse, both R and S precursors must be situated in a blebbed membrane system. Therefore, most probably, the precursors of the R and S phases have to be located on one bleb. We can thus infer that the true threshold field to produce S is indeed smaller than the one for R. This difference in threshold field would be even more pronounced if the S precursors reside in the patches attached to the bleb wall, as is discussed below. We estimate the intramembrane threshold field of S to be at the most 40 mV. The threshold of R varied between 50 and 100 mV. The difference in threshold and the fact that R depends more steeply on the external field strength are consistent with the conclusion that the two phases are created by different precursors. The apparent contradiction of our results with previous observations [5,7,9] that S saturates at much lower fields than R, is explained by the fact that in the latter studies, S was defined at some arbitrary point along the decay of the actual S phase. This decay is accelerated at higher fields causing an apparent saturation in the extent of S.

In earlier work [5], it was proposed that the R and S phases could also be characterized by the half-time of the field-off decay, i.e., it was found that for R and S, the respective half-times were 15 and 38 μs. We found, however, that for a situation where a pure S signal can be distinguished (e.g., at pH 4.5 for field duration longer than 50 μs), the half-time of the field-off decay of the S phase substantially increased with the pulse duration. For example, after 400 μs, the half-decay-time is 30 μs, which is twice as long as the half-time after 100 μs. This implies that the field-off decay does not provide a reliable parameter for the differentiation of the two luminescence phases.

The fact that R and S behave independently during bleb formation (see Figs. 2 and 7) strongly suggests that they belong to different regions of the blebbed membrane system. The most likely attribution is that R is located in the bleb wall and S in the patches which are attached to the bleb. Although it is extremely difficult to evaluate the development of R and S during the initial stages of the blebbing process quantitatively, the fact that S develops before R suggests that the membrane system where the S precursors reside is closely related to the unblebbed chloroplast. The patches observed on the bleb wall undoubtedly stem from unblebbed chloroplast material. Furthermore, very mild treatment with a homogenizer of the bleb suspension in distilled water invariably increases R, while S is increased much less or sometimes even decreases. This could suggest that by this careful manipulation, the blebbing process is as-

sisted by donation of material from the patches. The observation that during the course of the blebbing process, S decays long before R reaches its maximum, also points in the same direction. Based on these lines of argument alone, one obviously cannot rule out completely the possibility that the R and S precursors are actually the same, having a different environment which is provided by the bleb wall and patches, respectively.

Acknowledgements

We would like to acknowledge Michael Eisenbach for providing us with the phase-contrast microscopy facilities and Daniel Farkas for stimulating discussions. The research was supported by the United States-Israel Binational Science Foundation.

References

1 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223–317, Academic Press, New York
2 Malkin, S. (1977) in Photosynthesis I, Encyclopedia of Plant Physiology, New Series, Vol. 5 (Trebst, A. and Avron, M., eds.), pp. 473–491, Springer-Verlag, Berlin
3 Amesz, J. and Van Gorkom, H.J. (1978) Annu. Rev. Plant Physiol. 29, 47–66
4 Malkin, S. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 351–431, Elsevier/North Holland Biomedical Press
5 Ellenson, J.L. and Sauer, K. (1976) Photochem. Photobiol. 23, 113–123
6 Arnold, W. and Azzi, J. (1971) Photochem. Photobiol. 14, 233–240
7 Farkas, D.L., Korenstein, R. and Malkin, S. (1981) in Proceedings Vth International Congress on Photosynthesis, Vol. I (Akoyunoglou, G., ed.), pp. 627–636, Balaban, Philadelphia
8 De Grooth, B.G. and Van Gorkom, H.J. (1981) Biochim. Biophys. Acta 635, 445–456
9 Farkas, D.L., Korenstein, R. and Malkin, S. (1984) Biophys. J. 45, 363–373
10 Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272
11 Farkas, D.L. and Malkin S. (1979) Plant Physiol. 64, 942–947
12 Ravid, S. and Eisenbach, M. (1983) J. Bacteriol. 154, 604–611
13 Arnold, W.A. and Azzi, J. (1977) Plant Physiol. 60, 449–451
14 Barber, J. and Malkin, S. (1981) Biochim. Biophys. Acta 634, 344–349
15 Farkas, D.L., Korenstein, R. and Malkin, S. (1980) FEBS Lett. 120, 236–242
16 De Grooth, B.G., Van Gorkom, H.J. and Meiburg, R.F. (1980) Biochim. Biophys. Acta 589, 299–314
17 Zimmermann, U., Pilwat, G., Beckers, F. and Riemann, F. (1976) Biolelectrochem. Bioenerg. 3, 58–83
18 Junge, W. and Jackson, J.B. (1982) in Photosynthesis: Energy Conversion by Plants and Bacteria, Vol. 1 (Govindjee, ed.), pp. 589–646, Academic Press, New York
19 Meiburg, R.F., Van Gorkom, H.J. and Van Dorssen, R.J. (1983) Biochim. Biophys. Acta 724, 352–358
20 Meiburg, R.F., Van Gorkom, H.J. and Van Dorssen, R.J. (1984) Biochim. Biophys. Acta 765, 295–300