Biochimica et Biophysica Acta 894 (1987) 578–582
Elsevier

BBA 40204

BBA Report

Electric-field-induced luminescence emission spectra of Photosystem I and Photosystem II from chloroplasts

Marc Symons [a,b], Shmuel Malkin [a] and Daniel L. Farkas [a]

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

(Received 31 July 1987)

Key words: Electroluminescence; Luminescence; Photosystem I; Photosystem II; Photosynthesis; (Lettuce chloroplast)

The electroluminescence induced by external electric fields in blebs prepared from chloroplasts consists of two kinetically different phases, rapid (R) and slow (S), which were shown to be linked to Photosystem I (PS I) and Photosystem II (PS II) activities, respectively (Symons, M., Korenstein, R. and Malkin, S. (1985) Biochim. Biophys. Acta 806, 305–310). In this report we describe conditions involving heat treatment of broken chloroplasts, which make it possible to observe R phase electroluminescence essentially devoid of any contribution by the S phase. This allowed the precise measurement of the emission spectrum of PS I electroluminescence. The emission spectrum of PS II electroluminescence was obtained using regular broken chloroplasts, which show only S-type emission. The latter emission spectrum is identical to the one obtained for ordinary prompt fluorescence, peaking at 685 nm with a bandwidth of about 25 nm. The PS I emission spectrum is symmetric around 705 nm and is much broader, about 60 nm.

Delayed luminescence from photosynthetic membranes originates presumably through thermally induced back-reactions of the photosynthetic charge separation caused by light [1–3]. Hence, the study of luminescence is a source of information on photosynthetic mechanisms, particularly on cross-membranal electron transfer [4,2]. Due to the complexity of luminescence phenomena, there is an advantage in studying luminescence transients following specific external perturbations to the system, whereby a limited range of relevant variables can be singled out [1,5,6,7]. This is particularly the case in external electric-field-stimulated luminescence (electroluminescence), based on the strong sensitivity of the radiative back reactions to membrane potentials (Ref. 8, cf. also Refs. 1, 2, 3 and 5 for reviews), because here the perturbation may be brief and well controlled [9–13]. Electroluminescence studies have been carried out mostly on blebs, i.e., spherical membrane vesicles derived from broken chloroplasts under extreme low salt conditions by stretching, unfolding and swelling of the thylakoid membrane. In phase-contrast microscopy blebs seem to consist of a unilamellar membrane, with patches attached to part of this surface [13,14]. These patches most probably are remnants of grana stacks [41].

Previous work has shown that in blebs, the electroluminescence consists of two kinetically distinct phases (see Fig. 1): the R (rapid) phase which stems for precursors created in PS I, and

Abbreviations: PS I, Photosystem I; PS II, Photosystem II.

Correspondence: S. Malkin, Biochemistry Department, The Weizmann Institute of Science, Rehovot 76100, Israel.

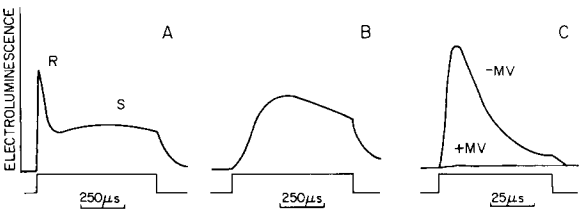

Fig. 1. Kinetics of electric-field-induced luminescence in blebs (A), broken chloroplasts (B) and heat-treated broken chloroplasts suspended in hypotonic medium (C). Blebs (A) were prepared by suspending chloroplasts for 15 min in 10 mM Tris (pH 4.85) and 1 mM $MgCl_2$. Chloroplasts (B) were suspended in the same medium supplemented by 0.4 M sucrose. Heat treatment (C) was performed by incubating the stock chloroplast solution (3 mg/ml chlorophyll) at 50.5 °C for 100 min. Subsequently blebs were formed by dilution of the stock suspension into 2 mM KCl. Final pH was 7.5. 50 μM methyl viologen (MV) was added as indicated. Applied electric field strengths were (A) 1.5 $kV \cdot cm^{-1}$, (B) 7.1 $kV \cdot cm^{-1}$ and (C) 1.2 $kV \cdot cm^{-1}$. The profile of the respective electric-field pulses is included at the bottom.

the S (slow) which originates in PS II [15,16]. The attribution of the two electroluminescence phases to the respective photosystems was substantiated by their relative action spectrum, sensitivity to electron acceptors, electron-transport inhibitors and heat treatment [16]. In regular broken chloroplasts suspended in isotonic medium, the electroluminescence is monophasic, similar to the S phase in blebs and therefore originates solely in PS II (Symons, M., unpublished results). Indeed, delayed luminescence in green plants predominantly arises in PS II [1–4]. Nevertheless, luminescence originating in PS I has also been reported (see Refs. 17–21 for an overview of the literature), although the evidence is not always clear-cut. The reason for the distinctly observable luminescence from PS I in blebs is probably due to the segregation of PS I into the unilamellar bleb wall in which the induced electrical field, being proportional to the radius [12] is much larger than for the attached patches [41]. The few published room-temperature emission spectra thought to arise in PS I were obtained mostly indirectly, involving subtraction of two similar spectra [28], analysis of decay components of picosecond fluorescence signals [29,40] or else by using PS I particles, obtained after elaborate procedures and which contain varying amounts of antenna pigments [30–34].

In this report we discuss the experimental conditions which make it possible to observe PS I electroluminescence devoid of PS II contamination. We present a well-resolved and accurate emission spectrum of PS I electroluminescence as well as that of PS II, with a comparison of the two.

Broken (class C) chloroplasts from lettuce were prepared as in Ref. 16. For long storage, the chloroplasts were resuspended in a medium containing 0.4 M sucrose, 10 mM Tris (pH 7.5), 1 mM $MgCl_2$ and 30% (v/v) of ethyleneglycol [22], and kept in a liquid nitrogen dewar. Blebs were formed by dilution (typically 500-fold) of the chloroplast stock suspension in various hypotonic media. All experiments were performed at room temperature (22 °C). The chlorophyll concentration of the samples was usually about 10 μg/ml.

Electric-field pulses were applied between a pair of parallel stainless-steel electrodes with an adjustable gap. The smallest gap (2 mm) allowed for pulsed rectangular fields of up to 12 $kV \cdot cm^{-1}$. The experimental protocol consisted of preillumination with a saturating 10 μs flash filtered by Corning 4-96 glass (approximate transmission wavelength band 390–600 nm), a delay of 10 ms, followed by application of the external-electric-field pulse. In kinetic experiments the resulting luminescence was passed through a RG 665 cut-off filter (Schott) to the photodetector (EMI 9558B) and was monitored on a fast oscilloscope (Tektronix 7623A). For measurements of the emission spectrum, the detection filter was replaced by a Jarrel Ash (model 82410) monochromator, with slits chosen to ensure a resolution of typically 3 nm. The luminescence was corrected for the spectral characteristics of the photodetector by separate elaborate measurements of the response to monochromatic light of known intensity.

Fig. 1A shows the typical biphasic kinetics of the electroluminescence from blebs suspended at low pH, where the rapid R phase, which originates in PS I, can be observed separately from the slower S phase, originating in PS II. The monophasic kinetic (S phase) of the electroluminescence of chloroplasts suspended in isotonic medium is illustrated in Fig. 1B. In these conditions no bleb formation is visible. The electric field necessary to induce a comparable extent of S phase electro-

luminescence in chloroplasts suspended in isotonic medium as in blebs is substantially higher in the former case. This probably reflects a structural change in the PS II centers during hypotonic treatment, causing an increase in the yield of electroluminescence [41]. Upon heat treatment, by incubation of the chloroplast stock suspension for 5 min at increasing temperatures, this S phase becomes gradually inhibited (Fig. 2). Surprisingly, however, at treatment temperatures from 40 °C on, a distinct R phase appears in these heat-treated chloroplasts, measured always in an isotonic medium. This heat-induced R phase has all the characteristics of the one observed in blebs, such as a much faster rise time and inhibition by methyl viologen (Fig. 1C), and can thus be attributed to PS I. Moreover, as can be seen from Fig. 2, while the S phase disappears completely after heat treatment at 60 °C, the R phase increases with the incubation temperature until it reaches a maximum after treatment at about 57 °C, then it decreases and is abolished at about 70 °C. These results are in general agreement with the inactivation temperatures for PS II and PS I as reported [23] to be 55 and 65 °C, respectively. A similar temperature dependence as the inhibition of the S phase electroluminescence is observed also for a considerable fraction of the unstimulated millisecond-delayed luminescence, consistent with the linkage of the normal delayed luminescence to PS II activity. Indeed, we also observed that this unstimulated delayed luminescence, is not inhibited by methyl viologen (not shown). This residual component of the delayed luminescence cannot be created by any active photosystem and thus likely consists of chemiluminescence of membrane-associated chlorophylls, as was proposed for the 73 °C peak observed in glow curves [24]. Similarly, the variable fluorescence, which is an indicator of PS II activity [5,35], is also completely abolished after incubation at 60 °C (not shown). The appearance of the R phase electroluminescence in heat-treated chloroplasts is probably caused by the limited amount of bleb formation (with an average radius of about 2 μm) which occurs under these conditions [25], in spite of the fact that the heat treatment is performed in isotonic conditions. This interpretation is in line with our previous observation that the extent of the R phase is strongly correlated with bleb formation [41]. It should be noticed in this respect that chloroplast swelling by heat treatment has been reported before [26]. It is also possible, however, that the R phase is caused by a more specific effect in PS I, possibly related to the 20 ms delayed luminescence component induced by heating in PS I particles [27].

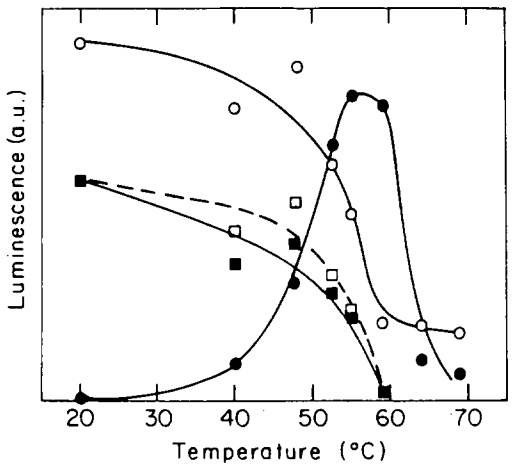

Fig. 2. Temperature sensitivity of the R and S components of electric-field-induced luminescence. Concentrated aliquots of chloroplasts (3 mg/ml chlorophyll) were incubated at the indicated temperature for 5 min and immediately afterwards suspended in 0.4 M sucrose, 10 mM Tris (pH 5.5) on ice. With this protocol, no bleb formation is visible under control conditions (incubation at 22 °C). However, blebs are induced by incubation at temperatures from about 45 °C on [25]. Applied field strength was 3 $kV \cdot cm^{-1}$. ●———●, R component, ■———■, S component, ○———○, background delayed luminescence; and □———□, background delayed luminescence minus the residual component at 65 °C, and subsequently normalized to the S component. The values of the R component were taken as the maximal extent of the R phase, at which time contribution by the S phase is negligible. The extent of the S component was measured in the presence of 100 μM methyl viologen.

In conclusion, from the data presented in Fig. 2, it is evident that, after a 5 min incubation at 60 °C, we can observe PS I electroluminescence devoid of any contamination by PS II. Similar results were obtained by incubating the stock chloroplast suspension at 50 °C for 90 min. We preferred the latter conditions because bleb formation is virtually completely inhibited after heat treatment at 60 °C (see Ref. 16, Fig. 6).

Fig. 3A shows the electroluminescence emission spectrum of broken chloroplasts. This spectrum

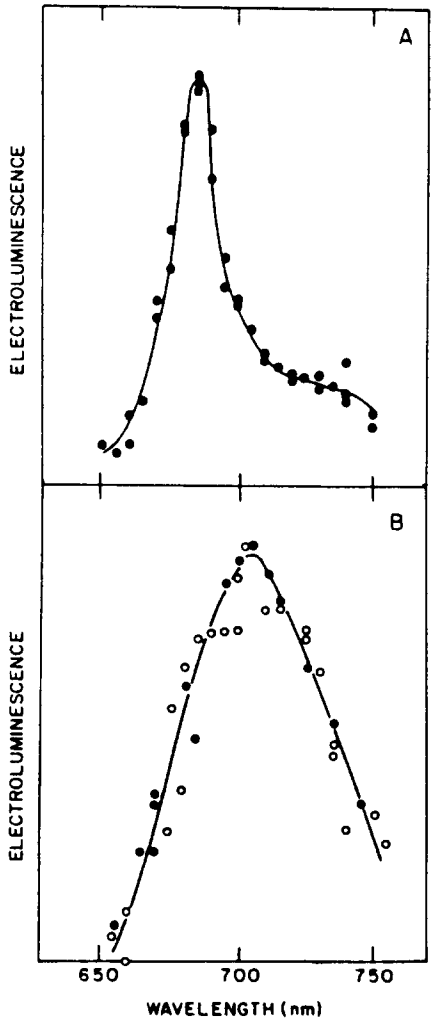

Fig. 3. Electric-field-induced luminescence emission spectra of broken chloroplasts (A) and heat-treated chloroplasts suspended in hypotonic medium (B). The emission peak, corrected for the detection photomultiplier spectral sensitivity, is plotted versus emission wavelength. (A) Chloroplasts were suspended in 0.4 M sucrose, 1 mM $MgCl_2$, final pH was 7.5. (B) ●———●, chloroplasts were heat treated and diluted in hypotonic medium as in Fig. 1C. Applied field strength was 4 $kV \cdot cm^{-1}$; ○———○, blebs were formed by dilution of the chloroplasts suspension in 5 mM Tris (pH 7.5) and 1 mM $MgCl_2$. The difference between the electric-field-induced luminescence in the absence and presence of 100 μM methyl viologen was plotted. Applied field strength was 1.4 $kV \cdot cm^{-1}$. Points represent an average of at least two measurements.

has all the characteristics of PS II fluorescence and luminescence spectra known in the literature [35]. Fig. 3B shows the emission spectrum of heat-treated chloroplasts, subsequently suspended in a hypotonic medium. This represents a pure emission spectrum of PS I, since, as is shown in Fig. 1C, under these conditions the emission can be completely abolished by the addition of methyl viologen. For comparison we also determined the PS I spectrum by subtracting the emission spectrum of normal blebs in the presence of methyl viologen from the emission spectrum in the absence of methyl viologen. The two spectra are indeed identical.

The PS I emission spectrum presented in Fig. 3B peaks at 705 nm. The 20 nm red-shift of this spectrum compared to that of PS II corroborates well with the wavelength shift characterizing the respective reaction center pigments [37].

The limited amount of information available in the literature on PS I emission spectra at room temperature is still very contradictory. Even in the case of PS I particles [28,30,31,33,34] there does not seem to be a consensus on the characteristics of the spectrum, which might be due to their strong dependence on the preparation procedure [38]. PS I emission spectra have been claimed to be resolved in several other ways, e.g., by analysis of decay components in time-resolved picosecond emission spectroscopy [29,40], analysis of long-term delayed luminescence (more than 0.3 s) in green algae and intact chloroplasts [21,39] or by measurement of the difference spectrum between phosphorylated and non-phosphorylated thylakoid membranes [28]. All these latter spectra, as well as some of those obtained from PS I particles [28,33] have in common that they are rather broad and red-shifted with respect to the 685 emission band attribute to PS II. The emission spectrum obtained of the LDL_1 component of long-term delayed luminescence [21] was most similar to the one presented in this report.

It should be emphasized again that the properties of R phase emission validate the attribution of this electroluminescence phase to PS I [16]. This R phase can be readily identified, even under conditions where there is no kinetic separation between the R and S phases, by its polarization [16] and by its sensitivity to methyl viologen (Ref. 16 and this report). However, it has been assumed in the literature that all measurable electroluminescence is produced in PS II alone [11,36]. The interpreta-

tion of these data [11,36] therefore should be revised.

Acknowledgement

This research was supported by the US-Israel Binational Science Foundation, grant no. 85-00369.

References

1 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223–317, Academic Press, New York.
2 Malkin, S. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 351–431, Elsevier/North-Holland Biomedical Press, Amsterdam.
3 Govindjee and Jursinic, P.A. (1979) in Photochemical and Photobiological Reviews (Smith, K.C., ed.), Vol. 4, pp. 125–205, Plenum, New York.
4 Amesz, J. and Van Gorkom, H.J. (1982) Annu. Rev. Plant Physiol. 29, 47–66.
5 Malkin, S. (1977) in Encyclopedia of Plant Physiology, New Series, Vol. 5 (Trebst, A. and Avron, M., eds.), pp. 473–491, Springer-Verlag, Berlin.
6 Lavorel, J., Lavergne, J. and Étienne, A.-L. (1982) Photobiochem. Photobiophys. 3, 287–314.
7 Inoue, Y. and Shibata, K. (1982) in Photosynthesis, Vol. 1 (Govindjee, ed.), pp. 507–533, Academic Press, New York.
8 Arnold, W. and Azzi, J. (1971) Photochem. Photobiol. 14, 233–240.
9 Ellenson, J.L. and Sauer, K. (1976) Photochem. Photobiol. 23, 113–123.
10 De Grooth, B.G. and Van Gorkom, H. (1981) Biochim. Biophys. Acta 635, 445–456.
11 Meiburg, R.F., Van Gorkom, H.J. and Van Dorssen, R.J. (1984) Biochim. Biophys. Acta 765, 295–300.
12 Farkas, D.L., Malkin, S. and Korenstein, R. (1984) Biochim. Biophys. Acta 767, 507–514.
13 De Grooth, B.G., Van Gorkom, H.J. and Meiburg, R.F. (1980) Biochim. Biophys. Acta 589, 299–314.
14 Barber, J. and Malkin, S. (1981) Biochim. Biophys. Acta 634, 344–349.
15 Symons, M., Malkin, S. and Korenstein, R. (1984) Biochim. Biophys. Acta 767, 223–230.
16 Symons, M., Korenstein, R. and Malkin, S. (1985) Biochim. Biophys. Acta 806, 305–310.
17 Goedheer, J.C. (1963) Biochim. Biophys. Acta 66, 61–71.
18 Björn, L.O. (1971) Photochem. Photobiol. 13, 5–20.
19 Shuvalov, V.A. (1976) Biochim. Biophys. Acta 430, 113–121.
20 Sane, P.V., Desai, T.S. and Tatake, V.G. (1980) Z. Naturforsch. 35c, 289–292.
21 Schmidt, W. and Senger, H. (1987) Biochim. Biophys. Acta 890, 15–22.
22 Farkas, D.L. and Malkin, S. (1979) Plant Physiol. 64, 942–947.
23 Doring, G., Renger, G., Vater, J. and Witt, H.T. (1969) Z. Naturforsch. 24b, 1140–1143.
24 Desai, T.S., Tatake, V.G. and Sane, P.V. (1982) Photosynthetica 16, 129–133.
25 Symons, M., Korenstein, R., Harris, C.M. and Kell, D.B. (1986) Bioelectrochem. Bioenerg. 16, 45–54.
26 Packer, L. and Murakami, S. (1972) Methods Enzymol. 24, 181–205.
27 Shuvalov, V.A., Klimov, K.U. and Krasnovskii, A.A. (1976) Mol. Biol. 10, 326–339.
28 Kyle, D.J., Baker, N.R. and Arntzen, C.J. (1983) Photobiochem. Photobiophys. 5, 79–85.
29 Holzwarth, A.R., Wendler, J. and Haehnel, W. (1985) Biochim. Biophys. Acta 807, 155–167.
30 Ikegami, I. (1976) Biochim. Biophys. Acta 449, 245–258.
31 Telfer, A., Barber, J., Heathcote, P. and Evans, M.C.W. (1978) Biochim. Biophys. Acta 504, 153–164.
32 Brinkmann, G. and Senger, H. (1981) in Photosynthesis, Vol. 3 (Akoyunoglou, G, ed.), pp. 337–346, Balaban, International Science Services, Philadelphia, PA.
33 Sonneveld, A., Duysens, L.N.M. and Moerdijk, A. (1981) Biochim. Biophys. Acta 636, 39–49.
34 Tripathy, B.C., Draheim, J.E., Anderson, G.P. and Gross, E.L. (1984) Arch. Biochem. Biophys. 235, 449–460.
35 Papageorgiou, G. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 320–371, Academic Press, New York.
36 Van Gorkom, H.J., Meiburg, R.F. and De Vos, L.J. (1986) Photosynthesis Res. 9, 55–62.
37 Govindjee and Govindjee, R. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 1–50, Academic Press, New York.
38 Nechustai, R., Naurizade, S.D. and Thornber, J.P. (1986) Biochim. Biophys. Acta 848, 193–200.
39 Schmidt, W. and Senger, H. (1987) Biochim. Biophys. Acta 891, 22–27.
40 Hodges, M. and Moya, M. (1986) Biochim. Biophys. Acta 849, 193–202.
41 Symons, M., Malkin, S., Korenstein, R. and Farkas, D.L. (1987) J. Photochem. Photobiol., in press.