

Photopotential of Chlorophyll *a* Containing Lecithin BLM Formed with and without Carotene

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Abstract. The potential of lecithin BLM with chlorophyll *a* and with and without carotene has been measured. The BLM containing both chlorophyll *a* and carotene have shown the highest value of the photopotential. Stronger fatigue effect during light puls excitation has been found on BLM with pigments than with isolated chlorophyll *a*-protein complex extracted from blue green alga.

Key words: Lecithin BLM — Chlorophyll *a* — Carotene

Introduction

Studies of black lipide membranes (BLM) containing photosynthetic pigments are useful not only for understanding the primary photochemical activity of the photosystems in vitro but also for the exploration of possible applications in the conversion of solar energy. For this reason, we can see an increasing interest in the problems of photoelectric properties of BLM in the last decade, e. g. (Tien 1976; Strauss 1976; Rich and Brody 1981; Kutnik and Lojevská 1981).

The presented work compares almost the photovoltaic effects obtained from a symmetric, (or asymmetric) chlorophyll *a* (ch *a*)-lecithin bilayers (containing or not containing β -carotene), formed by the Montal-Mueller method, with the observation of other authors, which are very often in mutual contradiction, (e. g. Hong 1976; Chen and Bears 1976; Mangel et al. 1975).

Material and Methods

The procedure for forming the bilayers from monolayers by means of the Montal and Mueller (MM) method has been described e. g. by Rich and Brody (1981). MM bilayers were formed on a 1 mm diameter hole drilled in a teflon cylinder situated inside a glass vessel. The membrane forming solution was applied to the hole with the aid of a syringe. After the membrane had been stabilized (a magnetic stirrer was used for the thinning process and the membrane resistance was monitored), the photopotential in a symmetric and asymmetric arrangement was measured.

The membrane solution was formed of 10 ± 2 mg of lecithin in 1 ml of the n-decane — n-octane mixture (5:1 ratio); lecithin was then extracted from eggs (Singleton et al. 1965). The photopotential, measured on fresh prepared membrane solutions, was very low; for this reason, we have always started measurements of the photopotential after one day formation of the membrane solution in the

refrigerator (oxidizing by air oxygen). This observation is, after all, in agreement with the results published by Lutz et al. (1974). To the membrane solution either chlorophyll *a*, or carotene, or chlorophyll *a*-protein complex was added. Chlorophyll *a* was extracted from a lyophilized blue green alga by means of absorption chromatography (Pančoška and Hladík 1981), carotene was separated using the method of Skorkovská and Vavřínek (1973). The chlorophyll *a*-protein complex was then isolated from the blue green alga *Plectonema boryanum* (Hladík 1981); the thylakoid membranes had been solubilized by Triton X-100 and the fractionation of these extracts was made on column chromatography with DEAE-cellulose. Only the chlorophyll-protein complex with apparent molecular weight of 260 KD (4.4 mg of protein on 1 mg of chlorophyll *a*; 16.3 mg carotene on 100 mg of chlorophyll *a*) was used for our measurements. The bathing solution on both sides of the membrane contained either 100 mmol/l KJ (KCl) or 100 mmol/l KJ (KCl) with 10 mmol/l potassium ferrocyanide (reducing side) and with 10 mmol/l potassium ferricyanide (oxidizing side).

The photopotential measurements were performed by the digital electrometer Keithley 616 (with calomel electrodes) which was connected to the registration millivoltmeter Servogor RE 511. The membranes were illuminated by a halogen lamp (150 W) in front of which were placed a set of heat and Schott interference filters, a camera shutter and corresponding lenses; the light output focussed on the membrane surface in the bathing solution was about $2 \cdot 10^{-2} \text{ W m}^{-2}$. All the measurements were made at room temperature.

The resistance of the membranes after the formation process was $10^2 - 10^5 \Omega \text{m}^2$, their capacity about $40 \mu\text{F} \cdot \text{m}^2$.

Results

Besides the volt-ampere characteristics of BLM with different concentrations of monomeric chlorophyll *a*, chlorophyll complexes, chlorophyll *a* and of β -carotene mixture and of chlorophyll *a*-protein complexes, our study was focussed on the conditions of a maximum photopotential production, on the influence of the light excitation pulse and on the regeneration ability of BLM during its repeated illumination on the value of the photopotential.

The volt-ampere characteristic of the membrane studies was linear up to 150 mV; the break-down voltage lies in the interval from 200 to 600 mV. The following Table 1 summarizes the typical photopotential values measured on different samples of BLM. The time dependence of the potential increase during the BLM illumination is shown by Fig. 1. From Fig. 1 it is evident that the photopotential has reached its saturation for illumination in time intervals longer than one minute. The measurements of the photopotential for shorter illumination times than 4 seconds were limited by the time constant of the experimental set. Knowing this result, we have started studying the influence of fatigue on the BLM photopotential during a repeated pulse white light illumination. The results obtained are shown in Fig. 2. It indicates that the fatigue effect of BLM with chlorophyll *a*-protein complexes, extracted from the blue green alga, has been considerably suppressed in comparison with BLM containing chlorophyll *a*. A similar fatigue effect on BLM with chlorophyll *a* was observed on BLM with chlorophyll *a* and carotene.

Discussion

The considerable increase in the photopotential in BLM, containing carotene, can be explained by the fact that in addition to the very fast singlet-singlet energy transfer from the carotene to the chlorophyll *a* described by Dallinger et al. (1981)

Table 1. Typical photopotential values (average values of 20 observations) measured on lecithin BLM with different pigment content at room temperature.

Sample	Pigment concentration mmol/l in BLM solution	Observed photopotential mV	Increase	Bathing solution mmol/l KCl	Remark
Monomer chlorophyll <i>a</i>	8	0,05	1	20	*
Monomer chlorophyll <i>a</i>	8	2	40	20	**
Chlorophyll complexes with H ₂ O	1.7	0.1	~2	1	
Chlorophyll <i>a</i> with carotene	8 (Chl <i>a</i>) 1 (carotene)	6	110	20	**
Chlorophyll <i>a</i> -protein complex	—	2	—	20	

* The threshold value for the photopotential observation was 0.7 mmol/l pigment concentration

** The bathing solutions contained 10 mmol/l of potassium ferro/ferricyanide

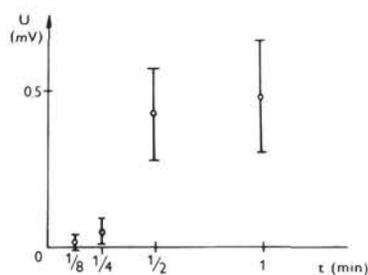


Fig. 1. The photopotential dependence of BLM (molar ratios: carotene: chlorophyll *a*: lecithine 1:10:16) on the time interval of the light illumination at room temperature in 100 mmol/l KCl bathing solution. Excitation performed with white light, energy output on BLM surface was $2 \cdot 10^{-2} \text{ W m}^{-2}$.

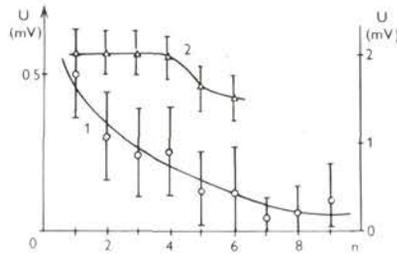


Fig. 2. The kinetics of BLM fatigue during the repeated pulse illumination. All measurements were taken at room temperature, curve 1 corresponds to the BLM with chlorophyll *a*, curve 2 corresponds to the BLM with chlorophyll *a*-protein complex extracted from the blue green alga. The photopotential (for the curve 1 on the left, for the curve 2 on the right) is plotted as a function of the number of repeated white light pulses n ; the light and dark time intervals of the pulse were 1 minute. The BLM were prepared from solutions which contained either 6 mg of chlorophyll *a* or 6 mg of chlorophyll-protein extract in 1 ml BLM stock solution.

the lower Fermi energy in carotene will participate in the increase in the photopotential observed in our lecithine BLM. In agreement with Mangel et al. (1975), the excited molecule of chlorophyll *a* can reach its ground state after the acceptance of the electron from the donor (carotene). This electron has, however, to penetrate through the potential barrier of Gouy's layer of the membrane and the most probable process of it is the tunnelling; carotene which has a lower Fermi energy than the potential barrier of the membrane can thus increase the probability of this tunnel process. Finally, we have also to take into consideration the specific influence of the carotene on the advantageous conformation of the chlorophyll *a* in BLM, so important for the effective energy transfer between them as it was observed (Pančoška and Hladík 1981) in chlorophyll *a*-protein complexes containing carotene.

The basis of the higher photosensitivity in BLM with chlorophyll *a* complexes (see Table 1) may be interpreted in agreement with Rich and Brody (1981) that two photoeffects may exist — one of a low quantum yield in monomers and the second one of a high quantum yield in aggregates. This conclusion also confirms the observations obtained from liposomes (Chen and Bears 1976) in which the chlorophyll *a* exists in an aggregated state.

The measurements of the influence of fatigue during the repeated illumination on the BLM photopotential seem to be very interesting. The evident decrease of fatigue in the BLM with chlorophyll *a*-protein complexes, extracted from the blue green alga *Plectomena boryanum*, in comparison with the BLM containing just chlorophyll *a* and carotene, may be explained by the presence of the carotene in chlorophyll *a*-protein complexes which can speed up the electron transport through

the BLM due to the specific orientation of the chlorophyll *a* and carotene mediated through the protein part of the complex, as was shown by Pančoška and Hladík (1981). This fact will then decrease the polarization, or other deactivation electron effects in the BLM without carotene.

In conclusion, it appears that the presented explanation of the important influence of carotene on the photopotential generation in lecithin BLM might not be considered as completely sufficient to exclude definitely the possible involvement of the lipid oxidation on the process discussed above. Studies on this subject are in progress.

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