Biochimica et Biophysica Acta, 345 (1974) 257—262

BBA Report

BBA 71198

THE EFFECT OF OXYGEN ON THE PHOTOCONDUCTIVITY OF LIPID BILAYERS CONTAINING MAGNESIUM-PORPHYRIN

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(Received March 18th, 1974)

Summary

Photoconductivity changes in lipid bilayers containing magnesium-octaethylporphyrin were measured in KCl solutions. Light flashes increase the total current to at least twice the dark current under anaerobic conditions. Under aerobic conditions the photocurrent decreases to less than a third of the dark current and approaches zero as the oxygen concentration is increased. ESR measurements on liposomes containing magnesium-porphyrin are used to show that magnesium-octaethylporphyrin is converted to a cation, accompanied by oxygen consumption.

Photoelectric effects in lipid bilayers containing chromophores have been used as model systems to increase the understanding of the reactions which may occur in membranes of photosynthetic organisms [1—8].

The present investigation deals with a simple synthetic system which appears analogous to the natural photosystem in that migration of charges takes place within the membrane rather than by formation of an electrical double layer at the interface of the membrane [8].

When chlorophyll is used as a chromophore, significant conductivity changes are only seen in the presence of lipophilic electron acceptors [7,8] or by the use of a lipid extract from photosynthetic organs which usually contain such acceptors [1,2] and different bathing solutions on both membrane surfaces. Mg-Et_8-porphyrin, however, does show conductivity changes without an acceptor being located in the membrane, as found by Hong and

Abbreviation: Mg-Et_8-porphyrin, magnesium-octaethylporphyrin.

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Mauzerall [5,6]. These authors assumed magnesium-porphyrin to react reversibly at the interface with either ferrocyanide on one side or ferricyanide on the other side of the membrane. Trissl [9] measured light-dependent conductivity changes in magnesium-porphyrin membranes separated by identical bathing solutions, containing KCl, upon applying a voltage across the membrane. The size of the observed photoeffects changed with different bathing solutions, suggesting that the oxygen content may play a role. We therefore investigated the effect of oxygen on the photoconductivity of magnesium porphyrin-containing membranes.

The membranes were formed on a teflon frame which had a circular hole of 3.4 mm diameter, separating two aqueous phases. The cuvette was mounted in an airtight plexiglas box to achieve anaerobic conditions. The box was equipped with gloves allowing the formation of the membranes. Anaerobic conditions were produced as follows: The teflon cuvette was repeatedly evacuated with a vacuum pump and gassed with argon. The KCl solution and the apparatus were also freed from air by a water suction pump and then gassed with nitrogen followed by argon. The cuvette was then taken from the dessicator inside the box and placed into the holder. It was filled with 10 mM KCl solution and again gassed with argon. The oxygen concentration in the cuvette reached a minimal value of less than 10^{-5} M after 2—3 h under anaerobic conditions. Oxygen concentration was measured with a Clark-type electrode. Reproducible aerobic conditions were achieved by bubbling a mixture of oxygen and nitrogen (20/80, v/v) through the aqueous solution before use. Under these conditions the oxygen concentration in the cuvette varied from $1.5 \cdot 10^{-4}$ to $2.4 \cdot 10^{-4}$ M O_2.

To watch the formation of the bimolecular state of the membrane (black state), weak red light was used above 640 nm. A xenon high pressure lamp XBO 150 W (Osram) was used as the light source. The light was focussed on the membrane with lenses and a mirror to cover 90% of the total area of the black film. In the plane of the membrane the intensity of the white light was $4.5 \cdot 10^{-2}$ W/cm^2. A black teflon sheet mounted on the rear wall of the cuvette prevented scattering of the light beam.

The membranes were formed from a 10 mM solution of synthetic dierucoyl-L-glycerophosphorylcholine (di-(22:1)-lecithin) [10] in decane to which Mg-Et_8-porphyrin was added up to the given molar ratios. The organic solvents and solutions were bubbled with argon before use.

The dark current—voltage characteristics were determined with an electrometer (Keithley 610 B) or by using a current amplifier (Keithley 427) and a storage oscilloscope (Tektronix 5103 N).

ESR studies were performed with a Varian E 3 instrument using an optical cavity. Using egg lecithin and Mg-Et_8-porphyrin at a molar ratio of 1/1 in KCl (100 mM), the liposomes were prepared by sonication for 20 min under argon.

Bilayer membranes containing Mg-Et_8-porphyrin in a molar ratio of 0.2 (Mg-Et_8-porphyrin/di-(22:1)-lecithin) show almost the same dark current

under anaerobic and aerobic conditions (Fig.1). The photocurrents induced by flashes of 1/60 s white light are several times higher under anaerobic than

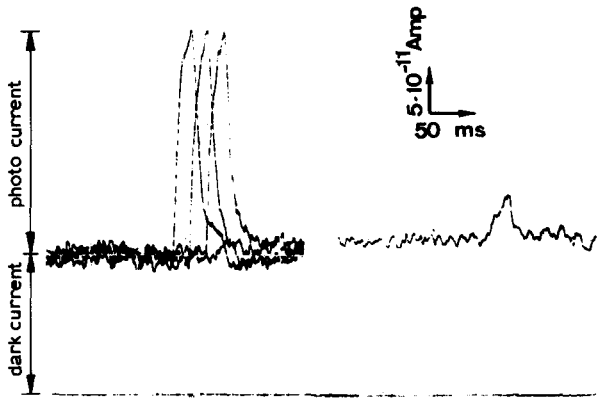

Fig.1. Photocurrents under anaerobic and aerobic conditions induced by white flashes of 1/60 s in a black lipid membrane containing Mg-Et_8-porphyrin in a molar ratio of 0.2 (Mg-Et_8-porphyrin/lecithin). Both aqueous phases contained 10 mM KCl. The applied voltage was 100 mV. Rise time of the amplifier: 10 ms. Left: Oscilloscope trace of three subsequently given flashes under anaerobic conditions. Right: One flash under aerobic conditions.

under aerobic conditions. With the same aqueous phases on both sides of the membrane the photocurrent—voltage dependence was linear up to 120 mV and symmetrical about the origin (Fig.2). The results shown in Fig.2 are the average from three to five different membrane preparations. Despite the large deviations observed from different membrane-forming solutions the two curves demonstrate a significant difference.

With care it is possible to increase the oxygen concentration in the cuvette without destroying the membrane and to measure the photocurrent before and afterwards. The resulting photocurrents of the same membrane at 130 mV were: $8.2 \cdot 10^{-10}$ A·cm^{-2} at $8.0 \cdot 10^{-6}$ M O_2 and $3.5 \cdot 10^{-10}$ A·cm^{-2} at $2.6 \cdot 10^{-5}$ M O_2 (dark conductivity $1.2 \cdot 10^{-8}$ $\Omega^{-1} \cdot$cm^{-2} in a membrane with a molar ratio of 0.13). The membrane was then destroyed and oxygen was bubbled through the aqueous phases and again a membrane was formed. The photocurrent decreased further to $0.73 \cdot 10^{-10}$ A·cm^{-2} at $2.0 \cdot 10^{-4}$ M O_2. Under all conditions the photocurrent—voltage dependence was linear up to 130 mV.

Under aerobic conditions the black state of the membrane was reached after 30—60 min in the dark without applying a voltage. Under anaerobic conditions, however, the membrane did not become black within 2 h and had 2—3 times lower dark conductivity. By flashing light on the membrane while voltage was applied, the membrane became black within less than one or two seconds total illumination time. Thereafter the conductivity was the same as in the aerobic system and did not change with time. In both systems the

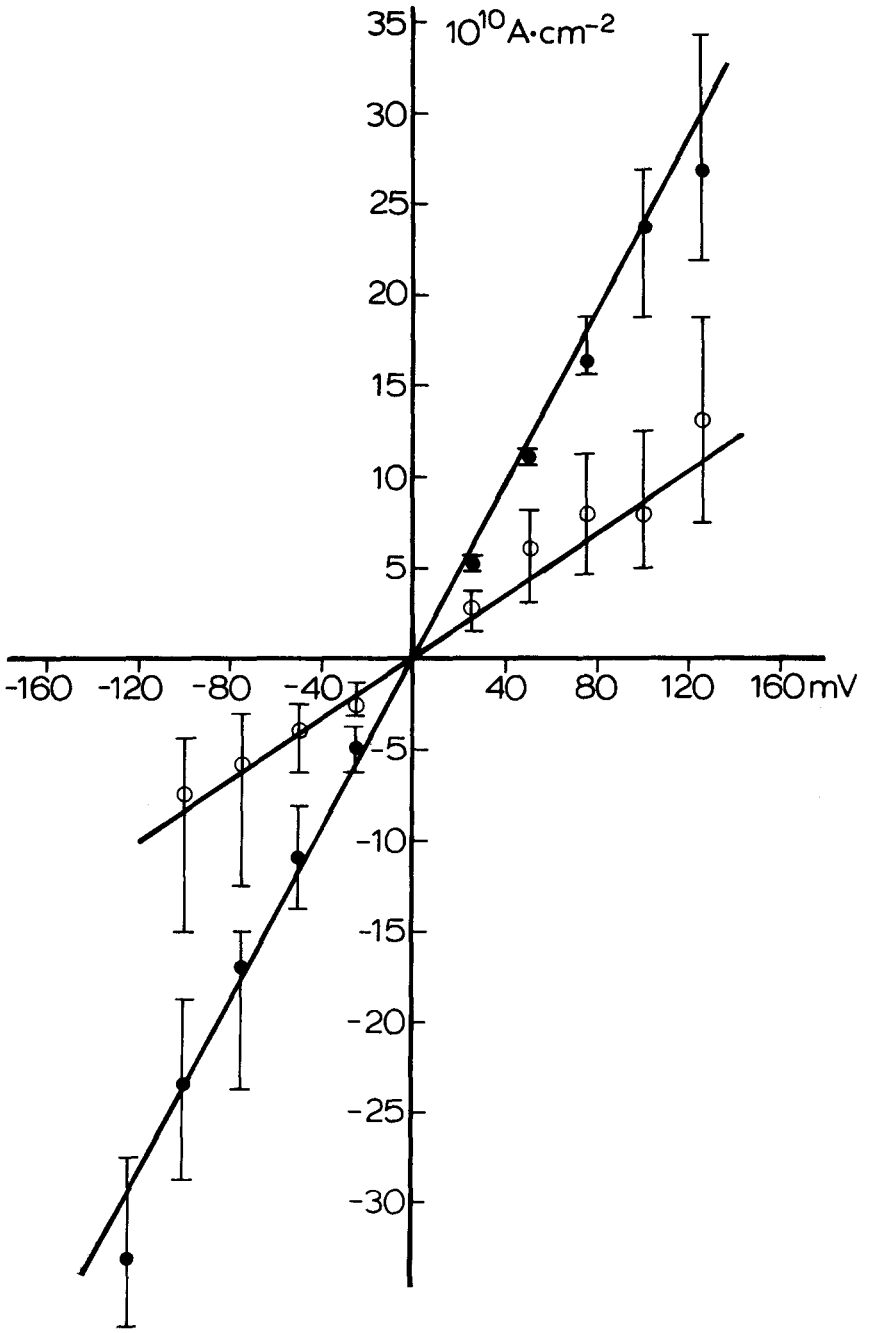

Fig. 2. Photocurrent—voltage dependence of black lipid membranes containing Mg-Et$_8$-porphyrin in a molar ratio of 0.2 (Mg-Et$_8$-porphyrin/lecithin). ●, anaerobic conditions; ○, aerobic conditions. The corresponding dark conductivities varied, for both conditions, from 1.0 to 2.0 · 10^{-8} $\Omega^{-1} \cdot cm^{-2}$. The bars indicate the range of photocurrent values observed with three to five different membranes.

current—voltage curves in the dark were identical and linear up to 100 or 120 mV.

In order to study the nature of the charged compound produced upon illumination we also measured the ESR signal in liposome suspension containing Mg-Et_8-porphyrin in a similar environment to that in a bilayer. In the absence of oxygen and also with oxygen in the dark, no ESR-signal was detectable. The anaerobic liposome preparations tend to precipitate, whereas oxygen bubbling through the suspension inhibits precipitation. Upon illumination of an oxygen-treated aqueous lecithin—porphyrin preparation a small ESR-signal arises which is stationary in the dark. Further illuminations increase the dark signal 2-fold reversibly at the given light intensity. The g value is in agreement with that of the cation radical of Mg-Et_8-porphyrin [12]. Moreover, the preparations show a pronounced oxygen consumption upon illumination. Within a few minutes the oxygen concentration falls to zero when the suspension is equilibrated with oxygen after sonication and the white light of a laboratory lamp is focused on it.

Hong and Mauzerall [5] demonstrated that a magnesium-porphyrin membrane in a molar ratio of approximately 0.25 was able to produce photocurrents which were not greater than 10% of the dark current if ferricyanide was added on both sides of the membrane. These results may be compared with our aerobic system in which the photocurrent was always less than 30% of the dark current and decreased to 4% of the dark current at $2.0 \cdot 10^{-4}$ M O_2. Much higher photocurrents could be achieved in a symmetric system without adding a redox system by decreasing the oxygen concentration. Since no net photocurrent can be produced across such a membrane, the measured current changes reflect reversible changes in the conductivity of magnesium-porphyrin-containing membranes.

A possible explanation for the unexpected effect of oxygen on the photocurrent of magnesium-porphyrin membranes might be the following: Under aerobic conditions the oxygen concentration is high enough so that Mg^{2+} formed upon illumination can react with oxygen to form uncharged (or impermeable) oxidation products. Although, under reduced oxygen pressure, there are enough oxygen molecules [11] to react with magnesium-porphyrin to form the cation upon illumination the subsequent irreversible oxidation is decreased by the lower oxygen concentration. Thus, under these conditions, the photocurrent is increased by the higher cation concentration.

Substantial support for this hypothesis comes from Fuhrhop and Mauzerall [12] who demonstrated the existence of a stable magnesium-porphyrin monovalent cation by ESR. The radical was formed in the dark in a reaction with iodine or a number of other oxidants. The formation of the radical was reversible and could be back-titrated with KI. From our findings we propose that the radical can be formed not only with iodine, ferricyanide or ferrichloride in the dark, but also with oxygen in the light using lipid interfaces. ESR studies with liposomes show that the small signal having the same g value as reported for Mg-Et_8-porphyrin by Fuhrhop and Mauzerall [12]

is produced in the dark and can be increased by illumination. Magnesium-porphyrin cations therefore appear to be responsible for the photocurrents observed in our system. The light-dependent ESR signal was partially reversible, but there was no detectable increase of oxygen after the light was turned off. Hence, although our experiments support the presence of a reversible reaction of the magnesium-porphyrin cation, it remains to be shown which reactant is responsible for the reduction of the cation.

Our results using liposomes agree with the bilayer studies in that black membranes containing Mg-Et_8-porphyrin or stable lipid—porphyrin vesicles appear to be formed only in the presence of certain oxidation products of Mg-Et_8-porphyrin.

These results might also explain the following effect observed by Hong and Mauzerall [6]. By scanning the magnesium-porphyrin membrane with a focused light beam the photocurrent under continuous illumination was shown to be larger towards the edges of the hole than in the center of the membrane. The increased photocurrent towards the torus could be caused by local anaerobic conditions due to geometrical restrictions in the diffusion of O_2. Similar oxygen effects have also been demonstrated in other systems; for example, Schadt [13] has shown that the photoeffect observed by others [14] in bilayers containing vitamin A, were produced by vitamin A acid, an oxidation product of vitamin A.

The authors would like to thank Professor Läuger and Dr G. Stark for their hospitality and for helpful discussions and Dr J. Fuhrhop for a gift of Mg-Et_8-porphyrin. The award of a grant to H. Lutz from the Swiss National Funds is gratefully acknowledged. This work was financially supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 138.

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