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Photoinduced changes in structure and function of hexadecyl merocyanine dyes incorporated into lipid membranes

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Abstract

In the purple membrane of *Halobacterium halobium*, bacteriorhodopsin (BR) acts as a proton pump leading to a transmembrane potential difference. A biomimetic system can be formed using merocyanine derivative dye which, when incorporated into a bilayer lipid membrane, creates a potential difference across the membrane. In this system, the dye molecules in combination with the lipid molecules act as a proton transport pump due to a light-induced conformational change of the dye. In this paper, we report the photovoltaic properties and crystal structure of hexadecyl merocyanine dye, together with the change in peak intensity in the X-ray diffraction pattern of the dye and dye–lipid system after illumination. We show that, at room temperature (~300 K), merocyanine dyes exhibit a crystalline form with a long-chain, fibre-like structure (diameter, 2.89 nm). However, on interaction with light, the crystalline form of the dye changes but the diameter remains the same.

Keywords: X-Ray diffraction; Photovoltage; Merocyanine dye

1. Introduction

Bacteriorhodoposin (BR), a protein molecule present in the purple membrane of *Halobacterium halobium*, acts as a proton pump and initiates photosynthesis in this bacterium [1]. Model membrane systems with BR probes have been used to mimic the natural process and photovoltage has been observed. The photovoltage is generated in the system as the protons are transported from one side of the membrane to the other due to the conformational changes of the BR molecules [2–5].

From an extension of this background knowledge, we have reported photovoltage generation in planar lipid membranes on incorporation of certain merocyanine dyes with similar photoinduced isomerization properties to BR [6]. In this paper, we report the photovoltage generation using hexadecyl merocyanine dye incorporated into lipid membranes. Scanning electron microscopy (SEM) reveals a long-chain, fibre-like structure of the dye, and the invariance of the peak numbers and positions in the X-ray diffraction pattern indicates that the unit crystal structure and size are unaffected by illumination. However, there is a change in the peak intensity, which arises from the conformational change of the dye.

2. Materials and methods

Hexadecyl merocyanine dye was synthesized according to Ref. [7]. AR grade HCl, KI, *n*-octane and *n*-decane, supplied by E. Merck, were used. Iodine (E. Merck) was purified by resublimation. Cholesterol was oxidized and recrystallized from *n*-octane.

The photoelectrochemical cell consisted of two L-shaped glass tubes, separated by a micropore glass filter with a porosity of 10 μm (for a detailed description, see Ref. [3]). A saturated solution of oxidized cholesterol in *n*-decane was added to the merocyanine dye. This mixture was sonicated in an Imeco sonicator (model no. 250P) for 15 min. The concentration of the dye was 3 mM. This solution was then used for membrane formation on the glass filter. One side of the membrane was filled with HCl (concentration, 0.1 mM) and the other with a saturated solution of iodine in 1 mM potassium iodide solution. The HCl solution acts as the proton source and I_2/I^- solution acts as the charge acceptor. Two platinum electrodes were placed across

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the membrane to measure the open-circuit photovoltage by a Keithley electrometer (model no. 614).

For X-ray diffraction experiments, we used a Philips PW X-ray diffraction unit with nickel-filtered Cu Kα radiation. Thin films of the dye and oxidized cholesterol were prepared by evaporation of saturated solutions in n-decane on a glass plate, in both light and dark conditions, and their X-ray diffraction patterns were taken. A Jeol 200 kV scanning electron microscope was used to obtain an electron micrograph of the merocyanine dye crystal.

3. Results and discussion

When the dye-probed membrane was illuminated with a light intensity of 700 lx cm^{-2}, photovoltage was generated which increased with time according to the relation [8]

$$V_t = V_0[1 - \exp(-\alpha_1 t)] \qquad (1)$$

where V_t is the photovoltage at time t after switching on the light, V_0 is the maximum or the saturation value of the photovoltage and α_1 is an empirical time constant of the growth curve. V_0 was reached within 90–100 min after turning the light source on. When the light was switched off, the magnitude of the generated voltage remained at its maximum for about 1 h and then started to decrease according to the relation

$$V_t = V_0 \exp(-\alpha_2 t) \qquad (2)$$

where α_2 is the empirical time constant for the decay curve. The photovoltage generated using the hexadecyl dye had a good storage time. On average it took 17 h to complete a cycle and all cycles were reproducible (Table 1).

At room temperature (~300 K), hexadecyl merocyanine dye is available in a crystalline form which has a long-chain, fibre-like structure; an electron micrograph is shown in Fig. 1. From the X-ray diffraction pattern of the dye, the d values and intensities of the peaks were calculated revealing its good crystalline form. The d values varied from 35.3 Å to 2.11 Å (Fig. 2). Using de Wolffe's method, the crystal structure was found to be orthorhombic [9]. The cell parameters obtained experimentally are listed in Table 2. A comparison of the diffraction patterns of the two films prepared in very bright light (40 °C) and dark conditions shows identical peaks at identical 2θ positions. The intensities of the peaks corresponding to the (010) plane with an interplanar spacing d = 23.37 Å differ markedly. The peaks are more intense for films prepared in the light, while in the dark the intensity is very low (Fig. 3).

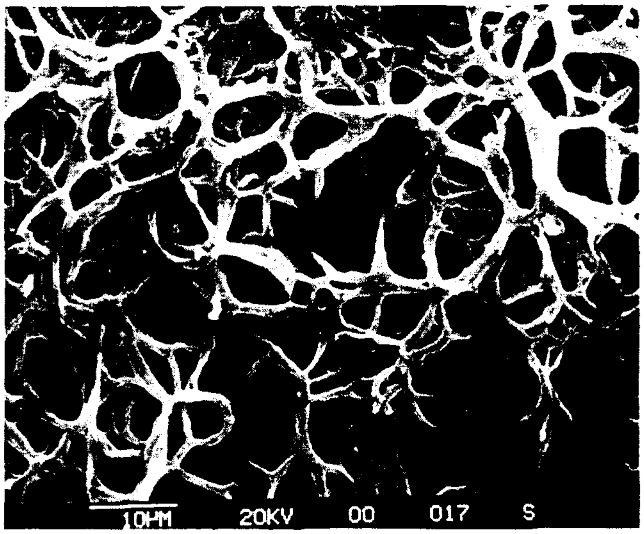

Fig. 1. Electron micrograph of hexadecyl merocyanine dye.

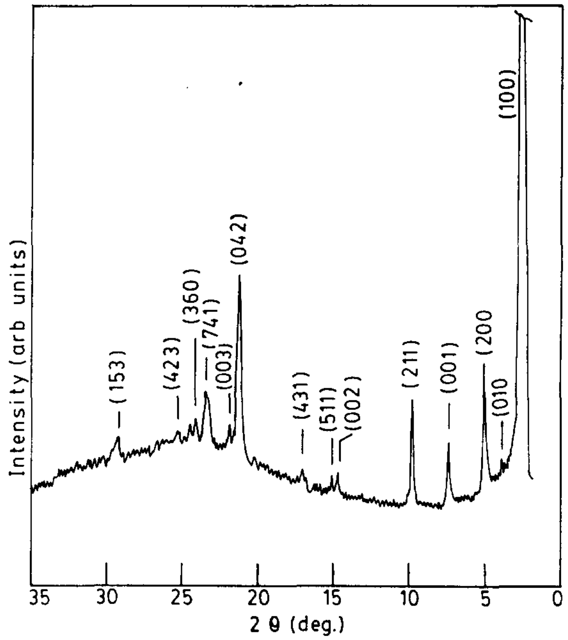

Fig. 2. X-Ray diffraction pattern of hexadecyl merocyanine dye.

Table 1
Photovoltaic properties of hexadecyl merocyanine dye

Peak voltage (mV)	Growth time (h)	Decay time (h)	Time for one cycle (h)
270	1.5	15.5	17.0

To calculate the crystal size of merocyanine dye, we used the Scherrer equation [10]

$$D = K\lambda/\beta \cos \theta$$

where K = 0.9, λ is the wavelength of Cu Kα, θ is the angle of diffraction and β is the line broadening of the (100) diffraction peak. For accuracy, the method of Klug and Alexander [10] was also employed. The calculated crystal size (2.89 nm) was the same in light and dark conditions. The increase in peak intensity

Table 2
Crystal structure of hexadecyl merocyanine dye

d (Å)	I/I_1	h k l	d (Å)	I/I_1	h k l
35.308	100	1 0 0	4.219	11	0 4 2
23.37	1	0 1 0	4.066	1	0 0 3
17.4	9	2 0 0	3.816	2	7 4 1
12.20	5	0 0 1	3.697	1	3 6 0
9.22	8	2 1 1	3.521	1	4 2 3
6.10	1	0 0 2	3.056	1	1 5 3
5.91	1	5 1 1	2.111	1	10 8 2
5.27	2	4 3 1			

Radiation, Cu $K\alpha_1$; $\lambda = 1.5408$ Å; filter, Ni; system, orthorhombic; $a_o = 35.286$ Å; $b_o = 23.37$ Å; $c_o = 12.199$ Å.

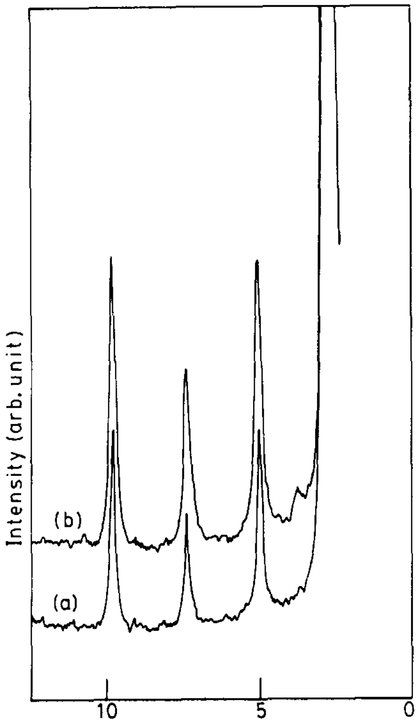

Fig. 3. X-Ray diffractogram of hexadecyl merocyanine dye: (a) in the absence of light; (b) in the presence of bright light.

corresponding to $d = 23.37$ Å indicates that more X-rays are reflected from the (010) plane, and this is only possible when the number of molecules in that plane increases. The presence of HCl and KI solutions at the experimental concentrations used did not affect the diffraction pattern of the dye. We also studied the diffraction pattern of the dye mixed with crystalline *o*-cholesterol dissolved in *n*-decane. The peak numbers, positions and intensities matched exactly those obtained earlier, indicating that the diffraction pattern is an inherent property of the dye (Fig. 4).

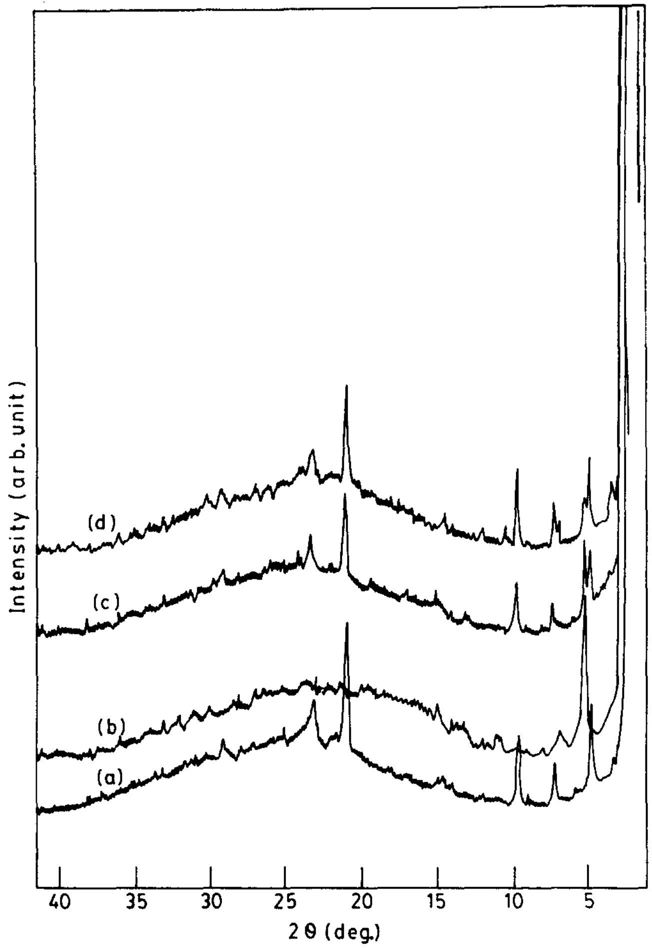

Fig. 4. X-Ray diffractograms: (a) hexadecyl merocyanine dye; (b) oxidized cholesterol; (c) hexadecyl merocyanine dye and oxidized cholesterol in the dark; (d) hexadecyl merocyanine dye and oxidized cholesterol in the light.

From the photovoltage study, we can conclude that merocyanine dye molecules, when incorporated into a lipid membrane, can induce photovoltage generation across the membrane with good storage capacity and high magnitude. From the X-ray diffraction pattern, we can conclude that the increase in intensity of the $d = 23.37$ Å peak is due to a light-induced change in the molecular orientation of the dye, so that more molecules are concentrated in that particular plane. Due to this photoisomerization of the dye, protons are transported across the membrane [6,11] giving rise to the observed photovoltaic effect. Moreover, the invariance of the crystal size and peak positions indicates that the unit crystal remains the same on illumination. We thus conclude that the photovoltage generation is caused by the change in molecular conformation of the dye.

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References

[1] A. Donan and W. Stoeckenius, Photophosphorylation in *Halobacterium halobium*, *Proc. Natl. Acad. Sci. USA, 71* (1974) 1234–1238.

[2] D. Oesterhelt and W. Stoecknius, Function of a new photoreceptor membrane, *Proc. Natl. Acad. Sci. USA, 70* (1973) 2853–2857.

[3] I.I. Severina, Nystatin induced increase in photocurrent in the system bacteriorhodopsin proteoliposome/bilayer planar membrane, *Biochim. Biophys. Acta, 681* (1982) 311–317.

[4] V.P. Skulachev, Conversion of light energy into electric energy by bacteriorhodopsin, *FEBS Lett., 64* (1976) 23–25.

[5] P. Ormos, Z. Dancshazy and B. Karvaly, Mechanism of generation and regulation of photopotential by bacteriorhodopsin in bimolecular lipid membrane, *Biochim. Biophys. Acta, 503* (1978) 304–315.

[6] T. Basu, S. Das and P. Nandy, Photoinduced proton transport mechanism in merocyanine dye-probed planar lipid membranes, *J. Photochem. Photobiol. B: Biol., 18* (1993) 155–159.

[7] M.J. Minch and S. Sadiq Shah, Spectroscopic studies of hydrophobic association of merocyanine dyes in cationic and anionic micelles, *J. Org. Chem., 44* (1979) 3252–3255.

[8] R. Datta and P. Nandy, Role of merocyanine dyes as proton pump in photovoltage generation, *Photochem. Photobiol., 52* (1990) 907–909.

[9] G. Roy, X-Ray study of a second, hydrated phase of copper ammonium sulphate hexahydrate, I, *J. Phys., 7* (1965) 307–312.

[10] P. Kundu, D. Pal and S. Sen, Preparation and thermal evolution of sol-gel derived transparent ZrO_2 and $MgO–ZrO_2$ gel monolith, *J. Mater. Sci., 23* (1988) 1539–1546.

[11] M.H. Abdel-Kader and U. Steiner, A molecular reaction cycle with a solvatochromic merocyanine dye, *J. Chem. Educ., 60* (1983) 160–162.