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Photo-Osmosis Through Liquid Membrane Bilayers

Studies on Mixture of Bacteriorhodopsin with Cytochrome-C, Myoglobin, or Hemoglobin

NIKKI GARG AND DATTA MADAMWAR*

Post-Graduate Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India

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ABSTRACT

The effect of cytochrome-C, hemoglobin, and myoglobin on photo-osmosis through liquid membrane bilayers generated by bacteriorhodopsin (BR) has been studied. The magnitude of photo-osmotic velocity was found to be much greater when BR was combined with any one of the three pigments than that of BR alone. This has been because of the exclusion of protons and electrons in the illuminated compartment by the action of light, where one acts as the acceptor for the others. The rate of light-induced volume flux of the combined system depends on temperature, intensity, and wavelength of incident light, and the nature and concentration of electron donors and acceptors.

Index Entries: Bacteriorhodopsin; cytochrome-C; hemoglobin; myoglobin; photo-osmosis; liquid membrane bilayers.

INTRODUCTION

Halobacteria live in salty, sunny, and hot places, such as brines having 20% NaCl concentrations. The retinal protein bacteriorhodopsin (BR) is found to be involved in energy production. On illumination, BR translocates protons into the exterior of the cell and a proton motive force develops

*Author to whom all correspondence and reprint requests should be addressed.

owing to charge separation, that is, an electrochemical gradient is created across the membrane (*1*). This is because the purple membrane of *Halobacteria* is oriented within the plasma membrane in such a way that release of protons will occur into the surrounding medium. This function of BR has been used to generate electrical potential difference across the membrane under the influence of light, and the phenomenon of photo-osmosis has been demonstrated (*1*). BR therefore acts as a photoelectric energy transducer.

It has been possible to develop systems of pigments for solar energy conversion into electrical power. Such systems containing chlorophyll or chlorophyll-like pigment and BR have gained worldwide acclaim (*2–4*). However, no viable system has as yet been developed. Therefore, experiments were carried out with a view to studying the phenomenon of photo-osmosis through liquid membrane bilayers generated by mixture of BR with hemoglobin, cytochrome-C, or with myoglobin, which are known for electron generation, so that H^+ ion liberation could be enhanced. This is to support the view that when BR is combined with substances that liberate electrons, the rate of photo-osmotic velocity is much greater than that generated by the individual pigments (*3,5*). Thus to provide a concept and experimental initiatives for establishing a viable system for conversion of solar energy into electrical power, our study possesses importance.

MATERIALS AND METHODS

Bacteriorhodopsin (Sigma [St. Louis, MO] cat no. B3636), hemoglobin (H 2500), cytochrome-C (C 3131), myoglobin (M 1882), and all other AR-grade chemicals prepared in doubly distilled water in an all-pyrex™ glass still were used in the present studies. The pH of all solutions was maintained at 5 using 0.1*M* sodium acetate buffer.

All pigment solutions were found to be surface-active in nature, and their critical micelle concentrations (CMC) in water as determined from the variation of surface tension with concentration were found to be: BR 9.5×10^{-2} ppm, hemoglobin 11.997 ppm, cytochrome-C 1.6×10^{-2} ppm, and myoglobin 8 ppm.

The experimental setup designed to demonstrate the phenomenon of photo-osmosis is depicted in Fig. 1, which has been labeled to make it self-explanatory. A Sartorius cellulose acetate microfiltration membrane M (cat no. 11107), average pore size 0.2 μm, of thickness $1 \times 10^{-4}m$ and area $5.53 \times 10^{-5}m^2$, which acted as a support for the liquid membrane, separated the transport cell into two compartments, C and D. The bright platinum electrodes E_1 and E_2 were fixed in both compartments. For these experiments, the external surface of both the compartments C and D of the transport cell was painted black except for a small window XY in the outer compartment, such that the solution in the compartment C in the vicinity of the membrane filter M could be illuminated, while the solution

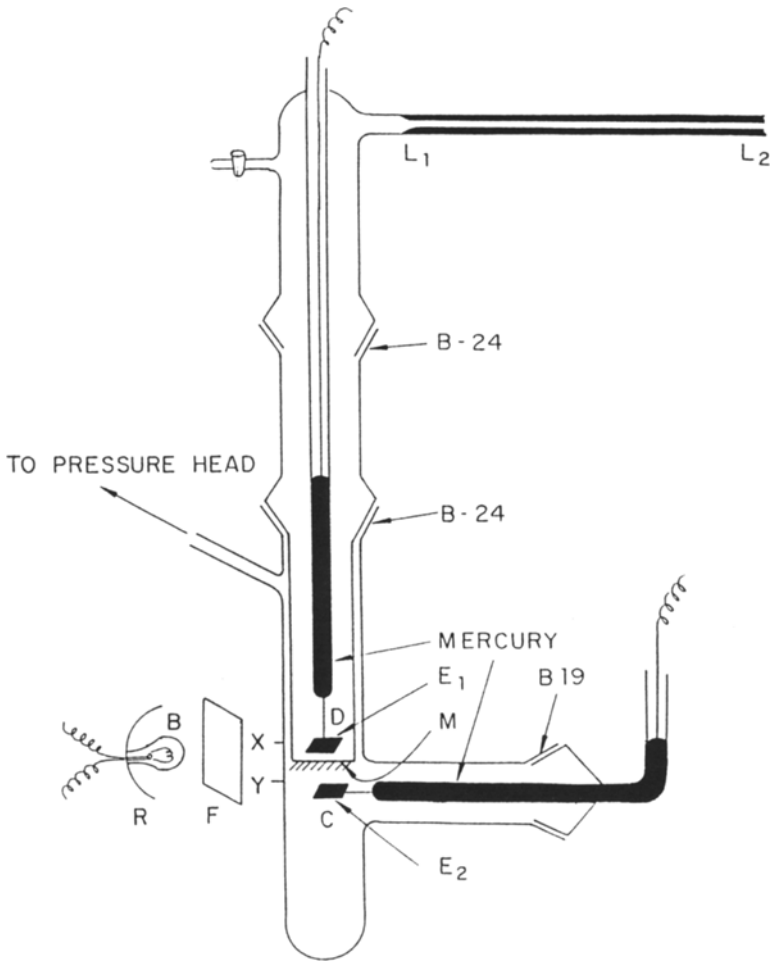

Fig. 1. Experimental setup for measurement of photo-osmotic velocity. R, reflector; B, 100-W bulb; F, filter; E_1 and E_2, platinum electrodes; M, cellulose acetate microfiltration membrane.

in compartment D was kept dark. A 20-ppm solution of BR and hemoglobin/cytochrome-C/myoglobin, in the ratio of 1:1 was filled into the two compartments of the transport cell and the condition of $\Delta P = O$ was imposed on the system by adjusting the pressure head; the pressure head was so adjusted that the liquid meniscus in the capillary L_1 L_2 remained stationary. BR, hemoglobin, cytochrome-C, and myoglobin were all surface-active in nature, and hence, according to Kesting's hypothesis (*6*) can generate surfactant layer liquid membrane. Therefore, it is expected that in both the compartments C and D, monolayers of a mixture of BR with the other pigment will be formed with the hydrophobic ends of the molecules preferentially oriented toward the hydrophobic supporting membrane filter M and the hydrophilic moieties drawn outward away from it.

The bulb B was then switched on, and the consequent movement of the liquid meniscus in the capillary L_1 and L_2 was noted with time using a cathetometer reading up to 0.001 cm and a stopwatch reading up to 0.1 s. During the measurement of light-induced volume flow, a constant and stabilized voltage of 220 from the AC main was fed to the bulb B, and the distance between the transport cell and the bulb B was kept fixed. To study the variation of the light-induced volume flow with intensity of the incident light, various voltages were fed to the bulb B to alter the intensity of the light. All measurements were made at constant temperature using a thermostat set at the desired temperature. Experiments were also done using BR alone as control with a concentration of 20 ppm in both the compartments.

RESULTS AND DISCUSSION

Data were obtained on light-induced volume flow generated by pigments—BR combined with hemoglobin or cytochrome-C or myoglobin. This flow is from the illuminated compartment C to the dark compartment D.

The magnitude of photo-osmotic velocity was found to be much greater when BR was combined with any one of the three pigments, that is, hemoglobin or cytochrome-C or myoglobin, than that for BR alone. This is because protons and electrons are extruded by the action of light in the illuminated compartment, where one acts as the acceptor of the other.

The fact that BR donates protons under the influence of light is well established by us as well as by others (*1,4,7*). Similarly, hemoglobin, cytochrome-C, and myoglobin have been shown to extrude electrons under conditions of illumination, indicating the phenomenon of photo-osmosis is de facto photo-electroosmosis (*8,9*), i.e., the volume flux is because of the light-induced electrical potential difference across the membrane and is owing to the light-driven electron pumping action of these pigments.

The data on photo-osmotic volume flux show the same trend as that of other pigments, i.e., increase in the magnitude of volume flow with increase in the intensity of incident light (Fig. 2) and with increase in temperature reaching maximum at 40°C (Table 1). Volume flow was seen instantaneously after switching on the bulb—the induction period was about 6–8 s, and as soon as the light was switched off, the flow stopped almost instantaneously, thereby ruling out the possibility of a thermal gradient. Such an induction period for establishment and decay of any significant thermal gradient is too short. In addition, the experiments were carried out under constant temperature conditions. The control experiments in which no pigment was used did not show any light-induced volume flow.

It was also observed that the magnitude of the volume flow, comparing all the filters used, was maximum in the wavelength region containing λ_{max} for BR (560 nm) (*10*) and hemoglobin (589 and 528 nm) in a mixture

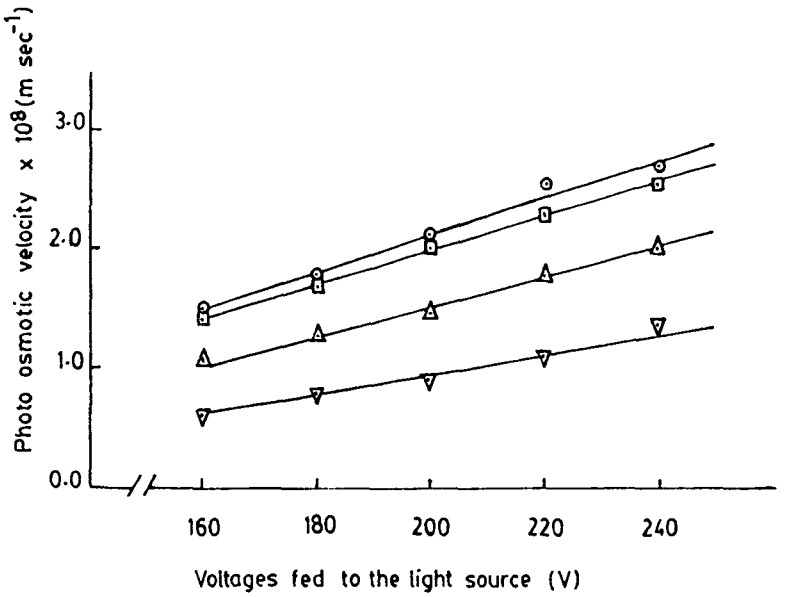

Fig. 2. Variation of photo-osmotic velocity Jv with intensity of light. The intensity was varied by feeding different voltages to the light source. ○, myoglobin with bacteriorhodopsin; □, hemoglobin with bacteriorhodopsin; △, cytochrome-C with bacteriorhodopsin; ▽, bacteriorhodopsin alone.

Table 1
Variation of Photo-osmotic Velocity
with Temperature Under Different Combinations

Temperature °C	Photo-osmotic velocity Jv × 10^8, m/s			
	BR[a]	BR[a] + myoglobin	BR[a] + cytochrome-C	BR[a] + hemoglobin
30	0.482 ±0.009	2.194 ±0.030	0.959 ±0.024	1.805 ±0.033
35	0.763 ±0.018	2.235 ±0.023	1.236 ±0.092	2.163 ±0.047
40	1.148 ±0.012	2.561 ±0.017	1.836 ±0.055	2.312 ±0.028
45	0.890 ±0.038	2.163 ±0.044	1.319 ±0.061	1.920 ±0.025
50	0.234 ±0.016	1.652 ±0.013	0.405 ±0.029	1.429 ±0.072

[a]BR = Bacteriorhodopsin
Jv = Light-induced volume flux per unit area of the membrane.

Table 2
Variation of Photo-osmotic Velocity
at Different Wavelengths Under Different Combinations at 40°C

Filter No.	Peak wavelength range, nm	Photo-osmotic velocity Jv × 10^8, m/s			
		BR[a]	BR[a] + myoglobin	BR[a] + cytochrome-C	BR[a] + hemoglobin
White light	—	1.148	2.561	1.836	2.312
		±0.012	±0.017	±0.055	±0.028
RG 2/4	644–1050	0.486	1.436	0.725	2.141
		±0.027	±0.062	±0.051	±0.021
VG 4/2	530–550	0.192	1.792	0.936	1.008
		±0.071	±0.098	±0.010	±0.018
BG 12/2	400–430	0.209	1.300	0.762	0.725
		±0.023	±0.018	±0.073	±0.097
GG 11/2	436–1050	0.768	2.321	1.539	2.214
		±0.036	±0.035	±0.029	±0.015
FGB 4/2	400–410	0.086	1.263	0.042	0.255
		±0.009	±0.019	±0.010	±0.040
BG 17/4	334–644	0.941	2.100	1.279	2.479
		±0.042	±0.092	±0.063	±0.072

[a]BR = Bacteriorhodopsin
Jv = Light-induced volume flux per unit area of the membrane.
Note: All filters were obtained from VEB Carl Zeiss JENA, Germany.

of BR and hemoglobin (Table 2). Similarly, volume flux values were constant with absorption spectra of cytochrome-C (Table 2), which exhibits bands at 550, 521, 416, and 407 nm (the peaks in the neighborhood of 400 nm are the most intense Soret bands) (*9*) and myoglobin (λ_{max} corresponds to 553 nm) along with BR.

Since the most intense absorption for porphyrins is also in the region of 400 nm, the present observation is consistent with the conclusion drawn from earlier studies (*8*) that absorption of light of BR and porphyrin-containing compounds (hemoglobin, myoglobin, or cytochrome-C) together are responsible for the development of electrical potential difference across the liquid membrane bilayers, causing the phenomenon of photo-osmosis where the rate of volume flow was much higher than when they were used alone.

As reported in earlier studies (*11*), the magnitude of electrical potentials developed across the liquid membrane bilayers generated by pigments when it was illuminated from one side was known to enhance many-fold in an asymmetric system. This happens when different redox

chemicals are present in two bathing solutions separated by liquid membrane bilayers, i.e., the stronger the electron acceptor in the illuminated compartment, the greater the magnitude of the photo-osmotic velocity. A similar explanation can be given when BR and porphyrin-containing pigments are used together. The magnitude of photo-osmotic velocity was found to be much greater than when they were used alone. The presence of BR and porphyrin-containing pigments forms an asymmetric system and results in enhanced volume flux. This may be because protons as well as electrons are extruded by the action of light in the illuminated compartment, where one acts as the acceptor of the other. In order to support this, electrodes E_1 and E_2 (Fig. 1) were short-circuited. Light-induced photo-osmosis did not stop completely, although the magnitude was reduced, which indicates that the light-induced electrical potential difference generated across the membrane was the result of both electron and proton pumping action of light in the illuminated compartment when BR and porphyrin-containing pigments were used together.

Under normal conditions, a negatively charged species should neutralize a positively charged species. For this, they should come in contact wiht each other. However, in this system, it seems that the positively charged domain of one species and negatively charged domain of another species are situated in such a way that they cannot come close enough to neutralize each other. This has resulted in the generation of system in which negatively and positively charged species can coexist on the supporting membrane.

Photo-osmotic velocity was also measured using different electron donors of varying donating strengths in the dark compartment of concentration $1 \times 10^{-3}M$ and electron acceptor (Na_2S) of varying concentrations in the illuminated compartment. It was observed from these experiments that magnitude of the photo-osmotic velocity increased with increase in concentration of electron acceptor and values of photo-osmotic velocity for various electron donors were in the order of their donating strengths (Table 3). However, increase in photo-osmotic velocity was not as significant by the use of electron donor and acceptor in the dark and illuminated compartments, respectively. This may be because the asymmetric system was already created by the presence of electron-generating and proton-releasing substances together on the supporting membrane. Photo-osmotic velocity using different electron donors in the dark compartment and different concentrations of electron acceptor in the illuminated compartment with BR alone, therefore, did not change. Values have not been given in Table 3 for BR alone. This is because of the fact that BR did not generate electrons.

Thus, in an attempt to develop a system of combination of pigments and to provide a concept for experimental initiatives for viable and potential energy-conserving method, we have performed these experiments.

Table 3
Variation of Photo-Osmotic Velocity Using Different Electron Donors in the Dark Compartment and Different Concentrations of Electron Acceptor in Illuminated Compartment Under Different Combinations

Electron donors in dark compartment $1 \times 10^{-3}M$	Electron acceptor in illuminated compartment Na_2S, M	Photo-osmotic velocity Jv $\times 10^8$, m/s		
		BR[a] + myoglobin	BR[a] + cytochrome-C	BR[a] + hemoglobin
NaI	1×10^{-5}	3.610 ± 0.014	2.435 ± 0.058	3.205 ± 0.061
	5×10^{-5}	3.843 ± 0.026	2.689 ± 0.005	3.460 ± 0.005
	1×10^{-4}	3.957 ± 0.044	2.969 ± 0.022	3.647 ± 0.056
	5×10^{-4}	4.282 ± 0.017	3.235 ± 0.080	3.836 ± 0.025
	1×10^{-3}	4.356 ± 0.019	3.459 ± 0.054	4.062 ± 0.058
$K_4Fe(CN)_6$	1×10^{-5}	3.266 ± 0.023	2.177 ± 0.062	3.031 ± 0.019
	5×10^{-5}	3.457 ± 0.047	2.384 ± 0.032	3.296 ± 0.011
	1×10^{-4}	3.656 ± 0.038	2.662 ± 0.061	3.478 ± 0.024
	5×10^{-4}	3.811 ± 0.022	2.889 ± 0.009	3.596 ± 0.036
	1×10^{-3}	3.917 ± 0.015	3.093 ± 0.064	3.793 ± 0.064
$Na_2S_2O_3$	1×10^{-5}	2.962 ± 0.026	1.993 ± 0.056	2.893 ± 0.092
	5×10^{-5}	3.259 ± 0.015	2.167 ± 0.013	3.069 ± 0.063
	1×10^{-4}	3.396 ± 0.024	2.329 ± 0.012	3.276 ± 0.013
	5×10^{-4}	3.471 ± 0.029	2.494 ± 0.092	3.403 ± 0.012
	1×10^{-3}	3.784 ± 0.031	2.605 ± 0.009	3.589 ± 0.013
$FeSO_4$ $(NH_4)_2SO_4$	1×10^{-5}	2.741 ± 0.026	1.905 ± 0.031	2.617 ± 0.061
	5×10^{-5}	2.893 ± 0.012	2.067 ± 0.061	2.801 ± 0.012
	1×10^{-4}	3.062 ± 0.014	2.175 ± 0.080	2.996 ± 0.022
	5×10^{-4}	3.291 ± 0.026	2.303 ± 0.066	3.184 ± 0.080
	1×10^{-3}	3.390 ± 0.027	2.445 ± 0.021	3.384 ± 0.054

[a]BR = Bacteriorhodopsin
Jv = Light-induced volume flux per unit area of the membrane.

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