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Photo-Osmosis Through Liquid Membrane Bilayers Generated by β-Carotene Coupled with Bacteriorhodopsin

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ABSTRACT

β-carotene, a photosynthetic pigment isolated from the blue green algae *Anabeana variabilis* has been shown to exhibit the phenomenon of photo-osmosis through liquid membrane bilayers. In our search for new pigments that could show this phenomenon, we found β-carotene also. When β-carotene was combined with bacteriorhodopsin extracted from the extreme halophile *Halobacterium halobium*, the rate of photo-osmotic velocity was much higher than for either of these pigments independently. The rate of light induced volume flux depends on temperature, intensity, and wavelength of incident light and the nature and concentration of electron donors and acceptors.

Index Entries: β-carotene; photo-osmosis; liquid membrane bilayers; bacteriorhodopsin.

INTRODUCTION

In order to harness inexhaustible flow of solar energy we use expensive crystalline silicone as receptors or traps (*1*). If we can use bacteriorhodopsin (BR) to generate electrochemical gradients by liberating H^+ across membranes, we may be able to generate energy. To increase the energy generating capacity, we tried to couple β-carotene with BR so that the H^+ ion liberation could be enhanced.

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In algae, β-carotene occurs as a major carotene. This pigment is a polyene consisting of isoprene residues so arranged that in middle of the molecule, 2 methyl groups are present in the 1,6 position whereas all other side chain methyl groups on the 1,5 position. The presence of this pigment in blue-green algae that gets invariably exposed to sunshine may have a role to play in the physiology of the organism and energy production.

β-carotene is found to be surface active in nature and hence according to Kesting's hypothesis (*2*), can generate surfactant layer liquid membrane. Therefore, it should be possible using the procedure used in earlier studies (*3,5*) to generate liquid membrane from β-carotene extract on either side of the hydrophobic supporting membrane. The β-carotene liquid bilayers thus generated have shown the phenomenon of photo-osmosis as a consequence of electrical potential difference which develops across the liquid membrane bilayers owing to the light driven electron pumping action of β-carotene. In our present communication we report a new observation on photo-osmosis through liquid membrane bilayers generated by β-carotene extracted from algae.

BR acts as a photoelectric energy transducer and generates electrical potential difference across the membrane under the influence of light by acting as a light driven proton pump and the phenomenon of photo-osmosis have also been demonstrated by using it (*6*).

The property of light-induced charge separation has made it possible to develop a system containing chlorophyll and BR as potential candidates for direct conversion of solar energy into electrical power (*7*). However, no viable system has yet been developed. Therefore, experiments carried out with a view to study the phenomenon of photo-osmosis through liquid membrane bilayers generated by a mixture of BR and β-carotene are also described in this communication.

MATERIALS AND METHODS

Bacteriorhodopsin (Sigma Cat. No. B 3636), β-carotene extracted from *Anabeana variabilis*, all other AR grade chemicals, and doubly distilled water in all Pyrex® glass still were used in the present studies. The pH of all solutions were maintained at 5 using 0.1*M* Tris HCl buffer.

The cell mass of Anabeana was collected by centrifugation and was crushed after addition of aqueous potassium hydroxide (1 mL of 60% W/V). Ethanol (10 mL) was added to the crushed mass. The tube was placed in a water bath at 40–50°C. After 5 min, the mixture was centrifuged and the supernatant decanted. The pigment is transferred to equal vol of ether and then water was added until the 2 phases separate. The ether phase is separated and dried with anhydrous sodium sulfate.

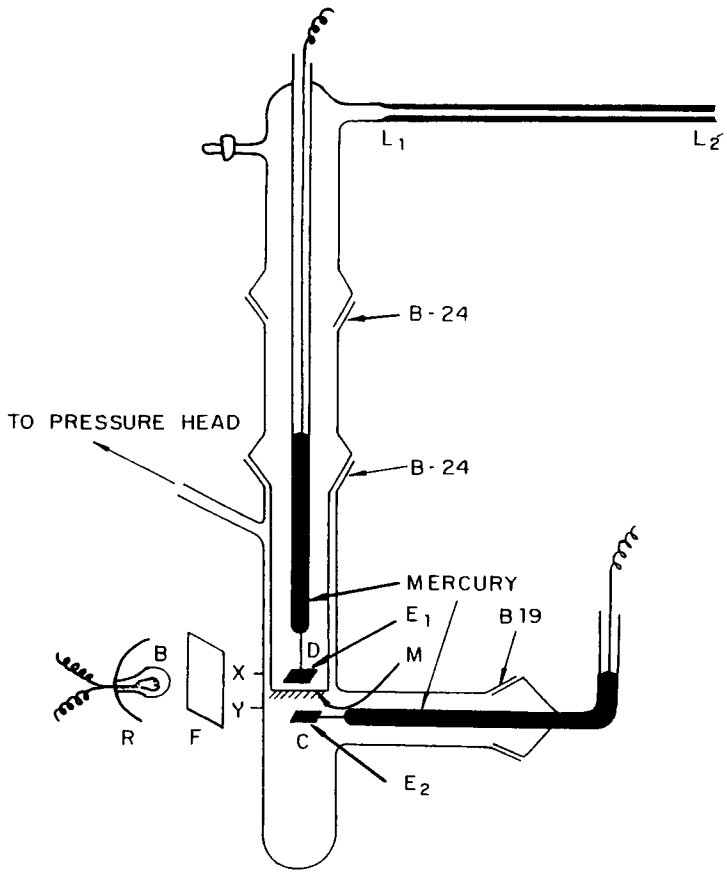

Fig. 1. Experiment setup for measurements of photo-osmotic velocity. R, reflector; B, 100 W bulb; F, filter; E_1 and E_2, platinum electrodes; M, cellulose acetate microfiltration membrane.

Bacteriorhodopsin and β-carotene are surface active in nature. Their CMC's in water were determined by hydraulic permeability data. To determine the concentration of β-carotene and BR to be used for the formation of liquid membrane bilayers on the cellulose acetate membrane support, their CMCs were calculated. The values were found to be 9.5×10^{-2} ppm for BR and 1 ppm for β-carotene. These values were further supported by electrical conductivity measurement data.

The experimental set up designed (*8*) to demonstrate the phenomenon of photo-osmosis is depicted in Fig. 1. A Sartorius cellulose acetate microfiltration membrane M (Cat. No. 11107, average pore size 0.2 μm) of thickness 1×10^{-4} m and area 5.53×10^{-5} m^2, which acted as a support for the liquid membranes separated the transport cell into two compartments, C and D. For these experiments the external surface of both the compartments C and D of the transport cell was painted black leaving a small

window XY in the outer compartment C through which the light could be allowed to fall on the solution in the vicinity of the membrane filter M in compartment C keeping the solution in compartment D dark. A 5 ppm solution of β-carotene was filled in both the compartments of the transport cell and the condition $\triangle P = 0$ 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. β-Carotene is surface active in nature and therefore, it is expected that in both compartments C and D, monolayers of β-carotene will be formed with hydrophobic ends of the molecule preferentially oriented towards the hydrophobic supporting membrane M (Fig. 1) 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 L_2 was noted with time using a cathetometer reading up to 0.001 cm and a stop watch reading up to 0.1 s. During the measurement of light induced volume flow that is expressed as photo-osmotic velocity (i.e., Jv—light induced volume flux per unit area of the membrane), a constant and stabilized voltage of 220 V from 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 thermostat set at desired temperature.

Various electron donors and acceptors were added to the solution to study their effect.

Experiments were repeated using mixture of BR and β-carotene (5 ppm solution of BR and β-carotene in the ratio of 1:1 in both the compartments).

RESULTS AND DISCUSSION

The data on photo-osmosis volume flux through liquid membrane bilayers generated by β-carotene and mixture of BR and β-carotene are recorded in Table 1, 2, and 3 shows the same trends as that of other photosynthetic pigments, i.e. increase in the magnitude of the volume flow with increase in intensity of light (Fig. 2), and with increase in temperature up to 40°C (Table 1). Light induced volume flow was observed from the illuminated compartment C to dark compartment D. Volume flow was seen instantaneously after switching on the light—the induction period was about 10 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 a induction period for establishment and decay of any significant thermal gradient is too short. In addition, the experiments were carried out under constant temperature condition.

Table 1
Variation of Photo-osmotic Velocity with Temperature

Temperature, °C	Photo-osmotic velocity $Jv \times 10^7$ (m s^{-1})	
	β-carotene	β-carotene + BR*
30	0.88 ± 0.008	1.93 ± 0.030
35	1.24 ± 0.008	2.78 ± 0.062
40	1.81 ± 0.006	2.96 ± 0.008
45	1.66 ± 0.002	1.81 ± 0.001
50	1.24 ± 0.006	1.35 ± 0.005

*BR = Bacteriorhodopsin.
Jv = Light induced volume flux per unit area of the membrane.

Table 2
Values of Photo-osmotic Velocity at Different Wavelength Ranges

Filter No.	Peak wavelength range (nm)	Photo-osmotic velocity $Jv \times 10^7$ (m s^{-1})	
		β-carotene	β-carotene + BR*
White light	—	1.81 ± 0.006	2.96 ± 0.007
BG 12/2	400–430	0.61 ± 0.002	0.88 ± 0.008
BG 7/3	480–509	0.66 ± 0.010	1.24 ± 0.008
BG 17/4	334–644	1.12 ± 0.001	1.52 ± 0.002
VG 4/2	530–550	0.75 ± 0.007	1.76 ± 0.001
GG 11/2	436–1050	1.30 ± 0.002	1.93 ± 0.01

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

As seen earlier in BR (*6*), in case of β-carotene, the volume of flow in the capillary L_1 L_2 continued as long as the light was on and ceased as soon as the light was switched off. 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 β-carotene (*9*) (Table 2). The control experiments in which no pigment was used did not show any light induced volume flow. It was observed that as soon as the electrodes, E_1 and E_2 (Fig. 1) were short circuited, the light induced volume flow stopped completely. Thus indicating the phenomenon of photo-osmosis is defacto photoelectroosmosis, i.e., the volume flux is on account of the light induced electrical potential difference across the membrane as sug-

Table 3
Variation of Photo-osmotic Velocity
Using Different Electron Donors in the Dark Compartment
and Different Concentration of Electron Acceptor in Illuminated Compartment

Electron donors in dark compartment ($1\times10^{-3}M$)	Electron acceptor in illuminated compartment Na_2S (M)	Photo-osmotic velocity $Jv\times10^{7}$ ($m\ s^{-1}$)	
		β-carotene	β-carotene + BR*
NaI	1×10^{-5}	3.18±0.001	3.59±0.001
	5×10^{-5}	3.43±0.002	4.12±0.001
	1×10^{-4}	4.16±0.001	4.68±0.003
	5×10^{-4}	4.74±0.002	5.56±0.001
	1×10^{-3}	5.47±0.001	5.81±0.002
$K_4Fe(CN)_6$	1×10^{-5}	2.59±0.008	3.14±0.009
	5×10^{-5}	2.64±0.009	3.99±0.001
	1×10^{-4}	2.89±0.003	4.28±0.004
	5×10^{-4}	3.31±0.002	4.52±0.005
	1×10^{-3}	4.12±0.001	5.29±0.002
$Na_2S_2O_3$	1×10^{-5}	2.10±0.002	2.34±0.001
	5×10^{-5}	2.74±0.004	3.07±0.002
	1×10^{-4}	3.05±0.004	3.45±0.001
	5×10^{-4}	3.92±0.001	4.27±0.004
	1×10^{-3}	4.79±0.001	5.12±0.001
$FeSO_4(NH_4)_2SO_4$	1×10^{-5}	2.00±0.001	2.15±0.004
	5×10^{-5}	2.24±0.002	2.58±0.004
	1×10^{-4}	2.71±0.011	3.22±0.003
	5×10^{-4}	3.43±0.002	3.94±0.009
	1×10^{-3}	3.85±0.010	4.31±0.007

*BR = Bacteriorhodopsin.
Jv = Light induced volume flux per unit area of the membrane.

gested by Tien (*7*). Electrical potential difference that develops across the liquid membrane bilayers is due to the light driven electron pumping action of β-carotene.

Explanation similar to that offerred by Tien in the case of chloroplast BLMs, can be extended in the present case also to account for the origin and direction of the photo-osmotic volume flux. That is to say the liquid membrane generated by β-carotene on excitation by light ejects electrons in the illuminated compartment. This leads to the formation of a double layer consisting of a negatively charged mobile phase and a positively charged fixed layer of the liquid membrane from the constituent molecules of which the electrons are knocked off by the action of light.

As reported in the earlier studies (*5*) magnitude of electrical potentials developed across the liquid membrane bilayers generated by photosyn-

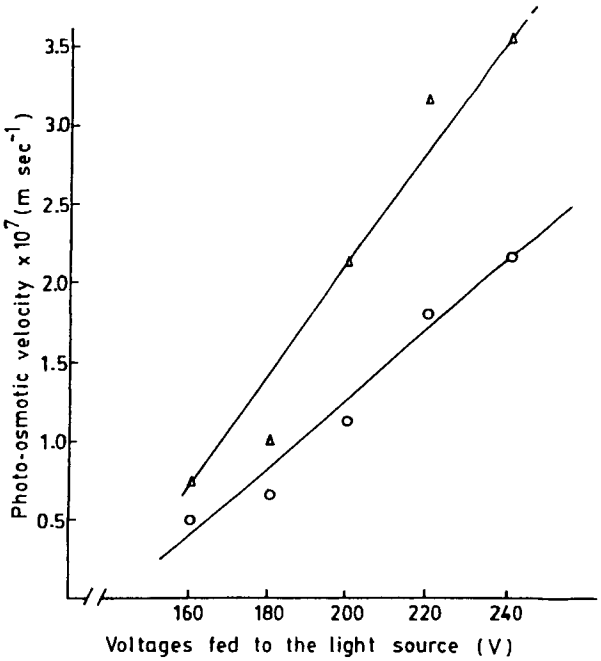

Fig. 2. Variation of photo-osmotic velocity Jv with intensity of light. The intensity was varied by feeding different voltages to the light source (○, β-carotene; △, β-carotene and Bacteriorhodopsin).

thetic pigment when it is illuminated from one side, are known to enhance manifold in asymmetric system. This happens when different redox chemicals are present in the two bathing solutions separated by liquid-membrane bilayers, i.e., stronger the electron acceptor in the illuminated compartment, greater should be the magnitude of the photo-osmotic velocity.

For this, photo-osmotic velocity was measured using different electron donors of varying electron donating strength in the dark compartment of concentration of $1 \times 10^{-3} M$ and electron acceptor (Na_2S) of varying concentrations in illuminated compartment. It was observed from these experiment that magnitude of the photo-osmotic velocity increases with increase in concentration of electron acceptors and values of photo-osmotic velocity for various electron donors are in the order of their electron donating strength. The electron donating strength of the various electron donors are in the following order:

$$NaI > K_4Fe(CN)_6 > Na_2S_2O_3 > FeSO_4(NH_4)_2SO_4$$

These experiments further support that volume flux is observed due to light induced charge separation across the β-carotene liquid membrane bilayers, i.e., generation of electrons in the illuminated compartment similar to Tien's observations on light induced water flow across chloroplast-BLM (*7*) in terms of semiconductor physics and classical electrokinetics.

Similar phenomenon of photo-osmosis have also been demonstrated using bacteriorhodopsin that acts as a photoelectric energy transducer and generate electrical potential difference across the membrane under the influence of light by acting as a light driven proton pump (*6,10*).

In order to develop a system containing β-carotene and bacteriorhodopsin due to their property of light induced charge separation, experiments have been carried out with a view to study the phenomenon of photo-osmosis through liquid membrane bilayers. Data are recorded in Table 1, 2, and 3.

The electrical potential difference developed across the bacteriorhodopsin-β-carotene liquid membrane bilayers, which is responsible for the observed photo-osmosis volume flux is a consequence of joint action of the light driven proton pumping action of bacteriorhodopsin and generation of electron in illuminated compartment by β-carotene.

When both, bacteriorhodopsin and β-carotene were used together, the magnitude of photo-osmosis velocity was found to be much greater than when they were used alone (Table 1, 2, and 3). This may be because of that protons as well as electrons are extruded by the action of light in the illuminated compartment, where one acts as the acceptor for the other.

The fact that bacteriorhodopsin donates protons under the influence of light is well established (*6,10*). Similarly, we have shown β-carotene extrudes electrons by the action of light. Under normal circumstances a negatively charged species should neutralize a positively charged species. For this, they should come in contact with each other. However, in this system it seems that positively charged domain of one species molecule and negatively charged domain of another species molecules are situated in such a way that they can not come close enough to neutralize each other. This has resulted in generation of system in which negatively and positively charged species can coexist on the supporting membrane. Presence of bacteriorhodopsin and β-carotene form an asymmetric system and result in enhanced volume flux.

Mixture of bacteriorhodopsin and β-carotene also shows the same trend of photo-osmotic velocity with intensity and wavelength of the exciting light, temperature, and concentration and nature of electron donors and acceptors in the system.

It was observed that the magnitude of the volume flow, comparing all the filters used was maximum in the wavelength region containing λ max for bacteriorhodopsin (560 nm) (*10*) and β-carotene (451 nm) (*9*). It indicates that absorption of light by bacteriorhodopsin and β-carotene together are responsible for the development of electrical potential difference across the liquid membrane bilayers causing the phenomenon of photo-osmosis in higher rate than when they are used alone.

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