CONVERSION OF LIGHT ENERGY INTO ELECTRIC ENERGY BY BACTERIORHODOPSIN

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According to the chemiosmotic theory of Mitchell [1], some membranous proteins should carry electrons or protons through the membrane against an electrochemical gradient. Studies along this line have resulted in an electrogenic activity of several membranous systems being directly demonstrated (for review, see [2]). Among them bacteriorhodopsin proved to be the simplest one. This protein, isolated from *Halobacterium halobium* by Oesterhelt and Stoeckenius, is a single polypeptide of 27 000 mol. wt. comprising 75% of the weight of special areas of the bacterial membrane (purple sheets) containing, in addition to bacteriorhodopsin, very stable saturated phospholipid and carotenoids [3]. No other proteins but bacteriorhodopsin were found in the purple sheets.

Our group has shown that illumination of the planar artificial membrane formed from a mixture of a decane solution of azolectin and purple sheets induces generation of a transmembrane electric potential difference which can be measured by a voltmeter [4]. Direction and magnitude of the potential varied from membrane to membrane being not higher than 50 mV. The photoeffect was found to increase if (1) an electric potential difference of the direction opposite to the photo e.m.f. was generated by an external battery, or (2) acid was added to the compartment which charges negatively in the light (or alkali was added to the positively-charged compartment). The photoeffect decreased if directions of external e.m.f. or ΔpH were reversed. In this way, bacteriorhodopsin was directly identified as an electrogenic system pumping H^+ (or OH^-) ions against their electrochemical gradient. The study of this model gave some indications of the existence of two bacteriorhodopsin pools in the planar membrane pumping protons in opposite direction. To obtain more regular arrangement of bacteriorhodopsins, we applied the method of bacteriorhodopsin proteoliposome reconstitution suggested by Racker and Stoeckenius [5]. Bacteriorhodopsin sheets and azolectin were dissolved by cholate with subsequent removal of cholate by dialysis. Vesicles reconstituted during dialysis were found to pump H^+ ions from the outside to the inside when the light was switched on [5,6].

As experiments showed [7], addition of the bacteriorhodopsin proteoliposomes to one of two compartments separated by a planar azolectin membrane results in the system demonstrating the photoeffect. To induce binding of proteoliposomes with a planar membrane a polyvalent cation (La^{3+}, Ca^{2+} or Mg^{2+}) was added. In this case 'plus' of the photoinduced potential difference was always in the proteoliposome-free compartment. The maximal photoeffect values were as high as 150 mV.

A difficulty arising always in experiments on planar artificial membranes consists in the fact that they are rather unstable. Apparently, the optimal thickness of the bacteriorhodopsin-containing artificial membrane should be about 70 Å since the natural function of bacteriorhodopsin is to carry H^+ ions through the bacterial membrane of this thickness. The diameter of the aperture covered by planar membrane in the model experiments is about 1 mm or 1×10^7 Å. This means that the ratio of the membrane thickness to its diameter is less than $1:10^5$. It is not surprising that such a film is unstable, especially when the membrane-forming mixture contains proteins and traces of detergents usually used to dissolve biological membranes and to prepare proteoliposomes.

To stabilize the planar membrane, L. A. Drachev et al. [8] used a millipore filter impregnated with phospholipid and treated on one side with bacteriorhodopsin proteoliposomes and Ca^{2+} ions. Such a filter covering a 1 cm aperture in the Teflon partition separating the two compartments of the experimental cell was found to generate a photocurrent after many

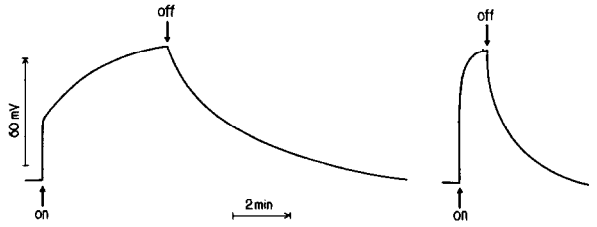

Fig.1. The light-induced generation of an electric potential difference across a millipore filter impregnated with azolectin solution in decane and treated on one side with bacteriorhodopsin proteoliposomes (after Drachev et al [8]). One of two solutions of equal composition (0.05 M Tris-HCl, pH 7.5, and 0.04 M $CaCl_2$) separated by the azolectin-impregnated filter, was supplemented with bacteriorhodopsin proteoliposomes (0.3 mg protein/ml). Millipore-type filter produced by Chemapol, Praha (Synpore) with 1.5 μm pores was used. After 45 min incubation required for proteoliposome association with filter, the light response was measured (the left-hand curve). Then solutions on both sides of the filter were substituted by fresh ones (without proteoliposomes) and the light response was measured once more (right-hand curve).

changes of the solutions on both sides of the filter. The data of a typical experiment are shown in fig.1. In such a system, a photoeffect can be observed during several days without changing the filter (fig.2).

Using the above techniques we made a three-compartment experimental cell with two bacteriorhodopsin-

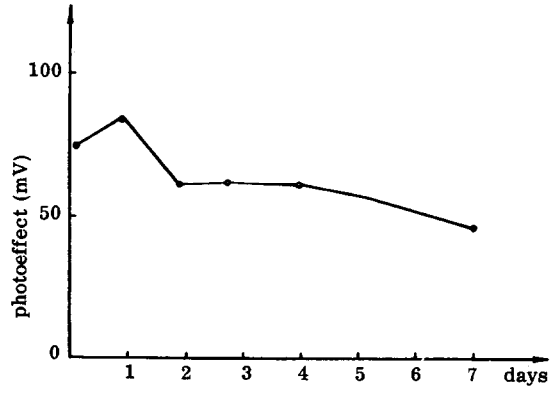

Fig.2. The light-induced responses of azolectin- and bacterio rhodopsin-treated millipore filter as a function of the time of filter storage [8]. The filter was stored at 21°C in a solution of 0.05 M Tris-HCl, pH 7.5, for the period indicated. For other conditions, see fig.1.

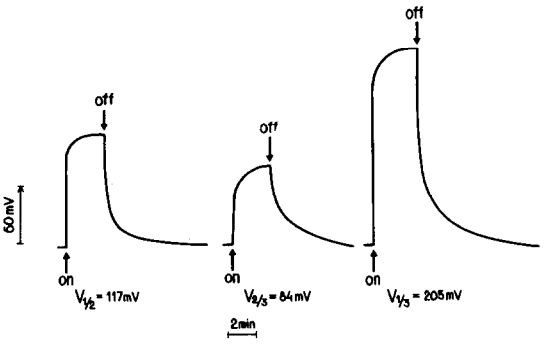

Fig.3. The light-induced response of two azolectin- and bacteriorhodopsin treated filters placed consecutively [8]. A three-compartment cuvette was used. $V_{1/2}$, $V_{2/3}$ and $V_{1/3}$:electric potential differences between 1st and 2nd, 2nd and 3rd, 1st and 3rd compartments, respectively. For other conditions, see fig.1.

containing filters placed consecutively. The total light-induced potential difference between the first and the third compartments was found to be equal to the sum of potential differences across each of the filters (fig.3). It should be mentioned that the stability of the photoeffect suggests that not only the phospholipid membrane is reinforced by the filter but also bacteriorhodopsin per se is very stable when incorporated into the membrane. It is not surprising since even boiling the purple sheets for one min could not inhibit the electrogenic activity of the planar membrane formed from the boiled sheets and azolectin.

The method based on association of proteoliposomes with a planar phospholipid membrane or a phospholipid-impregnated filter allowed us to demonstrate electric generation by cytochrome oxidase and H^+-ATPase from beef heart mitochondria [7] as well as by bacteriochlorophyll-containing lipoprotein complex of the photosynthetic reaction centers from *Rhodospirillum rubrum* chromatophores [9]. It was also observed that intact chromatophores can be associated with the planar membrane or the filter (in the latter case the highest value of membrane potential was 215 mV). All molecular generators other than bacteriorhodopsin are composed of several polypeptides and phospholipids. Their molecular weights were 3–20 times higher than that of bacteriorhodopsin. None of them was so stable as the bacteriorhodopsin generator.

Three possible ways to practical application of the bacteriorhodopsin photoelectric generator may be investigated. (1) First of all, attempts may be made to use bacteriorhodopsin to produce electric current at the expense of sunshine. To this end, further development of the method, described above, might prove to be useful. (2) Shunting the bacteriorhodopsin-containing artificial membrane by an external conductor, one can convert electric potential, generated in the light, to ΔpH across this membrane. The latter might be utilized to form O_2 in the alkaline compartment and H_2 in the acidic compartment using O_2- and H_2-electrodes, respectively. (3) Using the artificial membrane, containing both bacteriorhodopsin and H^+-ATPase, it seems to be possible to obtain an artificial system of photophosphorylation of ADP by inorganic phosphate. To do this, one should stabilize H^+-ATPase up to the level of the bacteriorhodopsin stability.

In all three cases, phospholipid-impregnated membrane filters can be used as a framework for molecular electric generators.

System (3) is of especial importance since the use of any anabolic enzyme to form a product of biological origin in vitro requires energy which is supplied by ATP (or some other nucleoside triphosphates). Therefore one will need large amounts of ATP to develop synthetic industry based on the usage of enzymes. So, search for any mechanisms of the ATP regeneration in vitro seems to be very interesting. Photophosphorylation in the reconstituted system, composed of H^+-ATPase and bacteriorhodopsin (or cyclic electron transfer in a photosynthetic redox chain), is very convenient since it has no other products but ATP, in contrast to respiratory and glycolytic phosphorylations coupled with oxidation of corresponding substrates.

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