BACTERIORHODOPSIN AS A LIGHT-DRIVEN ION EXCHANGER?

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Introduction

Some years ago, the retinal protein complex bacteriorhodopsin was discovered in Halobacteria [1] and its function recognized as that of a light-driven proton pump [2]. The outline given here describes the possible suitability of this chromoprotein to mediate an ion exchanging process driven by light which could serve for desalination purposes.

Occurrence, isolation and properties of bacteriorhodopsin

Bacteriorhodopsin is synthesized by Halobacteria which grow in saturated salt (NaCl) solutions e.g. in open ponds where salt is produced by evaporation of sea water. Such brines may contain large masses of Halobacteria. In the laboratory Halobacteria can be cultured easily even under unsterile conditions. Bacteriorhodopsin is maximally synthesized by the cells under conditions of illumination and limiting aeration. It then reaches intracellular concentrations of about 10^{-3} M.

Bacteriorhodopsin is organized in patches of a hexagonal lattice structure called the purple membrane [3]. These purple membrane patches are part of the cell membrane. They are easily isolated by simple centrifugation techniques after lysis of the halobacterial cell with water [4]. From a 10 litre culture 300–500 mg purple membranes may be isolated. A single purple membrane patch has an average diameter of 0.5 μm and a thickness of 48 Å. It is a two-dimensional crystalline array of about 10^5 molecules bacteriorhodopsin, all oriented in the same direction. The protein makes up 75% of the dry weight of the membrane, 25% consists of lipids. Two different faces of the membrane can be demonstrated, face A and face B that are different in surface structure and electric charge [3].

Purple membranes (≅ bacteriorhodopsin) are a very stable biological material. The chromophore is not destroyed between pH 1 and 10, will stand temperatures up to 80°C and proteases only attack the membrane protein with difficulty. Purple membrane samples may be kept for years without damage to their function.

The biological function of bacteriorhodopsin as a light-driven proton pump is mediated by the photochemical cycle of bacteriorhodopsin [5] (fig.1).

The formation and the decay of the intermediate of the cycle with the longest life time (412 nm complex) is accompanied by proton release and uptake. This photochemical cycle occurs as a vectorial process in the purple membrane, i.e. protons are released from one side of the membrane (B face) and taken up by the other side (A face). In the intact cell this results in an electrochemical gradient between inside and outside. Under natural conditions the photochemical cycle of bacteriorhodopsin will be light limited, i.e. depends on the extinction coefficient of bacteriorhodopsin (ϵ_M = 63 000 litre/Mol·cm), the quantum yield of the photochemical event (ϕ = 0.80) and the light intensity. Only very high light intensities saturate the system, so that the thermal reconversion of the 412 nm complex into the purple complex limits the cycle (k_2 = 200 sec^{-1} at physiological temperatures).

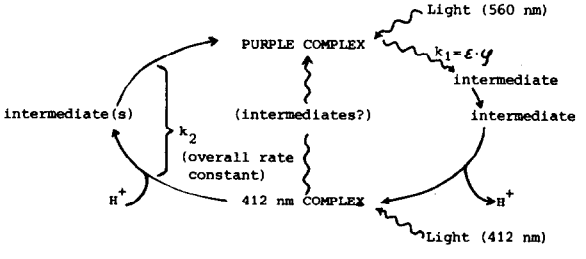

Fig.1. Photochemical cycle of bacteriorhodopsin. Purple complex and 412 nm complex designate two states of the chromophore.

This reaction can be photochemically accelerated. Further increase of the turnover number is therefore possible by concomitant illumination with blue and green light [5].

Because of all these properties the system represents a useful tool for the efficient conversion of quanta into an electrochemical gradient. Approximately one proton is electrogenically transported through the purple membrane per quantum. Out of the 50 kcal/Einstein absorbed light energy the proton carries an unknown percentage. Since the electromotive force of bacteriorhodopsin was estimated to be $\geqslant 300$ mV a free energy of $\geqslant 7$ kcal would be available [6]. If purple membrane sheets are joined in series an even larger amount of energy is obtained.

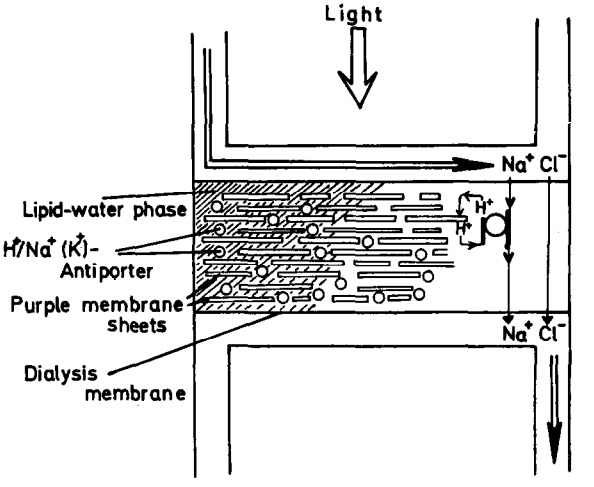

Fig.2. Schematic drawing of a desalination process coupled to the light-driven proton pump bacteriorhodopsin.

Steps towards a macroscopic pumping device

Since the purple membranes are too small to be handled as single sheets it is necessary to orient them parallel to each other, all A faces pointing to the same direction. The sheets should also be packed closely together. Such an arrangement of membranes would have to be stabilized and the interspace should be kept as small as possible in order to avoid back leakage of the pumped protons.

Orientation of purple membranes is possible by drying suspensions on mica [3] and by electrophoresis. It should also be possible by applying magnetic and electric fields which act through the anisotropy of the membrane sheets. Illuminated membrane suspensions consist of sheets with proton currents around the edges of the sheet and a magnetic moment could result, facilitating the orientation process.

It seems to be more difficult to orient the sheets with respect to their A and B faces. However, since one can assume that the two surfaces have different net charges, electrophoresis could induce this orientation. It has been shown, that the purple membrane sheets orient at water–air interfaces with their intracellular surface towards the aqueous phase [7]. In a three-dimensional oriented sample this type of orientation can be analyzed by the light induced net proton transport of the sample. The parallel orientation of the sheets is detected by X-ray diffraction or dichroism of bacteriorhodopsin.

Once an oriented bulk phase of purple membranes is obtained, this array has to be stabilized either by increasing viscosity (agarose or polyacrylamide gel) or by cross-linking agents. Glutaraldehyde cross-links purple membrane permanently without destroying its chromophore.

The last step towards an ion exchanger is the addition of a carrier that antiports H^+ for alkali ions, e.g. nonactin or monensin. Their action would be the conversion of the electrochemical gradient of the proton into that of an alkali ion gradient. The electrostatic force of this gradient can pull a chloride through the phase. Since chloride has a comparatively high permeability no additional carrier might be necessary. The properties of the water–lipid phase between the membrane sheets are of greatest importance. Its properties may have to be modified in order to compromise between H^+, Na^+ and K^+ permeabilities on one hand and that of chloride on the other hand.

Provided that all these steps can be successfully achieved one would expect that in light salt is transported from the upper compartment to the lower one. The desalination of sea water ($\sim$ 1 M) for production of fresh water (e.g. 0.01 M) could therefore be driven by sunlight. Although one cannot predict whether all steps can be biotechnologically solved it seems to be worthwhile to tackle this problem.

References

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