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Photoreactions of Bacteriorhodopsin* **

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Abstract. Bacteriorhodopsin is a membrane-bound light energy transducer which generates an electrochemical proton gradient. It undergoes a cyclic photoreaction in which five intermediates have been identified. During the cycle it releases a proton from one surface of the membrane and takes up a proton on the opposite surface. The active chromophore consists of retinal bound through a Schiff base to the protein. The Schiff base is deprotonized during the photoreaction cycle and appears to be involved in the transport of protons through the membrane. The retinal may also undergo an isomerization.

Key words: Light energy transduction – Purple membrane – Halobacteria – Proton transport – Spectroscopy.

Bacteriorhodopsin occurs as crystalline patches in the surface membrane of *Halobacterium halobium.* These patches, called the purple membrane, convert light energy into an electrochemical proton gradient across the membrane. The cell uses the energy stored in the gradient for the synthesis of ATP from ADP and P_i and for other energy-requiring processes.

Bacteriorhodopsin is the only protein present in the purple membrane. It has a molecular weight of 25,000–26,000, spans the width of the membrane and contains per mole protein one mole of retinal bound as a Schiff base to the ε-amino group of a lysine residue. Protonation of the Schiff base and further complexation of the protein induces a large red shift in the retinal absorption band to 560–570 nm. Bacteriorhodopsin undergoes a change in its absorption spectrum when it is kept in the dark for prolonged times; the absorption decreases slightly and its maximum shifts from 570–560 nm. We call this the dark-adapted form of the pigment and denote it bR(DA, 560) to distinguish it from the light-adapted form bR(LA, 570). This reversible photoreaction is too slow to account for the light energy conversion observed in

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intact cells. However, bacteriorhodopsin also shows a fast cyclic photoreaction which is accompanied by protonation changes and does account for its function as a light-driven proton pump. Both bR(DA, 560) and bR(LA, 570) show fast cyclic photoreactions. However, whereas bR(LA, 570) is completely recovered at the end of each cycle, part of bR(DA, 560) is converted to bR(LA, 570) and thus light adapted [1, 2].

We have investigated the photoreaction cycle of bR(LA, 570) with low temperature and flash spectroscopy. When bR(LA, 570) is illuminated at $-196°$ C, its absorption maximum shifts to longer wavelength due to formation of the photocycle intermediate K(LA, 610), which is stable at this temperature but is converted back to bR(LA, 570) by light. Because of this back conversion and the overlap of the absorption spectra, only photosteady state mixtures of bR(LA, 570) and K(LA, 610) can be obtained which maximally contain $\sim$ 50% K(LA, 610). The absorption spectrum of K(LA, 610) has been calculated; compared to bR(LA, 570) its extinction is slightly lower and the maximum shifted to 610 nm the isosbestic point occurs at 590 nm. When K(LA, 610) is allowed to warm up, it also returns to bR(LA, 570); however, transient spectral changes indicate that in this dark reaction at least two blue shifted intermediates occur which we denote L(LA, 550) and M(LA, 415), the subscripts indicating the estimated absorption maxima. They, too, cannot be obtained in pure form and their calculated absorption spectra involve some assumptions [2].

The same intermediates are also observed with flash spectroscopy at physiological temperatures. K(LA, 590) forms within a few picoseconds and decays to L(LA, 540) in $\sim$ 1 μs at room temperature [3]. M(LA, 412) forms from L(LA, 540) in $\sim$ 40 μs and decays with a half time of $\sim$ 7 ms. The small differences in the absorption maxima for the intermediates calculated from the flash and low temperature data may be due to errors in the calculation as well as a real effect of temperature. The flash data very clearly indicate the occurrence of at least two additional intermediates which we tentatively designate N(LA, 520), O(LA, 640) and place after M(LA, 412) in the photoreaction cycle. However, the temperature and pH dependence of the absorption changes attributed to these intermediates seems to preclude a simple linear reaction sequence; either a back reaction or branching must occur in this part of the photoreaction cycle. So far we have unambiguously established only that at least 5 intermediates are present in the reaction cycle [2]. Additional data are needed to describe their relation and kinetics.

For every bacteriorhodopsin molecule cycling, a proton is released and bound again, as indicated by the absorption changes of added pH indicator dyes. Under our conditions the proton appears shortly after M(LA, 412) forms and disppears with the time constant corresponding to the reformation of bR(LA, 570). In vesicle preparations which contain functional bacteriorhodopsin oriented in opposite directions, we have shown that the release of the proton occurs on the outer surface of the purple membrane and the uptake on the cytoplasmic surface, thus establishing that a pathway for protons through the membrane must exist which links the two sites and that the protonation change reflects the function of the membrane as a proton pump [1]. That protonation changes play a role in the function of the photoreaction cycle is further demonstrated by a rather large isotope effect on two time constants during the cycle when the membrane is suspended in D_2O. A fully deuterated membrane in

H_2O does show no significant difference to the normal membrane in its flash kinetics [2]. The Schiff base also undergoes a protonation change during the photoreaction cycle and it appears reasonable to assume that this light-induced reaction drives the translocation of protons across the membrane [4].

When the retinal is rapidly extracted from bR(LA, 570), only the all-trans isomer is obtained. It is not possible to extract the intermediates of the normal photoreaction cycle by the same technique because they are so shortlived. However, treatment of the membrane with high concentrations of NaCl and ether or with guanidine-HCl modify the kinetics of the reaction cycle so that during illumination relatively large concentrations of M(LA) accumulate and decay rather slowly in the dark to bR(LA). Extraction of these preparations during or immediately after illumination yield mixtures of 13-cis and all-trans retinal in the case of salt-ether or nearly exclusively 13-cis in the case of guanidine-HCl [5]. These findings indicate that an all-trans to 13-cis isomerization may take place during the photoreaction cycle. Consistent with this assumption is a movement of the chromophore during the photoreaction cycle which we have detected by following the decay of the dichroism induced in the membrane suspension by a flash of polarized light [2].

The data available so far allow one to speculate on possible mechanisms for the light-driven proton translocation and develop models of some heuristic value. Critical tests of such models, however, will have to await a better understanding of the photoreaction cycle.

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Discussion
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The analysis of proton release and binding in Bacteriorhodopsin revealed a biphasic process in both cases with a stoichiometry of total: 1.8 ± 0.4 indicating that more than one proton/Bacteriorhodopsin can be transferred during the photochemical cycle involving conformational changes of the protein. The proton release was time resolved at $-50°$ with the first order constants of 0.55 s^{-1} and 0.15 s^{-1} after laser flash activation at 580 nm. In the proton binding reaction the first phase correlates with a decay of a component absorbing at 435 nm whereas the second phase does not correlate. The reactivity of Bacteriorhodopsin was tested by its response to a transient electrical field in the order of the membrane potential of the native enzyme. In this analysis the response of protein absorption, the field indicator as well as umbiliferon could be observed in the purple membrane as well as its Apomembrane indicating field induced chemical changes correlating with the transients of the photochemical cycle of Bacteriorhodopsin (B. Hess, R. Korenstein, D. Kuschmitz, Katchalsky-Conference, Paris, 1976 and IUB-Congress Hamburg, 1976).