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PHOTOENERGY TRANSDUCTION IN HALOBACTERIUM HALOBIUM

FINAL TECHNICAL REPORT

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FINAL TECHNICAL REPORT

Summary of Project. This grant was awarded to study the structure and function of a rhodopsin-like pigment bacteriorhodopsin (bR), which we had discovered in cell membranes of the extreme halophile Halobacterium halobium and isolated in pure form. Its function was shown to be that of a light-energy transducer, which generates an electrochemical potential across the cell membrane, by translocating protons from the cytoplasm to the outside medium. The cell then uses this gradient to drive other metabolic processes and locomotion.

Under this grant we spectroscopically characterized intermediates which appear in the cyclic photoreaction that drives the proton translocation. For this we established time-resolved absorption spectroscopy which covered the time-range from 10^{-7} to 10^{0} s. For higher time resolution and resonance Raman experiments we collaborated with other groups. We established that the Schiff base which links the chromophore retinal to the protein undergoes a transient deprotonation during the photocycle, and also an all-trans to 13-cis isomerization. We further showed that after deprotonation of the Schiff base, a proton is ejected from the external side and later one proton taken up on the cytoplasmic side of the transmembrane protein, bR. We have also kinetically resolved the charge translocation in membrane monolayers and multilayers placed between electrodes. We have determined the angle between the chromophore transition movement and the plane of the membrane and shown that there is little if any change during the photoreaction cycle.

Bacteriorhodopsin occurs in the form of a crystalline two-dimensional lattice in the plasma membrane, which allows determination of its structure with near atomic resolution. Apparently, its polypeptide chain crosses the membrane seven times in α-helical configuration. We have used reconstitution of bR from the opsin and

retinal with one or the other component deuterated to obtain localization of retinal in the structure by neutron diffraction. It showed the β-ionone ring in the center of the membrane profile. The in-plane localization was ambiguous and has recently been superceded as a result of similar experiments carried out with the far superior neutron source available at Grenoble. In collaboration with R. Stroud, we have determined that an earlier determination of the binding site in the amino acid sequence of bR was erroneous and that the ε-amino group of Lys 216 is the correct site. This collaboration also led to a higher resolution structure which limited the possible assignments of amino acid sequence to the rod-like structures seen in the structural model.

Based on these data, we have developed a model for the proton translocation process, in which the isomerization of the retinal Schiff base decreases its pK to drive the proton off and simultaneously changes the connectivity from the cytoplasmic surface to the external surface. Proton conduction is assumed to occur via transmembrane chains of hydrogen-bonded groups. We have tried to follow the change in accessability of the Schiff base using nanosecond electron beam pulses to rapidly reduce the Schiff base and stably link it to the protein. We further hoped to disprove conclusively a postulated transient change in the Schiff base linkage from Lys 41 to 216 in the photocycle. These experiments, however, proved so time-consuming and costly that they did not progress beyond a feasibility study.

We have further investigated the stoichiometry of proton pumping in intact cells and the effect of the light-generated electrochemical potential on the kinetics of the photoreaction cycle and the synthesis of ATP. The latter experiments yielded strong evidence that in addition to a classical chemi-osmotic mechanism, a more direct energy-coupling must exist.

Finally, we have used the techniques developed for the study of H. halobium to investigate the occurence of bacteriorhodopsin and similar pigments in isolates from several natural sources, mainly the southern Sinai coast, the western Sahara and Baja California. Photoactive pigments similar or identical to bR or the closely related light-driven chloride pump halorhodopsin (hR) were found in many. For some, we have also demonstrated the corresponding light-driven ion movements and ATP-synthesis. In addition, similar pigments with a much slower photocycle were observed, which are apparently similar to the recently discovered sensory bacterial rhodopsins, which mediate phototactic responses.

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