$\Delta\tilde{\mu}_{H^+}$ determination in phospholipid vesicles reconstituted with aggregated and monomeric bacteriorhodopsin

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In the purple membrane of halobacteria the bacteriorhodopsin (BR) molecules are arranged as trimers in a two-dimensional hexagonal lattice. To determine the basic functional unit of the light-driven proton pump BR and to study possible effects of protein-protein and protein-lipid interactions on its molecular transport mechanism the electrochemical proton gradient $\Delta\tilde{\mu}_{H^+}$ generated by aggregated and monomeric BR in two different lipid environments was measured. For this purpose BR has been reconstituted into DMPC and Asolectin vesicles by means of detergent-dialysis and freeze-thaw sonication techniques. Whereas in the BR/Asolectin vesicles BR's state of aggregation was fixed due to the selected protein/lipid ratio, in the case of the BR/DMPC vesicles it could be reversibly changed by merely altering the temperature from below the lipid phase transition temperature to above. The exciton coupling effects in the visible circular dichroism spectrum and the extent of light adaptation were used to distinguish between monomeric and aggregated BR. The membrane potential $\Delta\Psi$ was determined by monitoring the light-induced uptake of $T\phi B^-$ with a liquid-membrane ion-selective minielectrode and ΔpH was evaluated from the light-induced quenching of fluorescence of 9-aminoacridine (9AA). In Asolectin and DMPC vesicles $\Delta\Psi$ did not exceed 10 - 20 mV and the $T\phi B^-$ uptake was completely abolished by adding a permeant ion such as NO_3^- to the assay medium. In most of our experiments we consequently measured only ΔpH in the presence of KNO_3 and/or KCl and valinomycin. The ΔpH values can be mathematically related to the quenching of fluorescence of 9AA according to the following equation: $\Delta pH = \log (Q/100-Q) + \log (V_o/V_i)$ where Q, V_o and V_i are respectively the fluorescence quenching of 9AA and the outer and inner aqueous compartments accessible to the probe. The V_o/V_i ratio was evaluated from a calibration curve obtained in the dark by causing a quenching of fluorescence of 9AA with acid to base transitions corresponding to ΔpH of known extent. Values of ΔpH ranging from 1.7 to 2.4 pH units were measured in Asolectin vesicles reconstituted with different techniques and different lipid/BR ratios; in DMPC vesicles somewhat lower ΔpH values (1.2-1.5 units) were obtained. In all the different types of vesicles tested, however, no significant dependence of the light-induced ΔpH values on BR's state of aggregation was observed. The finding that in these reconstituted systems BR monomers can generate a $\Delta\tilde{\mu}_{H^+}$ of the same size as BR molecules aggregated in a hexagonal lattice is a prove for BR monomers being the functional unit of this proton pump.