Light-dependent transport processes in isolated membranes of Rhodospeudomonas spaeroides

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S U M M A R Y

The phototrophic bacterium *Rhodopseudomonas sphaeroides* can obtain the energy for the uptake of amino acids from aerobic metabolism and from the absorption of light (Chapter II). The conversion of light energy into a chemical useful form is accomplished by a photosynthetic apparatus which is located in the cytoplasmic membrane and in invaginations of this membrane. In order to study light-dependent transport processes through the cytoplasmic membrane in more detail isolated membranes had to be used. Methods were developed for the isolation of membranes from cells. Chapter II describes the isolation of cytoplasmic membrane vesicles from cells by an osmotic shock of spheroplasts. These membrane vesicles can perform energy-dependent accumulation of amino acids. In vesicles isolated from cells grown aerobically in the dark transport of amino acids can be energized by aerobic oxidation of several electron donors. In membrane vesicles prepared from cells grown anaerobically in the light energy for active transport can be supplied by illumination.

In Chapter III is described a comparative study on structural and functional properties of the cytoplasmic membrane vesicles and of membrane particles which are obtained by breakage of cells by means of a French press. The latter membranes, the so-called chromatophores, are known to be mainly derived from intracytoplasmic membranes. It could be shown that qualitatively the composition of both membrane preparations is quite similar. Quantitatively, however, substantial differences were observed. The orientation of the membranes in both preparations was studied by freeze-etch electron microscopy, the localization of specific proteins and the direction of light-driven transport of amino acids and calcium ions. From these studies it was concluded that the orientation of the membrane vesicles is the same as that of the cytoplasmic membrane of intact cells, whereas the membrane of chromatophores has an inverted orientation.

In photosynthetic bacteria light energy is used to drive oxidation-reduction reactions in a cyclic electron transfer chain. This electron transfer is accompanied with the translocation of

protons across the membrane. Consequently, an electrochemical proton gradient is generated which is the driving force for processes such as ATP-synthesis and active transport. The proton gradient consists of two components: an electrical potential difference across the membrane ($\Delta\psi$) and a pH-gradient (ΔpH). Both components of the proton gradient have been measured in cytoplasmic membrane vesicles and chromatophores with several techniques (Chapter IV). Illumination of membrane vesicles results in the generation of a ΔpH, inside alkaline, and a $\Delta\psi$ inside negative. In chromatophores upon illumination a ΔpH, inside acid and a $\Delta\psi$ inside positive are generated.

The magnitude of the ΔpH and $\Delta\psi$ are strongly dependent on the composition and pH of the medium (Chapters IV and V). The effects of the ion composition of the medium on the ΔpH and $\Delta\psi$ suggest that chloride and potassium ions move, with different rates, through the membrane according to their electrochemical gradients. Furthermore, it is likely that phosphate and sodium ions are accumulated in membrane vesicles and chromatophores, respectively, at the expense of the ΔpH. Also Tris-molecules seem to distribute across the membrane according to the pH-gradient.

Electrical membrane potentials and pH-gradients can be measured by several methods. Measurements based on distribution of radioactively-labelled permeant ions and weak acids or bases, respectively, yield reliable results. Therefore, this technique has been frequently applied in the investigations described in this thesis. The fluorescent amine 9-aminoacridine has also been used to measure pH-gradients in inside-out oriented membrane particles. When, however, the ΔpH in chromatophores is calculated from the fluorescence quenching of this compound, values were obtained which are more than twofold larger than those determined from the accumulation of methylamine. For that reason the mechanism of fluorescence quenching of 9-aminoacridine at its interaction with chromatophore membranes has been investigated (Chapter VI). The fluorescence of the acridine is quenched upon its energy-independent binding to the membrane and by two light-dependent processes: a nigericin-insensitive interaction and a nigericin-sensitive interaction. The nigericin-sensitive quenching is an indication of the light-generated pH-gradient across the membrane.

It was shown, however, that this quenching could not be ascribed to entrapping of probemolecules in the chromatophores. Consequently, one is not allowed to calculate pH-gradients from this quenching based on such assumption.

Absorbance spectra of membrane-bound carotenoids are sensitive to changes in the electric field. The extent of this light-induced absorbance change is frequently used for membrane potential measurements. However, it can be questioned whether these changes really reflect this transmembrane potential. Potentials determined from these spectral changes are much larger than those calculated from ion-distribution (Chapter IV). The absorbance changes have been ascribed to electrochromism: a shift of the absorbance spectra of the pigment molecules under influence of alterations of the electric field. However, from detailed studies of the light-induced absorbance changes and changes induced by artificially generated membrane potentials it is concluded in Chapter VII that an electrochromic explanation is unlikely. The membrane potential induced spectral changes can be explained in terms of changes in the shape of vibrational splittings of the carotenoid main electronic transition.

The results described in this thesis clearly demonstrate that cytoplasmic membrane vesicles and chromatophores of *Rhodopseudomonas sphaeroides* are very useful systems for studies on energy-transducing biological membranes.