Functional reconstruction of photosynthetic bacterial membrane protiens

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SUMMARY

In bacteria primary H^+-pumps transform chemical, redox or light energy into an electrochemical potential difference of protons (Δp; PMF) across the cytoplasmic membrane. According to the chemiosmotic hypothesis for energy coupling introduced by Mitchell, the PMF is the driving force for ATP synthesis and secondary transport of solutes. To test this hypothesis, many experiments have been performed to study the relation between the magnitude of the PMF and the thermodynamic and kinetic properties of ATP-synthesis and solute transport. Most conclusions drawn from these experiments depend, however, heavily on the reliability of the methods used to determine the magnitude of the PMF.

To avoid uncertainties about the size of the PMF, a study in intact *Rhodobacter capsulatus* cells was performed with different types of inhibitors. By inhibiting the build up of a PMF, the relation between the PMF and the rate of two PMF consuming processes was studied *without* knowledge of the magnitude of the PMF (chapter 2). During illumination, myxothiazol, antimycin A (electron transport inhibitors) and CCCP (a protonophore) all had a more pronounced inhibitory effect on the rate of ATP synthesis than on the rate of alanine transport. CCCP had the most potent effect and antimycin A the least. The data suggest that the energy-input requirement for alanine transport is lower than for ATP synthesis. A new test for regulating factors in the process of PMF-generation and PMF-dissipation by the ATP synthase and the alanine transporter was applied to the data. Subject to reservations about the accuracy of rate measurements in intact bacterial cells and about the specificity of inhibition, the test revealed that the proton motive force is not the sole determinant of the rate of ATP synthesis and the rate of alanine transport.

Also when using this test (where estimations of PMF levels are not necessary) reservations have to be made with respect to the accuracy of registration of bioenergetic parameters in intact bacteria. An evaluation of the methods used to register the $\Delta\psi$ in intact bacterial cells was thus needed.

The electrical potential ($\Delta\psi$) across the cytoplasmic membrane of *Rhodobacter sphaeroides* has been measured by the two techniques that have been used most frequently: the distribution of TPP^+ and carotenoid band-shifts (chapter 3). Simultaneous measurements show that these two methods give different values for the $\Delta\psi$. Upon energization with either light or during respiration, TPP^+-distribution indicates a depolarization of the membrane, while electrochromic carotenoid band-shifts indicate a hyperpolarization. Treatment of the cells with venturicidin (an ATPase inhibitor) resulted -upon energization- in an increased light-induced membrane potential, as indicated by the carotenoid band-shift and led to a reversal in the polarity of the TPP^+ response. The presence of EDTA (a chelator) had no effect on the light-induced carotenoid absorbance change, but decreased the light-induced membrane depolarization indicated by TPP^+. These results show that at least one of these methods is seriously in error and/or that the intact cell system is too complicated to study properly energy coupling.

A model system was therefore developed with the advantage of light-induced PMF generation, but without the inherent complexities of intact cells (chapters 4 and 5). Reaction center complexes (RCs) were isolated from *Rhodopseudomonas palustris* with either one or both of the light-harvesting complexes attached ($RCLH_I$- or $RCLH_ILH_{II}$-complexes). Both complexes have been incorporated into liposomes made of phospholipids purified from *Escherichia coli*. Light-driven cyclic electron transport could be restored in these proteoliposomes upon the addition of the redox mediators cytochrome *c* and a water-soluble ubiquinone. During cyclic electron transport in this artificial system protons are extruded electrogenically from the liposomes. This leads to the generation of a PMF (maximally -180 mV). The RCs were subsequently introduced as a PMF generating system in membrane vesicles of two anaerobic bacteria. Liposomes containing $RCLH_I$ pigment protein complexes were fused with membrane vesicles of *Streptococcus cremoris* and *Clostridium acetobutylicum* by freeze-thaw/sonication. Illumination of these fused membranes resulted in the generation of a PMF of approx. -110 mV. The magnitude of the PMF in these membranes could be varied by changing the light intensity. As a result of this proton motive force amino acid transport into the fused membranes could be observed. In addition, accumulation levels and initial uptake rates could be varied by varying the light intensity. Thus, the introduction of bacterial

RCs in membrane vesicles by the fusion procedure yields a very attractive model system for the study of PMF consuming processes in membrane vesicles of (strict) anaerobic bacteria.

A large disadvantage of the RCs as a proton pump in hybrid membranes is the narrow pH range in which the pump can be used. Proton pumping is completely dependent on the (pH sensitive) chemical reaction between ubiquinol and cytochrome *c*. This pH range could, however, be extended by coreconstitution with the photosynthetic bc_1-complex (chapter 6). The interaction of purified RCs from *Rb. sphaeroides* and the purified bc_1-complex of *Rb. capsulatus* were studied in detergent solution and after co-reconstitution in liposomes. Under both conditions, the bc_1-complex increased light-induced cyclic electron transfer induced by RCs, with UQ_2 and cytochrome *c* as redox mediators. This effect was more pronounced at acid pH values. Light-induced cyclic electron transfer in these liposomes resulted in the generation of a PMF. Under conditions that the PMF was composed of a $\Delta\psi$ only, the highest (approx. -200 mV) was generated when UQ_{10} was used as redox mediator and when both electron transfer proteins were co-reconstituted in a 2:1 molar ratio. At acid pH values high, non-transient, membrane potentials could now be generated in liposomes, containing, besides RCs, the bc_1-complex. These observations show that the pH dependent direct oxidation of cytochrome *c* by ubiquinol in the liposomes is indeed catalyzed by the bc_1-complex and that this oxidoreductase participates in proton pumping. This could also be concluded from the stimulating effect of UQ_{10} on $\Delta\psi$ generation in liposomes containing both electron transfer complexes. Such a stimulation was not observed in liposomes containing only RCs.

A second aspect (besides the ability to pump protons) of *Rb. sphaeroides* pigment proteins, the carotenoid absorption change, could also be studied in the reconstituted liposomes (chapter 7). This electrochromic band shift of carotenoids, present in $RCLH_1LH_{II}$-complexes, was retained in proteo-liposomes upon generation of a potassium diffusion potential. The extent of the absorption changes increased with the amount of pigment-protein complex incorporated and was maximal at a ratio of 70 nmol BChl/mg lipid. Higher amounts of incorporated complexes led to a decrease in band-shift signal, due to increased membrane leakage. The carotenoid absorption change at 503-487 nm showed a linear dependency on the size of diffusion potentials, both negative as well as positive.

The spectrum of the absorption changes at a defined size of the diffusion potential for $RCLH_1LH_{II}$-liposomes had a similar shape as the spectrum found for chromatophores of *Rb. sphaeroides*, however with shifted maxima and minima. The spectrum found for LH_{II}-liposomes was inverted with respect to the spectrum of the $RCLH_1LH_{II}$-liposomes.

The shifted maxima and minima could be explained by a shift in the absorption peak of the field sensitive carotenoids, associated with B800 (chapter 8). The observed absorption changes can therefore fully be explained within common models for electrochromism. It is therefore allowed to use the carotenoid band-shift as a $\Delta\psi$ -registration method in these liposomal-membranes.

Reconstitution has been shown to be a powerful technique in the elucidation of the mechanism of (bacterial) energy transduction. The possibility to reconstitute both RCs and bc_1-complexes (even in combination) of photosynthetic bacteria supplies us with a strategy to study the function and molecular mechanism of these complexes in a simple, well-defined system. In addition, these liposomal systems can be used as PMF generating complexes to study partial reactions of bacterial energy transduction such as solute transport and ATP-synthesis. The electrochromic behaviour of carotenoids associated with the pigment-protein complexes of anoxygenic photosynthetic bacteria in the proteoliposomes, is an additional advantage of the reconstituted systems. It supplies a rapid, non-invasive technique to measure one of the most important parameters in bioenergetics - the membrane potential - in other systems than the native membranes of a photosynthetic bacterium.