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Energy-transducing processes in membrane vesicles from bacillus subtilis

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SUMMARY

For survival and growth bacteria need to take up and excrete different solutes. The uptake of solutes is necessary to supply the interior of the cell with substrates which are obligatory for growth as carbon and energy-sources and with other compounds which are necessary to survive but cannot be made by the cell itself. Excretion of solutes offers the cell the possibility to reduce high internal concentrations of solutes (for example metabolic end-products). These specific translocation processes enable the cell to regulate its internal environment which is essential for an optimal functioning of metabolism. A specific demand to achieve this goal is the presence of a selective solute barrier. In bacteria, this selective barrier is the cytoplasmic membrane. In this membrane specific transport systems are located which are responsible for the translocation of solutes across the membrane. Energy is required for the translocation process. This energy can be delivered in the form of electrochemical energy, which is generated by distinct membrane-bound components. In the Gram-positive bacterium, *Bacillus subtilis*, a linear series of electron transfer chain components convert redox-energy into electrochemical energy. The electron transfer chain components act as electrogenic proton pumps and couple the oxidation of high redox-energy intermediates to the vectorial translocation of protons across the membrane. Due to the relative impermeability of the membrane for protons and hydroxyl ions, this results in the generation of an electrochemical proton-gradient (Δp), consisting of a pH-gradient (ΔpH) and a membrane potential ($\Delta\psi$). They were related by the equation $\Delta p = \Delta\psi - Z\Delta pH$ (mV). The Δp acts as driving force for several uptake and extrusion processes but also regulates a variety of energy-requiring processes in bacteria. The knowledge on the molecular mechanism of the system(s) responsible for the conversion of redox-energy into electrochemical energy by the respiratory chain components is still limited. More detailed information of these components can be obtained by isolating these membrane-associated enzymes followed by reconstitution in artificial membranes in which their energy-transducing properties can be studied.

Cytochromes play an essential role in the electron transfer chain from *B. subtilis*. One of the terminal components of the chain is cytochrome *c*-oxidase (aa₃-type). This enzyme catalyzes the electron transfer of reduced cytochrome *c* to oxygen. It has been purified to homogeneity with two different techni-

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The oxid tron donors the generat genases are of physiolo identified ference in

ques. In the first approach cytochrome c-affinity chromatography is used (chapter 2). This technique enables rapid purification of the enzyme but in low quantities. Therefore an alternative procedure was introduced based on ammonium sulphate precipitation, anion-exchange and gel filtration chromatography which yields an active enzyme in high quantities (chapters 3 and 4). The purified enzyme shows spectral characteristics of an aa₃-type oxidase, reacts in the reduced form with CO and its activity is sensitive to cyanide and azide. This bacterial oxidase is composed of three subunits (57, 37, 21 kD). The energy-transducing characteristics were determined using proteoliposomes containing the purified oxidase (chapter 3).

Upon oxidation of reduced cytochrome c or the artificial electron donor phenazine methosulphate, a proton-motive force is generated in these proteoliposomes, consisting of a high membrane potential and a relatively low pH-gradient. This indicates that this bacterial cytochrome c oxidase acts as a Δp -generating site in the respiratory chain. Several factors influence cytochrome c-oxidase activity and Δp -generation in the proteoliposomes containing the oxidase. The phospholipid composition of the membrane has a marked effect on oxidase activity, incorporation and regulation of enzyme activity. The composition of the medium (ionic strength, pH) also affects the oxidase activity and the extent of both components of the proton-motive force. The effect of ionic strength on cytochrome c-oxidase activity can be explained in terms of disturbance of substrate-enzyme interactions and is not related to alterations in the aggregation state of the enzyme (chapter 4).

Additional cytochromes are present in the respiratory chain from B. subtilis. Potentiometric analysis reveals the existence of another cytochrome c-oxidase (o-type), consisting of two cytochrome b-components. This indicates the existence of a branching point in the respiratory chain at the level of cytochrome c. Furthermore b and c-type cytochromes have been identified and a tentative scheme of the operational sequence of cytochromes in the electron transfer chain is presented (chapter 5).

The oxidation of various physiological and artificial electron donors by membrane vesicles from B. subtilis results in the generation of a proton-motive force (chapter 6). Dehydrogenases are the primary components in the sequential oxidation of physiological electron donors. Several dehydrogenases are identified in B. subtilis membranes, but there is a marked difference in the capacity of Δp -generation via the dehydrogen-

ases. This can be explained by either low dehydrogenase activity or weak coupling of these dehydrogenases to the respiratory chain. For a critical evaluation of the coupling between Δp -generating and Δp -consuming processes it is essential to know the relation between oxidation rates of various electron donors via the respiratory chain and subsequent Δp -generation. The relation between oxidation rates and Δp -generation in membrane vesicles appears to be non-linear and can be explained by the non-ohmic conductance of the membrane. The individual components of the Δp are affected by the medium composition (ionic strength or composition, pH).

The role of the Δp in energy-consuming processes in B. subtilis membrane vesicles has also been studied (chapter 7). In B. subtilis membranes two Ca^{2+} -transport systems are identified: a low affinity electrogenic Ca^{2+} -uptake system and a high affinity Ca^{2+} /cation extrusion system. The divalent cation Ca^{2+} can also be translocated via a inducible divalent cation/citrate transport system. All three systems are dependent on the proton-motive force for translocation of the specific solute.

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