Active transport in cutoplasmic membrane vesicles of Bacillus subtilis

Bisschop, Arnold

SUMMARY

A rapid procedure for the isolation of cytoplasmic membrane vesicles of *Bacillus subtilis* is described in Chapter II; the method circumvents the protoplast formation step and consists in essence of treatment of the cells with lysozyme in a hypotonic medium. Membrane vesicles obtained in this way have a low endogenous oxygen consumption and a low endogenous activity for transport of several solutes. The membrane vesicles are devoid of other cell components as judged by electron microscopical examination of thin sections of the vesicles. Freeze-etch studies of intact cells, protoplasts, and membrane vesicles show that the orientation of the vesicle membrane is identical to that of the cytoplasmic membrane in intact cells and protoplasts.

Active transport of solutes in membrane vesicles from B. subtilis is coupled to electron transfer in the respiratory chain with oxygen as terminal electron acceptor. The best electron donors for the energization of transport are reduced β-nicotinamide-adenine-dinucleotide (NADH) and the non-physiological electron donor ascorbate+phenazinemethosulfate (Asc-PMS). With Asc-PMS as electron donor the transport activities of the membrane vesicles are comparable with those of intact cells and these activities are retained for a prolonged period of time.

Electron transfer from NADH to ferricyanide under anaerobic conditions also can generate energy for active transport in membrane vesicles of the predominantly aerobic bacterium *B. subtilis*. The membrane-impermeable electron acceptor ferricyanide, accepts electrons from cytochromes at the terminal oxidase-side of the respiratory chain and not at all from NADH dehydrogenase directly (Chapter III). Conclusions about the localization of dehydrogenases in the membrane and about the orientation of the vesicle membrane based on activity measurements with ferricyanide are therefore not justified.

Membrane vesicles of *B. subtilis* perform active transport of amino-, mono-, di-, and tricarboxylic acids. Transport of the dicarboxylic *amino* acids glu and asp in these vesicles is mediated by a single, highly specific, high affinity system. The system has no affinity for other natural amino acids or for several mono- and dicarboxylic acids (Chapter IV).

The dicarboxylic acids L-malate, fumarate and succinate are also transported by one highly specific, high affinity transport system in *B. subtilis* W23 (Chapter V). This system seems to be constitutive and has affinity for glu and asp, but these dicarboxylic *amino* acids are not transported by this system. It is suggested that glu and asp bind at a site on this carrier distinct from the site where the transport solutes are bound.

Membrane vesicles isolated from cells at different stages of growth oxidize NADH at different rates. During the exponential growth phase, a 3-4 fold increase is observed and maximal levels are found in vesicles isolated from stationary phase cells. Initial rates of amino acid transport, energized by NADH, closely parallels the increase in NADH oxidation rate. At all rates of NADH oxidation the transport of one molecule of a given amino acid requires the oxidation of a constant number of NADH molecules (Chapter VI). This number varies from 150-260 for the different amino acids.

Menaquinones are essential electron carriers functioning between dehydrogenases and cytochromes in the respiratory chain of *B. subtilis*. Membrane vesicles from the menaquinone-deficient mutant *B. subtilis aro D* oxidize NADH at a low rate. In these vesicles the NADH oxidase activity can be reconstituted by the incorporation of menadione (a menaquinone-analogue) to even higher

rates than observed in wild type vesicles. The NADH oxidase activity of the membrane vesicles increases linearly with the menadione content of the membrane and a 35-fold stimulation is obtained in fully reconstituted vesicles (Chapter VII).

In agreement with these observations membrane vesicles from *B. subtilis* aro *D* accumulate amino acids in the presence of Asc-PMS, but not with NADH. However, membrane vesicles from this mutant, reconstituted with menadione, display NADH-driven transport activity. This activity increases linearly with the NADH oxidase activity, but maximal transport activities are reached under conditions where the NADH oxidase activity is not yet maximal. This indicates that the rate of energy supply is the limiting factor for transport at low NADH oxidase activities and that the transport systems themselves become limiting under conditions of high NADH oxidase activities. Under energy-limiting conditions 135-235 molecules of NADH have to be oxidized in order to transport one molecule of amino acid. At all levels of energy supply no competition by the different amino acid transport systems is observed for the available energy. These observations indicate that only a fraction of the energy, generated by the respiratory chain, is used for the transport of an amino acid and that the bulk of the energy dissipates *via* other channels in the membrane vesicles.