Energy supply for active transport in anaerobically-grown escherichia coli
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SUMMARY

Membrane vesicles of anaerobically grown *Escherichia coli* perform active transport of amino acids and lactose under anaerobic conditions. The energy for this active transport is supplied by electron transfer in anaerobic electron transfer systems (Chapter 2), in which nitrate or fumarate serve as terminal electron acceptors. Therefore, these systems are termed nitrate respiration or fumarate reductase system, respectively. The presence of an anaerobic electron transfer system depends upon the growth conditions of the cells: the nitrate respiration system is derepressed under anaerobic conditions and repressed under aerobic conditions; the fumarate reductase system is derepressed under anaerobic conditions and repressed under aerobic and anaerobic conditions in the presence of nitrate.

In Chapter 3 is shown that active transport of amino acids by membrane vesicles of *E. coli*, grown anaerobically in the presence of nitrate, can be energized under anaerobic conditions by electron transfer from formate to the nitrate analogue chlorate or to the membrane-impermeable electron acceptor ferricyanide. Ferricyanide accepts electrons from at least two sites of the nitrate respiration system. One of these sites appears to be nitrate reductase, because cytochrome b, reduced by formate, is completely reoxidized by ferricyanide and L-glutamate transport energized by formate plus ferricyanide or by formate plus nitrate is affected by the same electron transfer inhibitors. The second site appears to be located prior to nitrate reductase, since formate is oxidized at a higher rate with ferricyanide than with nitrate, while formate/ferricyanide energizes transport of amino acids less effectively than formate/nitrate. Moreover, electron transfer inhibitors block electron transfer from formate to nitrate to a significantly higher extent than from formate to ferricyanide. Irradiation of the membrane vesicles with near ultra-violet light blocks completely formate dependent nitrate and ferricyanide reduction, suggesting that quinones play an essential role in the electron transfer from formate to nitrate or to ferricyanide.

Similar effects of ferricyanide are observed in membrane vesicles of *E. coli*, grown anaerobically in the presence of fumarate.

In Chapter 4 is shown that electron transfer from formate to nitrate in membrane vesicles of *E. coli*, results, at low formate concentrations, in
the generation of a membrane potential (interior negative) of about -90 mV, as indicated by the accumulation of the lipophilic cation triphenylmethylphosphonium. Moreover, this electron transfer leads to the generation of a transmembrane pH gradient which is about 1.3 pH units (interior alkaline), at a medium of pH 6.6, as indicated by the accumulation of the weak acid acetate in flow dialysis experiments. Thus, under anaerobic conditions, the total proton-motive force generated by nitrate respiration is at least -160 mV.

The undissociated form of formate is membrane-permeable and high external formate concentrations (around 10 mM) dissipate the pH gradient. In addition, it has been shown that the formate oxidation rate is inhibited by nigericin, indicating that formate is oxidized at the inner surface of the cytoplasmic membrane.

Active transport of L-glutamate in these membrane vesicles is energized by both the membrane potential and the pH gradient, as is demonstrated by the effects of the ionophores valinomycin and nigericin under aerobic and anaerobic conditions.

The role of the membrane-bound Ca$^{2+}$, Mg$^{2+}$-stimulated ATPase in the energization of active transport of amino acids was studied in mutants of *E. coli* deficient in ATPase activity (Chapter 5). The transport activities of the mutants, *E. coli* DL 54 and *E. coli* NR 70, were compared with those of the wild-type strains. Membrane vesicles from aerobically grown mutants exhibit defective amino acid transport, which can be restored by treatment with dicyclohexylcarbodimide. In contrast, vesicles prepared from anaerobically grown mutants exhibit normal transport activities when assayed under aerobic and anaerobic conditions. The suppression of the transport defect is not due to the isolation procedure of the vesicles or to changes in the basic genetic lesion. Furthermore, transport activities in membrane vesicles from aerobically grown mutants are relatively resistant to a treatment with chaotropic agents, while vesicles from anaerobically grown mutants are totally refractory to such a treatment. The real mechanism of the suppression of the transport defect is not yet elucidated, but it is clear that the ATPase plays a structural role in maintaining the proton-motive force across the membrane.

In Chapter 6 is shown, that *E. coli*, grown anaerobically on glucose, possesses a fumarate reductase system in which electrons are transferred from formate or NADH via menaquinone and cytochromes to fumarate reductase.
Measurements of maximal growth rates, growth yields and succinate production of mutants deficient in electron transfer components or a functional ATPase, indicate that the fumarate reductase system plays an important role in the bioenergetics of the cells during anaerobic growth on glucose.

Information about the generation of a proton-motive force and the synthesis of ATP in cells grown anaerobically on glucose, was obtained from studies of the proton-motive force dependent uptake of proline and of the phosphate-bond energy dependent uptake of glutamine. In the ATPase mutant a proton-motive force is generated by electron transfer in the fumarate reductase system, while in cytochrome-deficient cells this proton-motive force is generated by ATP hydrolysis. These observations clearly demonstrate that ATP can supply the energy for active transport processes. In wild-type E. coli cells, a proton-motive force can be generated by electron transfer in the fumarate reductase system or by ATP hydrolysis. The growth parameters of ATPase and electron transfer mutants strongly indicate that in wild-type E. coli, during anaerobic growth on glucose, this proton-motive force is preferably generated by electron transfer in the fumarate reductase system.