ENERGIZATION OF PHENYLALANINE TRANSPORT AND ENERGY-DEPENDENT TRANSHYDROGENASE BY ATP IN CYTOCHROME-DEFICIENT ESCHERICHIA COLI K12

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SUMMARY

E. coli SASX76 does not form cytochromes unless supplemented with 5-aminolevulinic acid. Uptake of [^{14}C]phenylalanine into cytochrome-deficient cells of this mutant was energized by glucose but not by endogenous substrates or D-lactate with or without fumarate. In contrast, uptake of this amino acid was supported in cytochrome-containing cells of this strain by oxidation of D-lactate or endogenous substrates. It is concluded that ATP can energize phenylalanine transport in cytochrome-deficient cells. Cytochrome-deficient cells lacked energy-dependent transhydrogenase activity driven by oxidation of NADH but ATP-driven transhydrogenation was unimpaired. Both transhydrogenase activities were present in cytochrome-containing cells.

INTRODUCTION

It has been reported that under anaerobic conditions active transport of sugars (including lactose) and amino acids in Escherichia coli could be coupled to the hydrolysis of ATP mediated by the Ca^{2+}, Mg^{2+}-activated ATPase (1-8). However, in a very recent study Konings and Kaback (9) found that, even under anaerobic conditions, active transport of lactose was coupled primarily to electron flow through an anaerobically-induced electron transport chain where either fumarate or nitrate acted as the ultimate electron acceptors. They found no evidence for the involvement of ATP in the transport process. We report here experiments with cytochrome-containing and cytochrome-deficient cells of a hem A$^-$ mutant of E. coli K12 which show that ATP

generated by glycolysis is directly involved in the transport of phenylalanine when electron transport through the cytochromes cannot occur. Furthermore, we show that ATP can energize the energy-dependent transhydrogenation of $NADP^+$ by NADH in membrane particles lacking cytochromes.

METHODS

E. coli SASX76 (previously SHSP18) (10), a hem A^- mutant of E. coli K12, was a generous gift of Dr. A. Sasarman, University of Montreal. For transport studies cells were grown in tryptic-soy broth in the presence or absence of 5-aminolevulinic acid (ALA) (50 µg/ml) at 37°C with aeration. For other experiments bacteria were grown in a minimal salts-glucose medium (11) containing L-methionine, L-tryptophan, and ALA, when present, all at a concentration of 50 µg/ml. The cells were grown at 37°C with aeration following the addition of a 10% inoculum which had been prepared by growing cells in tryptic-soy broth, harvesting the cells by centrifugation, and washing them aseptically with minimal medium. In all cases cells were harvested in the late exponential phase of growth, washed twice by centrifugation from either 0.05 M potassium phosphate buffer, pH 6.8 ("phosphate-washed cells") or 0.05 M Tris-HCl buffer, pH 7.8, containing 10 mM $MgCl_2$ ("Tris-washed cells"). Membrane particles were prepared from Tris-washed cells as previously described (12).

Uptake of [^{14}C]phenylalanine was measured with phosphate-washed cells. Cells (2.7 mg protein) were incubated at 30° in 8 ml 0.1 M phosphate buffer, pH 6.8, with chloramphenicol (100 µg/ml), 10 µM phenylalanine containing 1 µCi [^{14}C]phenylalanine, and an energy source, when present, at a final concentration of

20 mM. The assay mixture was shaken at 200 rpm (New Brunswick Scientific Co. Gyratory water bath shaker) in a 50 ml Erlenmeyer flask. Samples (1 ml) were removed at intervals and filtered through Millipore membrane filters (25 mm diameter; pore size, 0.45 μm). Each filter was washed three times with 2 ml portions of the phosphate-chloramphenicol buffer, dried under an infra-red lamp, and dissolved in Bray's scintillation fluid. The radioactivity of the sample was measured in a scintillation counter. [^{14}C]phenylalanine (477 Ci/mole) was supplied by Amersham-Searle Corporation.

Protein, Ca^{2+}-activated ATPase, D-lactate dehydrogenase, NADH oxidase, aerobic- and ATP-driven and energy-independent transhydrogenase activities were measured as described previously (11,13). Dithionite reduced minus hydrogen peroxide oxidized difference spectra were obtained with a Perkin-Elmer 356 spectrophotometer.

RESULTS AND DISCUSSION

When E. coli SASX76 was grown either aerobically or anaerobically in tryptic soy broth in the absence of 5-aminolevulinic acid (ALA) no cytochromes could be detected in reduced minus oxidized difference spectra of the cells. A normal cytochrome content was found in cells grown with ALA (Fig. 1). As expected from these results, cyanide-sensitive NADH oxidase activity was present in the cytochrome-containing cells but was absent from cytochrome-deficient cells (Table 1).

Phenylalanine is transported in E. coli both by the general aromatic amino acid system and by a specific permease system (14). Its transport can be coupled to the oxidation of D-lactate (15). The uptake of phenylalanine into cytochrome-containing cells of

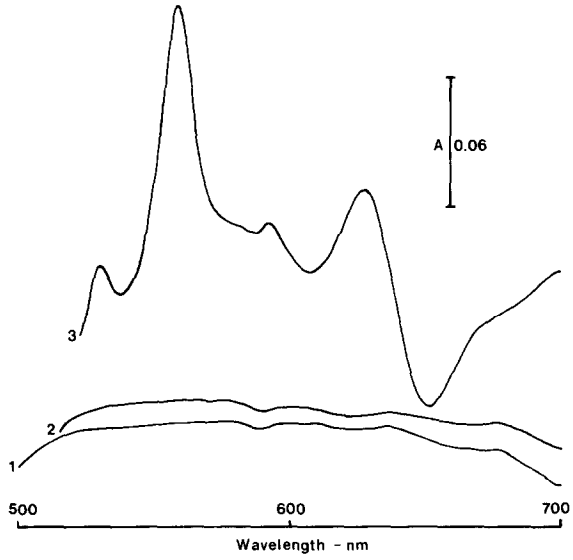

FIGURE 1. Reduced minus oxidized difference spectra of whole cells of E. coli SASX76 grown in the absence (curve 2) or presence (curve 3) of 5-aminolevulinic acid. Curve 1, base-line. Concentration of cells, 0.1 g wet weight/ml.

TABLE I. Specific activities of several enzymes in membrane particles of E. coli SASX76 grown in the presence or absence of 5-aminolevulinic acid (ALA)

Enzyme	With ALA	Without ALA
NADH oxidase		
-KCN	1896	32
+10 mM KCN	82	30
D-lactate dehydrogenase	272	609
ATPase	308	398
Transhydrogenase		
Aerobic-driven	38	0
ATP-driven	63	24
Energy-independent	427	146

Specific activity is expressed as nmoles/min/mg protein.

E. coli SASX76 energized by the oxidation of endogenous substrates was comparable to that of wild-type E. coli (15) and was stimulated further by oxidation of D-lactate (Fig. 2). With cytochrome-

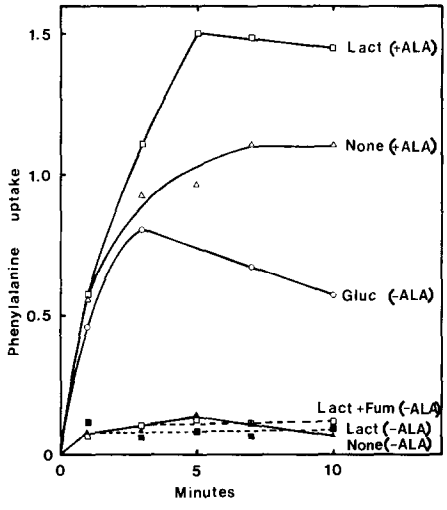

FIGURE 2. Uptake of [^{14}C]phenylalanine by whole cells of E. coli SASX76 grown in the presence (+ ALA) or absence (- ALA) of 5-aminolevulinic acid. The energy sources were endogenous (none), 20 mM D-lactate (Lact), 20 mM glucose (Gluc), or 20 mM D-lactate in the presence of 10 mM fumarate (Lact + Fum). Phenylalanine uptake is expressed as nmoles/mg protein.

deficient cells uptake of phenylalanine under aerobic conditions was negligible both in the absence of an exogenous energy source and in the presence of D-lactate (Fig. 2). The ineffectiveness of D-lactate as an energy source with cytochrome-deficient cells was not due to the absence of D-lactate dehydrogenase (Table 1). Presumably, an intact cytochrome chain is required for the oxidation of D-lactate, and in the absence of this phenylalanine transport cannot occur. Konings and Kaback (9) showed that under anaerobic conditions the oxidation of D-lactate could be linked to the reduction of fumarate to energize the transport of lactose. D-lactate in the presence of fumarate did not stimulate

the uptake of phenylalanine in cytochrome-deficient cells of E. coli SASX76 (Fig. 2). In contrast, glucose could drive phenylalanine transport in the cells (Fig. 2).

These results indicate that ATP generated during glycolysis was involved directly in the active transport of phenylalanine in cytochrome-deficient cells and support the hypothesis (1-8) that ATP is the energy donor for active transport of amino acids in E. coli under anaerobic conditions.

The involvement of cytochromes in the energy-dependent transhydrogenation of $NADP^+$ by NADH was examined. Since the formation of the transhydrogenase system was repressed by amino acids (11), E. coli SASX76 was grown in a minimal salts-glucose medium. In agreement with the results of Wulff (16) growth was poor in the absence of ALA but the procedure described in METHODS gave satisfactory results although the transhydrogenase system was still repressed to some extent.

Aerobic-driven transhydrogenase activity was completely absent from cytochrome-deficient cells although it was normal in cytochrome-containing cells (Table I). The loss of aerobic-driven transhydrogenase in cytochrome-deficient cells could be correlated with the loss of NADH oxidase activity. Oxidation of this substrate generates the energized state required for transhydrogenation. The transhydrogenation of $NADP^+$ by NADH energized by ATP was present in both cytochrome-deficient and cytochrome-containing cells. The lower level of activity in the former cells was probably due to repression of the transhydrogenase since the energy-independent transhydrogenase activity was also lower in these cells whereas the same level of membrane-bound ATPase was found in both types of cell (Table I).

We conclude that in cytochrome-deficient cells of E. coli

ATP is the energy source for active transport of amino acids and for energy-dependent transhydrogenation.

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