TRANSPORT OF α-METHYL GLUCOSIDE IN MUTANTS OF ESCHERICHIA COLI K12 DEFICIENT IN Ca^{2+}, Mg^{2+}-ACTIVATED ADENOSINE TRIPHOSPHATASE

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SUMMARY

The initial rate of uptake of methyl α-D-glucopyranoside by Escherichia coli is inhibited by respiration. The inhibition is more pronounced in mutant strains which cannot use the energy-rich state of the membrane to form ATP because of a defective Ca^{2+}, Mg^{2+}-activated ATPase. In both mutant and normal strains, the inhibition of glucoside uptake is not accompanied by an increase of the ATP content of the cells and is abolished by carbonyl cyanide m-chlorophenylhydrazone, a drug which dissipates membrane energy. It appears, therefore, that the inhibitory effect of respiration is mediated by the energy-rich state of the membrane and that ATP does not participate in the inhibition.

The mechanism of energy coupling for several processes of active transport has been investigated recently using respiration-deficient mutants of Escherichia coli. Some of these works have suggested that while β-galactoside and proline transports are driven by an energy-rich state of the cell membrane (1, 2), the uptake of glutamine is apparently driven directly by the phosphate-bond energy of ATP (2). In contrast with these processes of active transport, the uptake of αMG* through the glucose--phosphotransferase system is inhibited by the energy derived from respiration, which causes an increase of the apparent K_m for αMG (3). In order to investigate whether the inhibitory effect of respiration is exerted by the energized state of the membrane or by ATP, we have studied the transport of αMG in E. coli mutants which cannot perform oxidative phosphorylation because of a defective ATPase (4, 5). The results,

*Abbreviations used: αMG, methyl α-D-glucopyranoside; CCCP, carbonyl cyanide m-chlorophenylhydrazone; ATPase, Ca^{2+}, Mg^{2+}-activated ATPase (EC 3.6.1.3).

summarized in this report, suggest that the energy-rich state of the membrane is responsible for the inhibition of αMG uptake and that the ATP produced in oxidative phosphorylation does not play a direct role in the effect.

MATERIAL AND METHODS

Bacterial strains. All strains used were of E. coli K12: AN180 and its derivative AN120 were given by Dr. F. Gibson (4). A428 and its derivative N144 were obtained from Dr. D. L. Gutnick (5). The strains AN120 and N144 contain about 5% of the ATPase activity present in the respective parent strains due to mutations which probably affect the same gene (5, 6). The ATPase mutants do not grow aerobically with Krebs cycle intermediates as carbon source; the growth yields with limiting concentrations of glucose in aerobiosis is smaller on the mutants than on their parents (4, 5).

Cells were grown aerobically at 37°C in a basal salt medium (7) supplemented with 0.6 μM thiamine hydrochloride and 30 mM glucose. Proline, histidine or arginine were included at 0.2 mM when required by the strain.

Assays. Estimations of the rates of oxygen and of αMG uptake were performed as already described (3). The ATP content of cell suspensions was determined with the aid of a luciferin-luciferase assay. Samples, containing about 0.4 mg dry weight of cells per ml, were extracted with 0.9 M HCl, chilled for 10 min and centrifuged in the cold (10 min at 27 000 x g). The supernatants were collected, carefully adjusted to pH 7.4 ± 0.02 with NaOH and kept at 0°C. A luciferin-luciferase preparation was obtained by extraction of dried firefly lanterns (Sigma Chemical Co.) with 0.1 M sodium arsenate, pH 7.4 (4 mg of lanterns per ml) in a glass homogenizer, followed by centrifugation of the resulting suspension at 35 000 x g for 15 min. 0.5 ml of the supernatant were added to a 2.6 ml mixture containing 2.0 ml of the neutralized culture extract, 80 mM sodium arsenate, pH 7.4, and 10 mM $MgSO_4$. The light emitted was measured and compared with that produced by known amounts of ATP. The luciferin-luciferase extract was prepared near 0°C and the ATP assay was run at room temperature (20-22°C).

RESULTS AND DISCUSSION

Previous work from this laboratory showed that the initial rate of αMG uptake by E. coli K12 (NCIB 9483) was inhibited by substrates which stimulated the rate of oxygen consumption and which were not transported by the glucose-phosphotransferase system (3). Since the inhibition was relieved by CCCP, a drug which dissipates membrane energy (8), and the net rate of respiratory electron transport was not altered by the same drug, it was proposed that the inhibition was produced by some

Table I. Effect of respiration on the uptake of αMG by ATPase mutants and by their parental strains.

	αMG uptake			oxygen uptake		
addition / strain	none	succinate	arabinose	none	succinate	arabinose
	(nmoles/min per mg dry wt of cells)					
AN180 ($ATPase^+$)	2.5	1.6	2.3	13	34	20
AN120 ($ATPase^-$)	1.7	0.7	1.5	10	27	13
A428 ($ATPase^+$)	2.7	1.6	2.7	8	41	14
N144 ($ATPase^-$)	0.9	0.5	0.8	7	32	12

Succinate and arabinose were added 16 min before the assays (3). The concentrations of the compounds used were: sodium succinate, 22.5 mM; D-arabinose, 18 mM and [U-^{14}C] αMG, 0.05 mM (1.5 μCi/μmole).

form of energy derived from respiration (3). A similar inhibition of αMG uptake by respiration is observed in the ATPase -defective mutants, AN120 and N144, and in their respective parental strains, AN180 and A428. Table I shows that succinate, which clearly enhances the rate of oxygen consumption by cultures of the four strains, decreases in all cases the initial rates of αMG uptake. Arabinose stimulates respiration to a much lesser extent and has little effect on αMG transport. The inhibition by succinate does not take place when CCCP is included in the assay mixtures even though the rate of oxygen consumption is increased by succinate to about the same extent whether CCCP is present or not (Table II).

Table I also shows that the activity of αMG uptake is lower in the ATPase-defective

Table II. Effect of succinate on the uptake of αMG and of oxygen by ATPase mutants and by their parental strains in the presence of CCCP.

strain \ addition	αMG uptake		oxygen uptake	
	none	succinate	none	succinate
	(nmoles/min per mg dry wt of cells)			
AN180	4.1	4.4	13	35
AN120	5.6	5.4	9	32
A428	4.6	4.7	8	48
N144	5.7	5.1	7	33

CCCP, 10 μM, was added 14 min before the assays. All other conditions were as in Table I.

mutants than in their respective parental strains, both in the presence and in the absence of succinate. The lower rates of the mutants appear to be caused by an enhanced sensitivity of the glucose-transport system towards respiration because both mutant and normal strains incorporate αMG at similar rates when the effect of respiration is removed by CCCP (Table II). The enhancement of the inhibition by the ATPase deficiency is clearly observed even in the absence of succinate, when respiration is supported only by endogenous reserves and proceeds at a low but significant rate (Table I).

The ATPase-defective strains, AN120 and N144, grow on carbon sources which support ATP formation at the substrate level but not on those carbon sources which can be used to produce ATP at the level of the respiratory chain only (4, 5). In addition, these strains do not carry out oxidative phosphorylation (4, 9). As it was expected

Table III. Effect of CCCP and of succinate on the ATP content in ATPase mutants and in normal strains.

addition \ strain	AN180	AN120	A428	N144
	ATP (nmoles/mg dry wt of cells)			
none	3.9	1.6	7.3	7.0
CCCP	0.8	1.5	2.4	7.8
succinate	3.7	1.9	4.8	6.2

Cell suspensions were incubated with succinate or CCCP before the assays, as in Table II.

from these properties and in contrast with what happened in the normal strains, the steady-state concentrations of ATP in cultures of the mutants did not decrease after incubation with the uncoupler CCCP (Table III). Incubation with succinate, as it was also expected, hardly changed the levels of ATP in the mutant cultures. However the fact is not meaningful since succinate failed to increase the ATP levels in cultures of the normal strains too (Table III). This is not surprising because the steady state concentrations of the nucleotide depend both on the rate of ATP formation and on the rate of ATP utilization, and both are probably altered when succinate is added to cultures of normal strains. In any case the examination of the data of Tables I-III indicates that the rate of αMG uptake is not related to the steady-state concentrations of ATP of any of the four strains under different respiratory conditions.

Respiratory energy can be used to drive a number of metabolic processes in E. coli without the direct participation of ATP, such as the reduction of NADP by NADH

(10) and the accumulative transport of different substrates (11). Reactions of this type are carried out by the ATPase-defective strains used in this work. The respiratory electron flow of strain N144 sustains the energy-linked NADH-NADP transhydrogenation (12), and strain AN120 performs the accumulative transports of inorganic phosphate, of β-galactosides, of proline and of serine under aerobic conditions (6, 13). Other independently obtained mutants which show a similar ATPase deficiency have been reported to transport β-galactosides (1) and proline (2) during the operation of respiratory electron flow. The properties of these ATPase-defective mutants indicate that the cell membrane can be energized by respiration in the absence of a functional ATPase, a conclusion which is supported by studies of membrane preparations of strain N144 with a fluorescent probe (14). Our observation that the inhibition of αMG uptake by the energy derived from respiration (3) occurs in the mutants suggests that the inhibition is mediated by the energized state of the cell membrane and that the phosphate-bond energy of ATP does not participate in the effect. This proposal is supported by the additional observation that the inhibition of αMG uptake is enhanced in the ATPase-defective strains. As membrane energy cannot be used by these mutants to form ATP, it accumulates to a higher degree than in the normal strains in which it is spent in oxidative phosphorylation. However the mechanism through which membrane energy controls the uptake of αMG by the glucose-phosphotransferase system still remains unknown.

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