TRANSPORT OF α-METHYL GLUCOSIDE IN A CYTOCHROME-DEFICIENT MUTANT OF *ESCHERICHIA COLI* K-12

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1. Introduction

Glucose, and its non-metabolizable analogue α-methyl glucoside (αMG), are transported in *Escherichia coli* by the phosphoenolpyruvate phosphotransferase system [1]. In contrast to the active uptake of other solutes which is energized either by substrate oxidation or by ATP hydrolysis [2], the transport of αMG was inhibited by substrate oxidation and this was reversed by uncouplers [3–6].

Recent studies by Hernandez-Asensio et al. [7] and del Campo et al. [8] using wild-type and ATPase-deficient mutants of *E. coli* have shown that the inhibition of αMG uptake was dependent on the formation of an energized state of the membrane. However, they suggested that ATP was not involved in the inhibition.

In this paper we report that αMG uptake in a cytochrome-deficient mutant of *E. coli* was inhibited by the formation from ATP of an energized state of the membrane. This process was reversed by uncouplers and by inhibitors of the Ca^{2+}, Mg^{2+}-activated membrane ATPase.

2. Materials and methods

E. coli SASX76 (F^-, hem A^-, met^-, trp^-, lac^-, str^-) [9] was kindly supplied by Dr A. Sasarman, University of Montreal. The cells were grown aerobically at 37°C on a minimal-salts medium [10] containing 0.5% bactotryptone and 0.5% (w/v) D-galactose (unless indicated otherwise). 5-Aminolevulinic acid (ALA) (25 μg/ml) was added when cytochrome-containing cells were desired. The cells were harvested at 22°C in the early exponential phase of growth, washed twice with minimal salts medium lacking a carbon source at 22°C, and resuspended to a concentration of 2–3 mg/ml in the same medium containing chloramphenicol (100 μg/ml). Generally 20–30 μl of cell suspension was used for the measurement of αMG or proline uptake at 22°C by the method of Cox et al. [11]. Inhibitors were preincubated at 37°C with the cell suspension for 5 min before uptake was measured. Methyl α-D-[U-^{14}C]glucoside (3.0 mCi/mmol) and [U-^{14}C]proline (290 mCi/mmol) were used at final concentrations of 70 μM and 13.6 μM, respectively.

3. Results and discussion

E. coli SASX76 did not form cytochromes unless the growth medium was supplemented with 5-aminolevulinic acid (ALA) [12,13]. As shown in fig.1 uptake of αMG in cytochrome-deficient (ALA^-) cells was supported by endogenous substrate while D-lactate had little effect on uptake under these conditions. In contrast, the uptake of αMG by cytochrome-containing (ALA^+) cells due to endogenous substrates was markedly inhibited by the addition of D-lactate. As we have shown previously D-lactate is not oxidized in the absence of cytochromes [13]. These results provide support for the hypothesis that αMG uptake is inhibited by the energized state of the membrane formed by substrate oxidation and that this is reversed when substrate oxidation is blocked by inhibitors or by lack of oxygen [3–6], or in our case by an incomplete respiratory chain.

In cytochrome-deficient cells the uptake of αMG was inhibited by the addition of D-galactose whereas

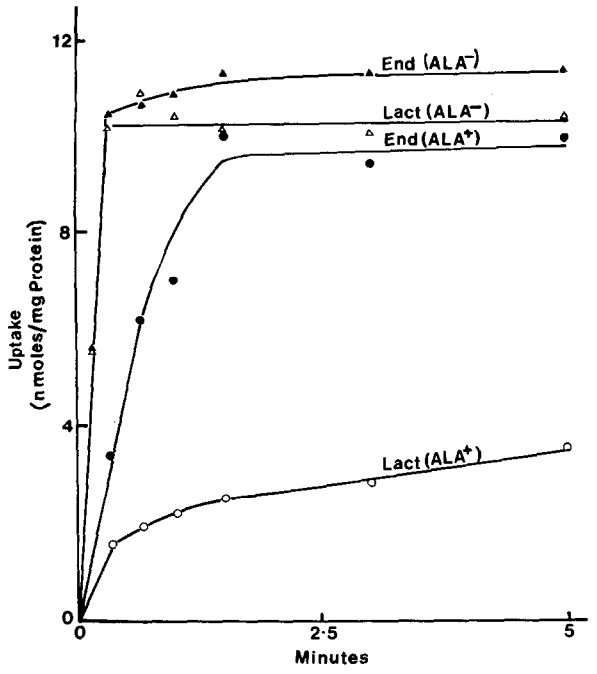

Fig.1. Effect of D-lactate on αMG uptake by cytochrome-containing (ALA^+) and -deficient (ALA^-) cells. Uptake of αMG was measured in the absence (End) and presence of 20 mM D-lactate (Lact). The cells were grown on a glucose medium.

the uptake of proline was markedly stimulated (fig.2). Since galactose and αMG are transported by different systems [14], the effect of galactose on αMG uptake cannot be due to competition for the same transport carriers. Moreover, similar results were obtained when glycerol in the presence of fumarate was used as an energy source with cells grown anaerobically on these compounds in the absence of ALA. Under these conditions the uptake of amino acids was stimulated [15].

The inhibitory effect of galactose on αMG uptake might be due to the formation of ATP from this substrate by glycolysis. The ATP formed in this way could energize the membrane, which in turn might inhibit the uptake of αMG in contrast to its role in driving the uptake of proline [2]. The involvement of ATP through the Ca^{2+}, Mg^{2+}-activated ATPase, and of the energized state, was shown by the effects of the ATPase inhibitors, dicyclohexylcarbodiimide (DCCD) and azide [16], and by the uncouplers 2,4-dinitrophenol and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) [17], respectively. Azide or DCCD reversed the inhibitory effect of galactose on αMG uptake (fig.3). A similar result was obtained with 2,4-dinitrophenol (fig.4). However, the effect of CCCP was more complex (fig.4). Thus, instead of reversing the effect

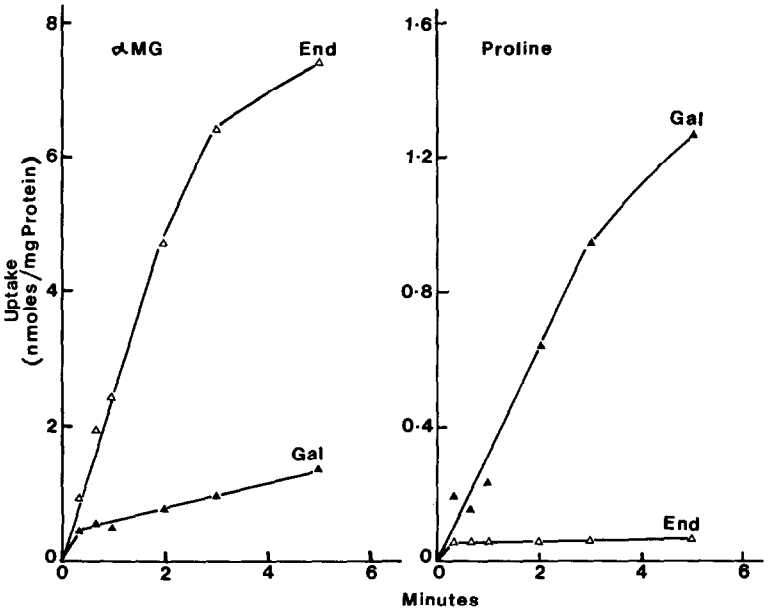

Fig.2. Effect of galactose on αMG and proline uptake by cytochrome-deficient cells. Uptake was measured in the absence (End) or presence of 50 mM D-galactose (Gal).

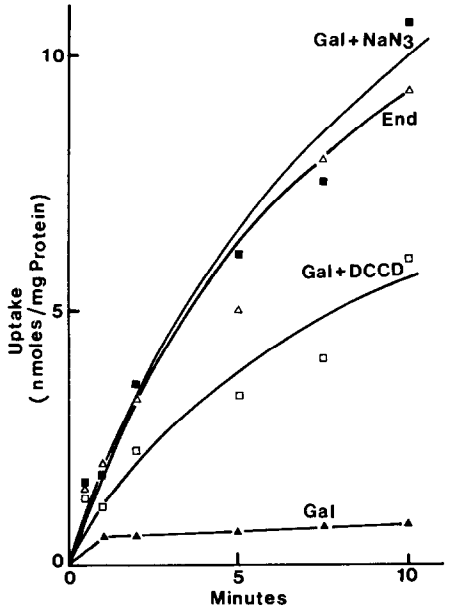

Fig.3. Effect of ATPase inhibitors NaN_3 and DCCD on D-galactose inhibition of αMG uptake by cytochrome-deficient cells. Uptake was measured in the absence (End) or presence of 50 mM D-galactose (Gal). The concentrations of DCCD and NaN_3 were 0.5 mM and 5 mM. respectively.

of galactose on αMG uptake further inhibition was observed. This effect was due to the reaction of CCCP with sulfhydryl groups since it was prevented by dithiothreitol. In the presence of this compound CCCP behaved like an uncoupling agent and reversed the inhibitory effect of galactose in a similar manner to 2,4-dinitrophenol. The inhibitory effect of sulfhydryl reagents on αMG uptake, and the ability of CCCP to act as a sulfhydryl reagent, have been reported before [18,19]. The reversal of the galactose inhibition of αMG uptake in cytochrome-deficient cells by DCCD, azide, 2,4-dinitrophenol and CCCP was probably not due to inhibition of the uptake of galactose since oxidation of this substrate by whole cytochrome-containing cells was unaffected by these agents at the concentrations used.

We conclude that the uptake of αMG in *E. coli* can be regulated by the energized state which may be generated both by substrate oxidation and by ATP hydrolysis.

Acknowledgement

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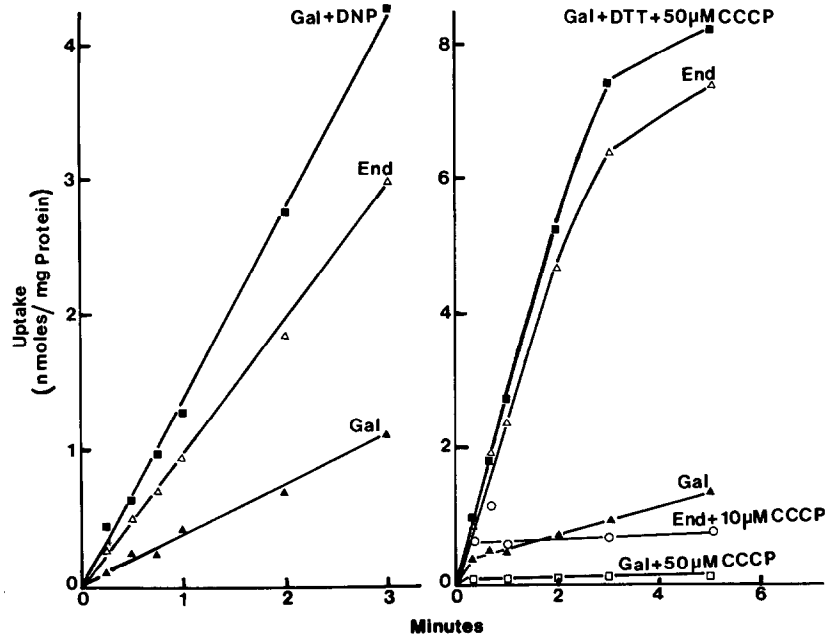

Fig.4. Effect of 2,4-dinitrophenol (DNP) and CCCP on D-galactose inhibition of αMG uptake by cytochrome-deficient cells. Uptake was measured in the absence (End) or presence of 50 mM D-galactose (Gal). The concentration of DNP was 1 mM.

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