THE α-METHYLGLUCOSIDE EFFECT ON ADENYLATE CYCLASE ACTIVITY AND MEMBRANE ENERGIZATION IN *ESCHERICHIA COLI* K12

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1. Introduction

Glucose, when added to bacterial cells, lowers intracellular cyclic AMP levels due to inhibition of adenylate cyclase activity [1]. Glucose, as well as its non-metabolizable analogue methyl α-glucoside is transported into Enterobacteria by means of the PEP-linked phosphotransferase system [2].

The MG transfer is followed by its phosphorylation catalysed by the membrane component of the PTS – the glucose specific enzyme II, specified by the *ptsG* gene. In this reaction, the phosphate donor is a soluble phospho~factor III protein, which is phosphorylated at the expense of phosphoenolpyruvate with participation of an enzyme I and a small protein HPr, specified by the *ptsI* and *ptsH* gene, respectively. As a result, MG-6-phosphate and pyruvate appear in the cell in a 1:1 ratio [3].

Therefore the MG uptake is accompanied by:

(i) Binding of substrate by enzyme II;
(ii) Its translocation across the membrane with simultaneous phosphorylation;
(iii) Equimolar formation of pyruvate.

Here we report studies on the role of each of the above-mentioned processes in the inhibition by MG of the AG activity in intact cells of *E. coli* K12.

2. Experimental

The following mutant strains defective in different PTS components were used: JD3 (*gpt*), deprived of the enzyme II activity for MG binding and phosphorylation [4]; J624 (*tgl*), defective in the binding, but not in phosphorylation of MG [5,6]; P34621 (*ptsI,H*), lacking enzyme I and HPr, where enzyme II is able to bind MG without further phosphorylation [7]. All the strains have the J621 genetic background [7].

Since the AC substrate ATP could not enter the bacteria, we rendered the cells permeable by EDTA treatment [8] and [^{3}H]ATP was added to the culture thus treated. After paper chromatography [9] radioactive cyclic [^{3}H]AMP was determined.

β-Galactosidase (EC 3.2.1.23) was induced by isopropylthio-β-D-galactopyranoside over 5 min.

3. Results

The results presented in table 1 indicate that MG considerably inhibits the AC activity as well as β-galactosidase synthesis in the wild type cells. The mutants with different PTS lesions do not display such effects.

The experiments show that for MG to exert a repressive effect, it must both be bound by its enzyme II as well as undergo transmembrane phosphorylation. Even being transported into the *tgl* cell and phosphorylated in the cytoplasm, MG still cannot repress the enzyme induction and the AC activity (table 1), since it does not interact with the 'binding' site of the enzyme II [5].

The following experiments indicated that pyruvate generation during MG transport is also necessary for the inhibition of the AC activity.

Abbreviations: AC, adenylate cyclase (EC 4.6.1.1); MG, α-methylglucopyranoside; PTS, phosphoenolpyruvate-dependent phosphotransferase system (EC 2.7.3.9); CCCP, carbonylcyanide *m*-chlorophenylhydrazone

Table 1
The α-methylglucoside effect on the adenylate cyclase activity and β-galactosidase synthesis

	J621 (*pts*$^+$)			J624 (*tgl*)	JD3 (*gpt*)	P34621 (*ptsI,H*)
		CCCP (0.01 mM)	N_2			
	AC activity (pmol cyclic AMP.μg protein^{-1}.min^{-1}, 37°C)[a]					
–	2.4	0.75	–	1.18	1.52	0.78
MG (0.5 mM)	0.63	0.8	–	1.3	1.48	0.62
	β-Galactosidase synthesis (units of activity.mg protein^{-1}.min^{-1}, 37°C)[b]					
–	18.38	17.37	4.53	7.21	10.85	3.25
MG (0.5 mM)	9.37	19.31	3.84	10.33	9.26	2.18

[a] The incubation mixture contained EDTA-treated cells in 0.12 M Tris–HCl (pH 8.05); 0.05 M $MgCl_2$; 1 mM cyclic AMP; 1 mM [^{3}H]ATP. The cells were pretreated with CCCP for 3 min

[b] The cells growing in M9 salt medium, supplemented with 0.4% casamino acids, were induced by isopropylthio-β-D-galactopyranoside (1 mM). CCCP and MG were added simultaneously with the inducer. Anaerobiosis was obtained by passing N_2 through the cell suspension 5 min before the addition of inducer

We inferred that intracellular accumulation of pyruvate and its subsequent oxidation would lead to the additional membrane energization.

The $\Delta\tilde{\mu}_{H^+}$ changes in the presence of MG were defined by assaying the [^{14}C]proline transport rates.

The results obtained (table 2) show that MG stimulates the [^{14}C]proline uptake by wild type cells and by the J624 (*tgl*) mutant (which retained the ability to phosphorylate MG); proline enters the cell by active transport [5,6]. In the JD3 (*gpt*) and P34621 (*ptsI,H*) mutants, deprived of pyruvate generation in the process of MG uptake, the addition of MG did not accelerate proline uptake. Control experiments revealed that lactate stimulates the amino acid transport in all the strains used as well (data are not shown). Similar changes in membrane potential influenced by

Table 2
Influence of α-methylglucoside on [^{14}C]proline accumulation (μmol/g protein at 37°C)

Strains	Initial rate (1 min)		Stimulation (%)	Steady-state (5 min)		Stimulation (%)
	–	MG		–	MG	
J621 (*pts*)$^+$	3.11	4.85	56	4.81	6.66	39
J624 (*tgl*)	1.64	2.49	52	4.28	5.27	23
JD3 (*gpt*)	2.13	1.99	0	5.31	5.67	7
P34621 (*ptsI,H*)	2.08	2.12	2	6.25	6.31	1

Washed cells were resuspended in M9 salt medium with chloramphenicol (50 μg/ml). The concentrations were: MG, 0.5 mM; [^{14}C]proline, 0.013 mM. Blanks, obtained with cells pretreated with 0.01 mM CCCP for 3 min, were subtracted

MG were registered using fluorescence quenching of 1-anilino-8-naphthalenesulfonate (data are not shown).

The necessity of the membrane hyperpolarization for the manifestation of MG repression is verified by the experiments described in table 1. The AC in J621 cells is not affected by MG in the presence of the uncoupler CCCP. (The EDTA-treated cells retain sufficient transmembrane potential H^+ [10].) Concomitantly, β-galactosidase synthesis becomes resistant to MG in the presence of CCCP or in anaerobic conditions. The discrepancy in the extent of the CCCP action upon the AC and β-galactosidase activities may be the result of different treatment of the cells used for these two enzyme assays (see footnotes for table 1).

The results obtained correlate with the data of other authors, denoting that glucose repression could be 'switched off' by exposing bacteria to anaerobiosis [11] and that under these conditions the cyclic AMP levels are considerably increased [12].

The data reported suggest that changes in AC activity brought about by MG are caused by:

(i) Conformational changes of the glucose enzyme II during the transmembrane phosphorylation of MG;

(ii) Hyperpolarization of the membrane, probably as a result of the oxidation of pyruvate, generated by the PT reaction.

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