THE COMPETITIVE INHIBITION OF α-METHYLGLUCOSIDE UPTAKE IN ESCHERICHIA COLI*

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The concentrative uptake of α-methyl-D-glucopyranoside (α-MG) by Escherichia coli has been reported by Cohen and Monod (1957) and by Hoffee and Englesberg (1962). The former authors presented evidence that α-MG was transported by the same system as that which mediated the uptake of glucose. Although α-MG is not used for growth by E. coli, Rogers and Yu (1962) have reported that part of the α-MG is converted to α-MG-6-phosphate. We wish to extend the data on the kinetics of α-MG uptake by E. coli and to report a competitive inhibition of α-MG uptake by several compounds.

MATERIALS AND METHODS

E. coli strains ML 30 and ML 308 were grown with aeration at 37° on the mineral salts medium "56" (Cohen and Monod, 1951) containing the carbon source specified at a concentration of 10^{-2} M. Exponentially growing cultures were harvested by sedimentation, washed twice, and resuspended to a density of approximately 5 X 10^8 bacteria per ml in ice-cold medium "56" without a carbon source but containing 50 μg per ml of chloramphenicol. For the measurement of α-MG uptake the bacterial suspensions were brought to 37°, aerated, and α-MG-U-^{14}C added. One half milliliter samples were filtered through membrane filters (Millipore HA,

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0.45 μ pore diameter) and the bacteria on the filter washed with five 0.2 ml portions of ice-cold medium "56". The filter disks were then glued to planchets, air dried, and the radioactivity determined in a gas flow counter. The glycerol and fructose used were chromatographed and found to be glucose free.

RESULTS AND DISCUSSION

Fig. 1 demonstrates that the intracellular and medium α-MG were in complete equilibrium. It also shows that glucose competed for uptake and displaced intracellular α-MG. The eventual escape from inhibition presumably reflected utilization of the glucose. The inhibition by glucose was competitive (Fig. 2).

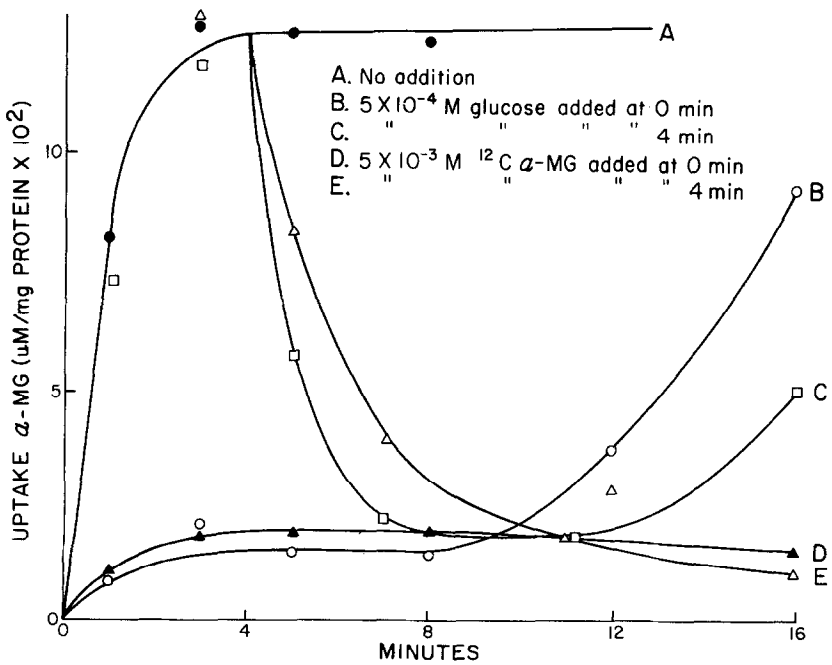

Fig. 1. Uptake of 5 X 10^{-4} M α-MG by glucose grown E. coli ML 30. Inhibition and displacement by ^{12}C α-MG and glucose.

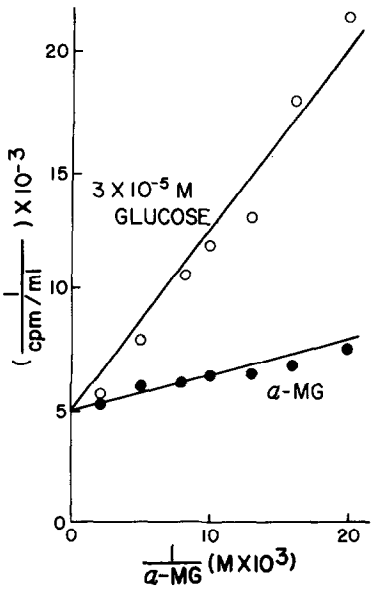

FIG.2

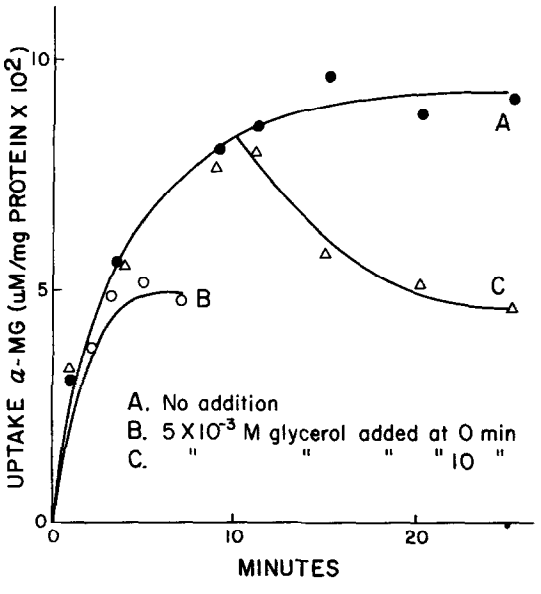

FIG.3

Fig. 2. Competitive inhibition of α-MG uptake by glucose in glucose grown E. coli ML 30. Samples for filtration were withdrawn 45 sec. after the addition of the bacterial suspension to tubes containing ^{14}C α-MG (350,000 cpm/μM) and the inhibitor, when tested.

Fig. 3. Uptake of 5×10^{-4} M α-MG by glycerol grown E. coli ML 30. Inhibition and displacement by glycerol.

Glycerol and fructose did not inhibit α-MG uptake by cultures grown on glucose. However these compounds did inhibit α-MG uptake by bacteria grown on glycerol and fructose, respectively. Fig. 3 shows that in a culture grown on glycerol, glycerol inhibited the uptake and caused the displacement of intracellular α-MG. The inhibition was competitive (Fig. 4). Similarly fructose competitively inhibited α-MG uptake by cultures grown on fructose (Fig. 5) and displaced intracellular α-MG in such cultures. Table 1 gives the uptake constants, $K_{\alpha\text{-MG}}$, which may be taken as a measure of the dissociation of the "bacterium - α-MG" complex, for cultures grown on glucose, glycerol, and fructose. The inhibition constants, K_i, describe the ability of these compounds to inhibit α-MG uptake competitively. Preliminary experiments indicate that lactose, maltose, lactate, and succinate also inhibit α-MG uptake competitively in cultures grown on these substrates.

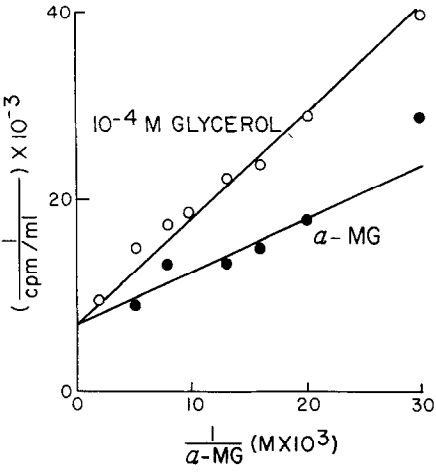

FIG. 4

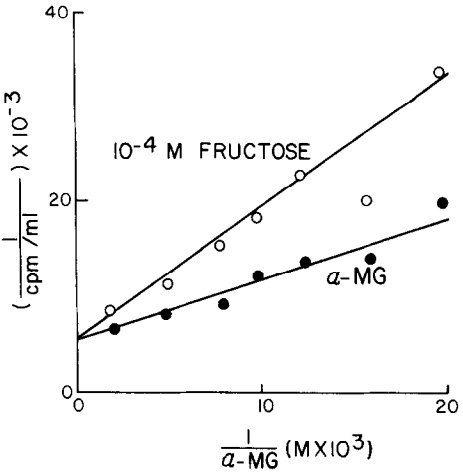

FIG. 5

Fig. 4. Competitive inhibition of α-MG uptake by glycerol in glycerol grown E. coli ML 30. See legend of Fig. 2 for procedure.

Fig. 5. Competitive inhibition of α-MG uptake by fructose in fructose grown E. coli ML 30. See legend of Fig. 2 for procedure.

TAPLE 1

α-MG uptake by E. coli ML 30. Uptake (K) and inhibition (K_i) constants as a function of the growth substrate.

Carbon source during growth	$K_{\alpha\text{-MG}}$	K_i growth substrate
Glucose	2.9×10^{-5} M	6.7×10^{-6} M
Fructose	13.2×10^{-5} M	1.0×10^{-4} M
Glycerol	7.8×10^{-5} M	1.0×10^{-4} M

To determine if this situation was a general phenomenon, E. coli ML 308, which forms the galactoside transport system constitutively, was grown on glucose as a source of carbon, and the ability of glucose to inhibit the uptake of thiomethylgalactoside (TMG) was tested. TMG uptake was inhibited competitively by glucose with an inhibition constant, $K_{i\ glucose}$ of 5×10^{-4} M; the uptake constant K_{TMG} was 2.4×10^{-4} M

(Fig. 6). Neither glucose nor glycerol inhibited the uptake of TMG if the culture had been grown with glycerol as a carbon source.

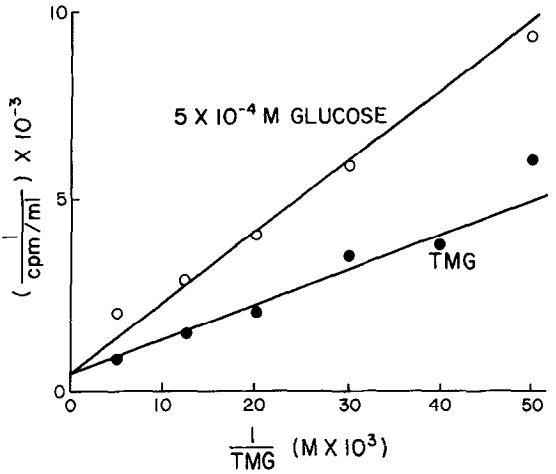

Fig. 6. Competitive inhibition of TMG uptake by glucose in glucose grown E. coli ML 308. Specific radioactivity 364,000 cpm/μM TMG. See legend of Fig. 2 for procedure.

These findings are best explained by the assumption of at least two steps in the transport of metabolites and related compounds. The first step may well be catalyzed by the specific "permeases" (Rickenberg et al, 1956; Cohen and Monod, 1957). Competition for this first step by, for example, glycerol and α-MG, or glucose and TMG seems to be excluded. It appears likely then that derivatives formed from these compounds in the first (highly stereospecific) step compete for a common second step.

A discussion of the possible contribution of the exit reaction to the kinetics of α-MG transport and its inhibition has been ignored deliberately in this communication. This aspect of α-MG transport as well as the likelihood of the existence of at least two glucose uptake systems will be discussed at a later time.

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