Biochimica et Biophysica Acta, 440 (1976) 403–411

BBA 47142

EFFECTS OF COLICINS K AND E1 ON THE GLUCOSE PHOSPHOTRANSFERASE SYSTEM

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(Received February 2nd, 1976)

SUMMARY

1. Glycerol-grown cells of *Escherichia coli* and its mutant *uncA*, treated with colicin E1 or K, exhibited a several-fold higher level of α-methylglucoside uptake than untreated cells. This stimulation was independent of the carbon source present during the uptake test. In a mutant strain that has elevated levels of α-methylglucoside accumulation the addition of colicin E1 or carbonylcyanide *m*-chlorophenylhydrazone (CCCP) did not further enhance the uptake.

2. Colicins K and E1 decreased the apparent K_m for α-methylglucoside uptake significantly and increased the V about twofold. The exit of the glucoside was severely inhibited by the colicins.

3. In the presence of colicins, α-methylglucoside is still accumulated via the phosphoenolpyruvate-phosphotransferase system since no accumulation or phosphorylation occurs in an enzyme I mutant. The colicins increased the relative intracellular concentration of phosphorylated α-methylglucoside, possibly by inhibiting the dephosphorylation reaction, and caused an excretion of this compound.

4. The results are interpreted as indicating that energization of the membrane has an inhibitory effect on the phosphotransferase system. Possible modes of action are discussed.

INTRODUCTION

Colicins K and E1 belong to a class of protein antibiotics that adsorb to specific receptors on the cell surface and kill sensitive cells of *Escherichia coli* by affecting various metabolic functions [1]. It has recently been proposed [2] that the main biochemical action is the deenergization of membrane systems such as the transports of β-D-galactosides and proline, which are driven by the energized state of the membrane [2, 3] as well as the transport of glutamine [2], which requires phosphate-bond energy. These colicins have been reported to have only minor effects on the uptake of α-methylglucoside in glucose-grown cells [3]. Recently Gilchrist and Konisky [4] have reported that colicin Ia, which in several respects resembles colicins K and E1 in its mode of action, increases the uptake of α-methylglucoside by *E. coli* cells.

The uptake of α-methylglucoside occurs by vectorial phosphorylation via the phosphoenolpyruvate-phosphotransferase system, which involves several cytoplasmic and membrane-bound proteins as well as lipids [5, 6]. As more is learned about the in vitro activity of the phosphotransferase system, its in vivo activity appears to be more complex than originally believed. Neither the dephosphorylation step following α-methylglucoside-phosphate accumulation by the phosphotransferase system, nor the effects of inhibitors and of uncouplers of oxydative phosphorylation [7–11], which stimulate the uptake of α-methylglucoside, are as yet understood. Furthermore, it has become apparent that the phosphotransferase system may have additional physiological roles such as regulation of catabolite repression [12] and interference with entirely unrelated transport systems [13, 14].

In the present study, we have investigated the effects of colicins K and E1 on the accumulation of α-methylglucoside in normal cells of *E coli* and in two mutants that are defective in $(Mg^{2+}+Ca^{2+})$-dependent ATPase activity. The results indicate that the uptake of α-methylglucoside is under partial control of the energized state of the membrane.

MATERIALS AND METHODS

Bacterial strains and media The bacterial strains used throughout this study were *E. coli* G6 his$^-$ and its derivative G6 *uncA* [2], and *E. coli* 7 and its mutant NR70 [15, 16], which were obtained from Dr. B. Rosen. Strains G6 *unc*A and NR70 are defective in $(Mg^{2+}+Ca^{2+})$ATPase activity. The bacteria were grown aerobically a 37 °C in Ozeki medium base [17], supplemented with 3 μM thiamine, 1 mM L-histidine and 0.5 % glucose or 0.5 % glycerol as carbon source. *E. coli* CHE 30, an enzyme-I deficient mutant, was obtained from Dr. B. Tyler and was grown with 0.5 % gluconate as carbon source.

Colicin preparation and assay. Colicins K and E1 were prepared and assayed as described previously [18].

Uptake of α-methylglucoside. Cells were harvested in the exponential phase, washed and resuspended at $5 \cdot 10^8$ C.F.U./ml in standard buffer consisting of 50 mM Tris · HCl, 1 mM $MgSO_4$, 10 mM potassium phosphate (pH 7.0), 100 μg chloramphenicol per ml and a carbon source if required. After addition of L-[U-^{14}C]-α-methylglucoside (5.5 Ci/mol, 40 μM final concentration) (0.2-ml) aliquots were removed at various time intervals, filtered on nitrocellulose filters (pore size 0.45 μm, Matheson-Higgins, Inc., Woburn, Mass.) and washed with 7 ml of standard buffer. The filters were dried and counted in a liquid scintillation counter.

Total phosphorylated α-methylglucoside was determined by the barium bromide precipitation technique [19]. The cells were resuspended in standard buffer plus glycerol as carbon source, and L-[U-^{14}C]α-methylglucoside was added. Samples (of the same size as above) were taken and diluted into 10 vols. of ice-cold 90 % ethanol containing 37 mM $BaBr_2$. After 20 min at 0 °C the mixtures were filtered, washed with ice-cold 80 % ethanol, and the filters dried and counted. The extracellular phosphorylated α-methylglucoside was determined by filtering a sample and collecting the filtrate, which was subsequently diluted into 10 vols. of ethanol-$BaBr_2$ solution. To measure the rate of exit of α-methylglucoside, 2 ml of a cell suspension were allowed to accumulate the glucoside as just described. After 5 min the suspension

was rapidly diluted into 50 vols. of standard buffer and kept at 37 °C. Aliquots were removed at different time intervals, filtered, and the filters dried and counted.

Reagents. [U-^{14}C]α-methylglucoside was purchased from Calatomic, Los Angeles, Cal. Chloramphenicol was obtained from Parke-Davis and Co., Detroit, Mich. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was a gift from Dr. P. Heytler of DuPont de Nemours.

RESULTS

Colicins E1 and K are known to inhibit the active transport of several substrates, including β-D-galactosides and proline, which are driven by the high-energy state of the membrane [2]. As shown in Fig. 1, colicins E1 and K stimulate several-fold the uptake of α-methylglucoside by glycerol-grown cells of both *E. coli* G6 or its derivative G6 *unc*A, which is deficient in the (Mg^{2+}+Ca^{2+})ATPase activity. A similar increase was observed in cells of *E. coli* 7 after the addition of colicin E1. At higher colicin concentrations (less than 1 % survival), α-methylglucoside uptake starts to decrease, due to an increase of the permeability of the membrane. In *E. coli* NR70, which lacks the (Mg^{2+}+Ca^{2+})ATPase molecule and whose membrane is highly permeable to protons [15], no increase was evident after the addition of colicin E1 (Fig. 1). Results similar to those given by colicin E1 were produced by the uncoupler CCCP, as also shown in Fig. 1. Colicin K had no effect on the accumulation of α-methylglucoside by cells of *E. coli* 7 or NR70, since these strains are resistant to that colicin.

It has been reported that drastic inhibition of respiration increases the uptake of α-methylglucoside [8]. This is not the cause of the enhancement of α-methyl-glucoside uptake by colicins since simultaneous measurement of the respiration evidenced only a 25 % inhibition of oxygen uptake during the first 5 min after colicin

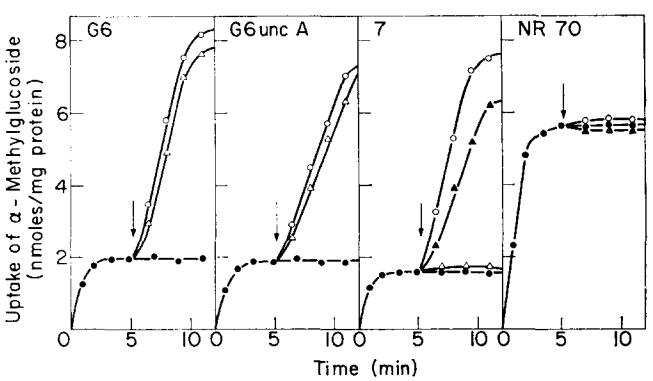

Fig. 1. Effect of colicins K and E1 and of CCCP on the accumulation of α-methyl-glucoside in cells of different strains of *E. coli*. The cells were grown on Ozeki-medium base with glycerol as carbon source until the exponential phase. Cells were gathered, washed and resuspended in standard buffer plus chloramphenicol (100 μg/ml) and 0.5 % glycerol. After 15 min, pre-incubation, the α-methyl-glucoside uptake was measured at various time intervals. After 5 min, either buffer or CCCP (20 μM final concentration) or colicin K or E1 (1 % survival at 5 min after trypsin treatment) was added and α-methylglucoside uptake was measured. ● — ●, control; ○ — ○, colicin E1 added; △ — △, colicin K added; ▲ — ▲, CCCP added.

TABLE I

EFFECT OF SEVERAL CARBON SOURCES ON THE RATES OF α-METHYLGLUCOSIDE UPTAKE IN CELLS OF *E. COLI* G6 IN THE PRESENCE OR ABSENCE OF COLICIN E1.

Cells were grown in Ozeki medium base with glucose or glycerol as carbon source. The cells were centrifuged, washed and resuspended in the same medium and no carbon source, 30 mM glycerol or 25 mM succinate were added. The α-methylglucoside uptake was measured in the absence or in the presence of colicin E1.

Carbon source in growth medium	Carbon source in assay	Addition	α-methylglucoside uptake (nmol/min per mg protein)
Glycerol	Glycerol	None	1.3
	Glycerol	Colicin E1	4.2
	Succinate	None	1.1
	Succinate	Colicin E1	4.6
Glucose	None	None	7.9
	None	Colicin E1	9.5
	Succinate	None	4.0
	Succinate	Colicin E1	9.9

addition. Neither can such inhibition be the cause of the high level of α-methylglucoside accumulation in strain NR70 since, as reported by Rosen [16], this strain has a respiration rate almost twice as high as its parent strain 7.

The increase of the α-methylglucoside accumulation by the colicins is not limited to glycerol-grown cells. Table I shows the relation of carbon source to the rate of α-methylglucoside uptake in the presence and in the absence of colicin E1. The colicin enhanced the uptake in all cases tested. The increase in α-methylglucoside accumulation upon the addition of the colicin was smaller for glucose-grown cells tested without carbon source, a condition used by previous workers [3], than for the same cells in the presence of a carbon source. This is due to the much lower respiration rate of the cells in the absence of an external carbon source [8] resulting in an enhancement of the α-methylglucoside uptake. With succinate as a carbon source, the increase in uptake following colicin treatment could be due in part to inhibition of succinate transport resulting in a severe inhibition of respiration. The higher rates of α-methylglucoside uptake in glucose-grown cells than in glycerol-grown cells is in agreement with the proposal of Kornberg and Reeves [20] that some limiting factor of the phosphotransferase is induced to a higher extent by growth in glucose.

Like the addition of uncouplers of oxydative phosphorylation such as dinitrophenol, CCCP, or NaN_3 and inhibition of respiration [8, 10] the addition of colicin K or E1 creates a condition that stimulates the uptake of α-methylglucoside while de-energizing those membrane systems that are dependent on the high-energy state of the membrane. In strain NR70, which is highly permeable to protons a property that leads to severe inhibition of the energization of the membrane, colicin E1 or CCCP, though they uncouple in different ways, have no further effect on the α-methylglucoside uptake. All these results seem to indicate that α-methylglucoside uptake is under partial control of the energized state of the membrane.

Kinetic parameters. In order to examine how the colicins affect the kinetic parameters of α-methylglucoside transport, uptake was measured at different concentrations of the glucoside. Fig. 2 presents Lineweaver-Burk plots of results obtained

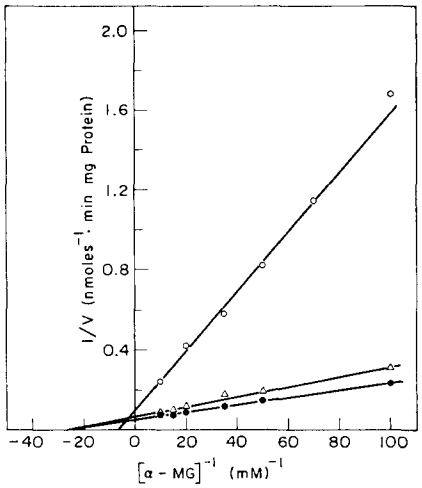

Fig. 2. Effect of colicins E1 and K on the initial rate of α-methylglucoside uptake in cells of *E. coli* G6 at different concentrations of the glucoside. Cells treated as described in Fig. 1 were pre-incubated with the colicin for 2 min (survival at 2 min was 3 % as measured after trypsin treatment). The cells were then assayed for α-methylglucoside uptake as described under Materials and Methods. ○—○, control; ●—●, colicin E1 added; △—△, colicin K added.

with glycerol-grown cells of *E. coli* G6. In the absence of colicin, the K_m and V were 148 μM and 9.4 nmol/min per mg protein, respectively. In the presence of colicin E1, the apparent K_m and V were 35 and 17.5, respectively, and in the presence of colicin K, 38.5 and 14.4. The effect of the colicins on the rate of exit of the glucoside from cells preloaded with the radioactive compound is shown in Fig. 3. The measured rate of

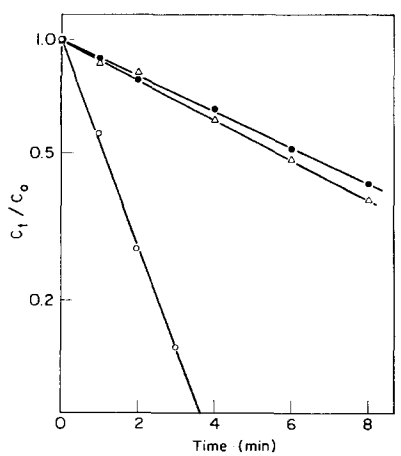

Fig. 3. Effect of colicin E1 or K on the rate of exit of α-methylglucoside. α-Methylglucoside was pre-accumulated in cells of *E. coli* G6 treated as described in Fig. 1, in the presence or absence of colicin E1 or K. The cell suspensions were then rapidly diluted, and at various time intervals, aliquots were taken and the amounts of intracellular glucoside were determined. The C_t/C_0 ratio was plotted against time (C_t, C_0 = intracellular concentrations of the glucoside at t and 0 min, respectively). ○—○, control; ●—●, colicin E1 added; △—△, colicin K added.

exit will approach the efflux since the external α-methyl glucoside concentration will be so low after dilution that the rate of α-methylglucoside uptake can be neglected as can be calculated from Fig. 2. The exit half-lives in untreated cells and in cells treated with colicin E1 or with K are 1.1, 6.3 and 5.7 min, respectively. Similar to conditions that abolish the energized state of the membrane, such as the addition of uncouplers [8, 10, 11] colicins increase the accumulation of α-methylglucoside by decreasing the apparent K_m and inhibiting the exit of the glucoside.

Phosphorylation and dephosphorylation reactions. During the translocation process, the glucoside is phosphorylated and the initial rate of uptake is identical to the initial rate of intracellular appearance of α-methylglucoside-phosphate. The accumulated α-methylglucoside-phosphate is then dephosphorylated by a still unknown reaction, which seems to be energy-dependent [9, 21]. The level of α-methylglucoside-phosphate reaches a plateau, due to the equilibrium between accumulation and dephosphorylation reactions. In order to find out whether the excess α-methylglucoside taken up in the presence of colicins is still transported via the phosphotransferase system and accumulated as α-methylglucoside-phosphate, the effect of colicin E1 on the level of α-methylglucoside-phosphate was determined in cells of *E. coli* 7. Figs. 4a and b show the time course of the internal (α-methylglucoside-phosphate plus α-methylglucoside) level, total and external α-methylglucoside-phosphate in untreated, glycerol-grown cells of *E. coli* 7 and in cells treated with colicin E1. From the curves the internal α-methylglucoside-phosphate and the ratio of internal α-methylglucoside-phosphate to internal (α-methylglucoside plus α-methylglucoside phosphate) can be calculated. This ratio is 0.51 and 0.95 for untreated and colicin-treated cells, respectively. In glucose-grown cells, this ratio is 0.5 and 0.75 for untreated and colicin-treated cells, respectively. Comparable results were obtained for colicin K and *E. coli* G6. In *E. coli* CHE 30, an enzyme-I mutant, there was no accumulation or phosphorylation either in the absence or in the presence of colicins (data not shown).

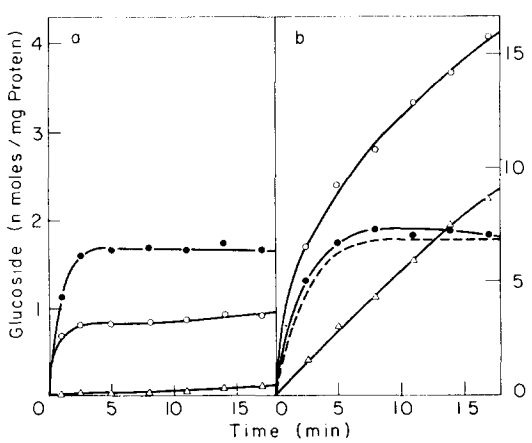

Fig. 4. Effect of colicin E1 on the phosphorylation and dephosphorylation reaction in *E. coli* 7. Cells were treated as described in Fig. 1. After 1 min pre-incubation with buffer (a) or colicin E1 (b), α-[^{14}C]methylglucoside was added and: (○—○), total α-methylglucoside-phosphate; (●—●), internal (α-methylglucoside plus α-methylglucoside-phosphate) and (△—△), external α-methylglucoside-phosphate were determined as described under Materials and Methods. The internal α-methylglucoside-phosphate (dotted line) was calculated from the results.

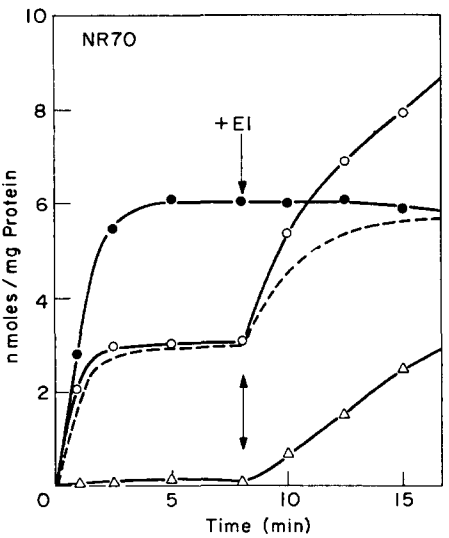

Fig. 5. Effect of colicin E1 on the phosphorylation and dephosphorylation reaction in *E. coli* NR70
Cells were treated as described in Fig. 1. At time zero α-[^{14}C]methylglucoside was added and 5 min later colicin E1. At various time intervals aliquots were taken and the total α-methylglucoside-phosphate (○—○), the internal (α-methylglucoside plus α-methylglucoside-phosphate) (●—●), the external α-methylglucoside-phosphate (△—△) and the internal α-methylglucoside-phosphate (– – –) were determined.

These results show that in the presence of the colicins, the α-methylglucoside is still transported by the phosphotransferase system. The effect of the colicins on the α-methylglucoside accumulation manifests itself in two ways. First, the relative intracellular concentration of α-methylglucoside-phosphate is much larger in colicin-treated cells than in untreated cells, indicating that the dephosphorylation reaction is inhibited by the colicins. Second, in contrast to untreated cells, which are rather impermeable to α-methylglucoside-phosphate, the colicin-treated cells excrete α-methylglucoside-phosphate. This finding may be related to the report [22] that colicin E1-treated cells excrete several phosphorylated compounds, such as glucose 6-phosphate. Yet the colicin-treated cells do not become generally leaky, since they are not permeable to a number of other substances, including protons, β-galactosides and several organic compounds [3, 22].

Cells of *E. coli* NR70 treated with colicin E1 do not have a higher level of α-methylglucoside uptake than untreated cells, but have a much higher ratio of internal α-methylglucoside to internal (α-methylglucoside plus α-methylglucoside-phosphate), equal to 0.96 and excrete α-methylglucoside-phosphate. Therefore, the enhanced accumulation of the glucoside induced by the colicins in the other strains may not be due to the inhibition of the dephosphorylation.

DISCUSSION

The results of this study show that colicins E1 and K enhance the accumulation of α-methylglucoside in normal cells of *E. coli* and in an ATPase-deficient mutant

*unc*A. These findings are in accordance with the reported stimulation of α-methylglucoside uptake in normal cells by colicin Ia in the presence of glycerol as a carbon source [4] and explain the failure of observing such stimulation in the absence of an exogenous carbon source by previous workers [3].

Colicins E1 and K act by decreasing the apparent K_m for α-methylglucoside uptake and decreasing the rate of exit. In strain NR70, which is highly permeable to protons and exhibits a severe reduction in membrane energization and an enhanced uptake of α-methylglucoside [16], colicin E1 does not increase further the uptake of α-methylglucoside. It is believed [2] that the primary action of colicins K and E1 is an uncoupling of the membrane systems that are driven by the so-called energized state of the membrane. The above results suggest that the uptake of α-methylglucoside is directly or indirectly under a partial control of the energized state of the membrane. This control resembles feed-back regulation and may be necessary for the cell to obtain the right balance between the supply of glucose and the membrane energization.

It is conceivable that interference by colicin with the utilization of membrane energy results in an alteration of the cellular level of an effector of the phosphoenolpyruvate-phosphotransferase system, such as increased production of phosphoenolpyruvate. However, the colicins decreased the level of phosphoenolpyruvate in glycerol-grown cells (unpublished results). Also, Klein and Boyer [23] reported that the levels of phosphoenolpyruvate are not limiting for the uptake of α-methylglucoside.

A more interesting interpretation is that the colicins K and E1, like colicin Ia and the uncouplers of oxidative phosphorylation such as CCCP, dinitrophenol and NaN_3 and the inhibition of respiration, create in cells a membrane-state that stimulates the phosphotransferase system while inhibiting those membrane systems that depend on the high-energy state of the membrane. The energized state may affect in opposite ways the binding of glucoside to enzyme-II protein and the binding of β-galactosides to the lactose-carrier protein [24]. For example, it may favor one of two alternative conformational states, one with the binding site available to the exterior of the membrane and the other with the binding site to the interior. De-energization would favor the exposure of the lactose-carrier with the binding site to the interior and favor the exposure of the binding site of enzyme II to the exterior. This hypothesis is supported by findings of Haguenauer-Tsapis and Kepes [25], who reported different sensitivities of enzyme II to sulfhydryl reagents under energized and de-energized conditions. Experiments to test this hypothesis further are being undertaken.

Although the effect of the colicins E1 and K on α-methylglucoside accumulation resembles that of uncouplers, two majors differences are evident. First, the colicins increase the relative intracellular concentration of α-methylglucoside-phosphate, possibly by inhibiting the dephosphorylation-reaction, whereas uncouplers have little effect on the ratio of internal α-methylglucoside-phosphate to internal (α-methylglucoside plus α-methylglucoside-phosphate) [9]. Second, in contrast to untreated cells and to cells treated with uncouplers, which are rather impermeable to α-methylglucoside-phosphate, colicin-treated cells excrete α-methylglucoside-phosphate. This may be an instance of the colicin-induced excretion of certain phosphorylated metabolites [22].

It has become apparent that the phosphotransferase system has, in addition

to sugar-transport function, several physiological roles, such as regulation of catabolite repression [12], and interference with entirely different transport systems, such as the one for glycerol. It has been reported [14] that the phosphotransferase system plays some regulatory role in glycerol permease. It is known that colicins E1 and K inhibit the uptake of glycerol [22]. The colicins might enhance the phosphorylation of α-methylglucoside leading to the inhibition of the glycerol-permease in a competitive way. The effect of colicins on the phosphotransferase system could then account for the effect colicins have on a number of related systems, such as the uptake of glycerol.

ACKNOWLEDGEMENTS

The author expresses his appreciation for the stimulation and guidance of Dr. S. E. Luria, in whose laboratory this work was conducted. Research was supported by Grant No. AI 03038 from N.I.H. and Grant No. BMS 71-01230 from N.S.F.

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