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Biochemistry

Escherichia coli adenylate cyclase complex: Regulation by the proton electrochemical gradient

(proton motive force/transport/carbonyl cyanide *m*-chlorophenylhydrazone)

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ABSTRACT **Sugars such as glucose are transported into *Escherichia coli* by a coupled phosphorylation mechanism (the phospho*enol*pyruvate:sugar phosphotransferase system, PTS). Transport of sugars through the PTS results in inhibition of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] activity by a mechanism involving a change in the state of phosphorylation of PTS proteins. Other sugars (e.g., lactose) are transported without modification by a mechanism involving proton cotransport, which requires a proton motive force across the cell membrane. We show here that uptake of sugars through the lactose transport system results in inhibition of adenylate cyclase activity if the proton symport mechanism is also active. The protonophore carbonyl cyanide *m*-chlorophenylhydrazone also inhibits adenylate cyclase activity. These data suggest that the steady-state electrochemical proton gradient regulates the activity of adenylate cyclase. We propose that sugar-dependent inhibition of adenylate cyclase activity may occur by either of two mechanisms. Sugars transported by the PTS inhibit adenylate cyclase activity by dephosphorylation of a regulatory protein, while sugars transported by the proton motive force system inhibit adenylate cyclase activity as a result of collapse of the proton electrochemical gradient.**

In *Escherichia coli*, cyclic AMP (cAMP) levels are controlled mainly by regulation of the expression of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] activity (1). The system that catalyzes the transport of glucose and some other sugars [the phospho*enol*pyruvate:sugar phosphotransferase system (PTS) (2)] plays an important role in controlling adenylate cyclase activity. Models for inhibition of adenylate cyclase activity by PTS sugars have been suggested involving the idea that the fraction of some PTS protein in the phospho form is proportional to the adenylate cyclase activity (3–5).

A mechanism for regulation of cAMP levels by interaction of the adenylate cyclase complex with the PTS does not account for the fact that sugars transported by another general mechanism, driven by the cellular proton motive force (PMF), also lower cAMP levels (6). The studies presented here deal with the mechanism by which such sugars (e.g., lactose) inhibit adenylate cyclase. We present evidence that transport through the lactose permease leads to an inhibition of adenylate cyclase activity, but only under conditions where there is a concomitant proton symport (which decreases the proton electrochemical gradient). In the absence of sugars, PMF can be decreased with a protonophore, which also leads to an inhibition of adenylate cyclase activity.

We propose that adenylate cyclase activity in *E. coli* is subject to a dual regulation mechanism. Sugars transported by the PTS inhibit adenylate cyclase activity by decreasing the phosphorylation of a regulator protein associated with the PTS; sugars transported by the PMF mechanism inhibit adenylate cyclase activity as a result of the conversion of a factor connected to the energy-transducing system to a deenergized state.

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MATERIALS AND METHODS

E. coli were grown in a New Brunswick gyrotory shaker at 37°C in salts medium (7) supplemented with 1% nutrient broth or with thiamine (5 μg/ml) and lactose (0.1%). At an OD_{650} of ≈0.3, they were centrifuged, washed, and resuspended in salts medium. For experiments using toluene-treated cells, the washed cells were resuspended in ≈1/50 the original volume (protein concentration ≈2.5 mg/ml). Adenylate cyclase in toluene-treated cells was measured as described (8). For experiments using intact cells, the cells were resuspended in one-fourth of the original volume (protein concentration ≈0.2 mg/ml). Adenylate cyclase activity in intact cells was measured by incubating cells suspended in salts medium with any indicated additions at 30°C. At designated times, aliquots were removed and held in a boiling H_2O bath for 2 min. After centrifugation, the supernatant solutions were adjusted to pH 4 and cAMP concentrations in the samples were determined (9, 10). ATP concentrations in such boiled samples were determined as described (11). Thiomethylgalactoside and α-methylglucoside uptake were measured at 30°C by mixing equal volumes of cells suspended in salts medium with a solution containing salts medium (7), chloramphenicol (50 μg/ml), and either β-[*methyl*-^{14}C]methyl-D-thiogalactoside (TMG) or methyl α-D-[*U-glucose*-^{14}C]glucopyranoside (1 mM, 1 Ci/mol; 1 Ci = 3.7×10^{10} Bq). At the indicated times, aliquots were deposited on Millipore membrane filters (HAWP 02500, HA 0.45 μm; 25 mm diameter) and the cells were washed with 10 ml of salts medium. The filters were then dissolved in Cellosolve and radioactivity was measured in scintillation fluor. The concentration of protein in uptake assays was ≈0.1 mg/ml.

E. coli strains were generously provided by the following colleagues: ML308 ($i^-z^+y^+a^+$), ML308-831 ($i^-z^+y^+a^-$), ML35 ($i^-z^+y^-a^+$), and ML308-22 ($i^-z^+y^{unc}a^+$) from T. H. Wilson (Harvard University); ML308-225 ($i^-z^-y^+a^+$) from H. R. Kaback (Roche Institute of Molecular Biology); ZSC112 (carrying mutations in enzymes II of the PTS for both glucose and mannose) from W. Epstein (The University of Chicago).

RESULTS

Adenylate Cyclase in Toluene-Treated Cells Is Coupled Only to PTS. We previously showed that, when wild-type *E. coli* were adapted to growth on lactose, adenylate cyclase activity measured in toluene-treated cells was inhibited by lactose (12). The present studies, using mutants in β-galactosidase or transport proteins for lactose or glucose, explain this effect: *in toluene-treated cells*, lactose can freely permeate the cells and be split to glucose if β-galactosidase is present. The glucose produced can diffuse out of the cells and then be phosphoryl-

Abbreviations: PTS, phospho*enol*pyruvate:sugar phosphotransferase system; cAMP, cyclic AMP; PMF, proton motive force; IPTG, isopropylthiogalactoside; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; TPMP, triphenylmethylphosphonium ion.

ated by the glucose-specific PTS. The transport of PTS sugars produces inhibition of adenylate cyclase activity (3, 13). Toluene-treated cells of strain ML308, which constitutively produce lactose permease, β-galactosidase, and glucose-specific PTS, contain adenylate cyclase that is inhibited by either glucose or lactose (Fig. 1A). The presence of lactose permease is not necessary for lactose to inhibit adenylate cyclase in toluene-treated cells since strain ML35, a lactose permease mutant, also contains adenylate cyclase that is inhibited by either glucose or lactose (Fig. 1B). The necessity for the presence of β-galactosidase activity for demonstrating lactose-dependent inhibition of adenylate cyclase in toluene-treated cells is shown in Fig. 1C. ML308-225, which has only a trace level of β-galactosidase, contains adenylate cyclase in toluene-treated cells that is almost completely resistant to inhibition by lactose, but that is substantially inhibited by glucose.

We previously showed (13) that a strain deficient in the membrane-bound PTS enzymes II for glucose was unable to catalyze the phospho*enol*pyruvate-dependent phosphorylation of glucose and also was insensitive to inhibition of adenylate cyclase by glucose. Fig. 1D shows that when this strain (ZSC112) is adapted to growth on lactose, it is also insensitive to the inhibition of adenylate cyclase by lactose in toluene-treated cells.

Adenylate Cyclase in Intact Cells Is Inhibited by Lactose:Proton Symport. Effective activity of lactose permease requires cotransport with protons (14). We show that *in intact cells*, in which the lactose is translocated in symport with protons, adenylate cyclase inhibition is dependent on lactose accumulation. Measurement of adenylate cyclase activity in toluene-treated compared with intact cells indicates that these two preparations respond differently to isopropylthiogalactoside (IPTG), a nonmetabolizable substrate of lactose permease (Fig. 2). *In toluene-treated cells*, neither strains containing normal (Fig. 2A) nor those containing mutant (Fig. 2B) lactose permease contain adenylate cyclase that is affected by IPTG. However, when adenylate cyclase is assayed *in intact cell preparations*, there is a partial inhibition of cAMP accumulation by IPTG in a strain containing normal lactose permease (Fig. 2C); no such inhibition by IPTG was observed when the lactose permease is not functional (Fig. 2D).

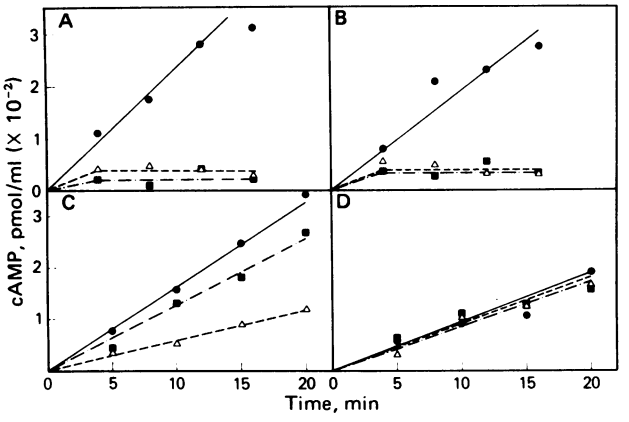

FIG. 1. Effect of glucose or lactose on adenylate cyclase in toluene-treated cells of strains carrying mutations in β-galactosidase or sugar transport proteins. Toluene-treated cells were prepared from various strains and tested for adenylate cyclase activity (8) in the absence or presence of 1 mM glucose or 1 mM lactose as indicated. (A) Strain ML308 ($i^-z^+y^+$) grown in nutrient-broth medium; each 0.1-ml assay point contained 0.05 mg of protein. (B) Strain ML35 ($i^-z^+y^-$) grown in nutrient-broth medium; each 0.1-ml assay point contained 0.058 mg of protein. (C) Strain ML308-225 ($i^-z^-y^+$) grown in nutrient-broth medium; each 0.1-ml assay point contained 0.055 mg of protein. (D) Strain ZSC112 (glucose phosphotransferase and mannose phosphotransferase negative) grown in lactose medium; each 0.1-ml assay point contained 0.042 mg of protein. Data are expressed as pmol of cAMP per ml of incubation mixture. ●, Control; △, glucose; ■, lactose.

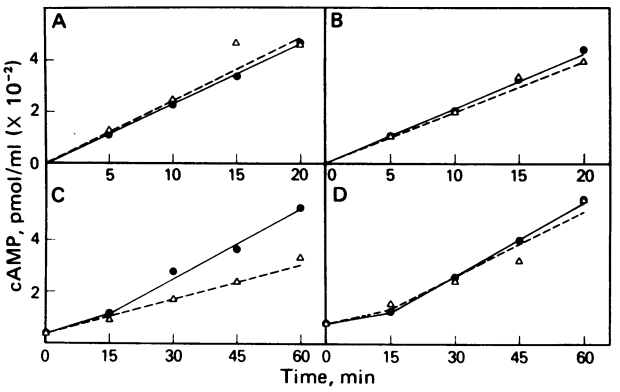

FIG. 2. Effect of IPTG on adenylate cyclase in toluene-treated compared to intact cells of strains differing by mutations in lactose permease. All cells were grown in nutrient-broth medium. Strains ML308 ($i^-z^+y^+$) (A) and ML35 ($i^-z^+y^-$) (B) were tested for adenylate cyclase activity in toluene-treated cells in the absence or presence of 1 mM IPTG. Each 0.1-ml assay point contained 0.035 (A) or 0.038 (B) mg of protein. Strains ML308 (C) and ML35 (D) were tested for adenylate cyclase activity in intact cells in the absence or presence of 1 mM IPTG. Incubation mixtures contained ≈0.2 mg of protein per ml. Data are expressed as pmol of cAMP per ml of incubation mixture. The specific activities calculated for adenylate cyclase in the absence of IPTG in toluene-treated compared to intact cells, respectively (expressed as pmol of cAMP formed per mg per hr) are: strain ML308, 3914 and 2780 (A and C); strain ML35, 3210 and 2890 (B and D). ●, Control; △, plus IPTG.

Inhibition of Adenylate Cyclase by Lactose Permease Substrates Requires Proton Symport. The following experiments support the idea that, in intact cells, the inhibition of adenylate cyclase activity by transport through the lactose permease depends on the capability of the permease to stimulate proton influx and leads to the conclusion that the transport of lactose is only incidental to the mechanism. We measured adenylate cyclase activity in intact cells in a strain carrying a mutation in β-galactosidase but with constitutively expressed lactose permease (Fig. 3A). In this case, the PTS substrate glucose confers complete inhibition, while the lactose permease substrates, lactose or thiomethylgalactoside, partially inhibit the activity. Increasing the concentration of thiomethylgalactoside does not result in a greater degree of inhibition (data not shown). Studies (15, 16) using membrane vesicles of this mutant (ML308-225) established that lactose uptake partially collapses membrane potential and $\Delta\bar{\mu}H^+$ as measured by the uptake of triphenylmethylphosphonium ion (TPMP) and permeant acids. The incomplete dissipation of PMF associated with lactose uptake suggests an explanation for the incomplete inhibition of adenylate cyclase coupled to lactose transport; the state of the PMF might regulate adenylate cyclase activity.

Wong *et al.* (17) isolated a strain of *E. coli* (ML308-22) that had an increase in the concentration of lactose carriers but was deficient in the capacity to accumulate nonmetabolizable substrates of the lactose permease. West and Wilson (18) showed that this strain had a high activity for the facilitated diffusion of β-galactosides. However, while a normal strain caused the medium to become more alkaline concomitant with the transport of thiomethylgalactoside, this mutant did not. It was therefore concluded that this strain has a lactose permease that is uncoupled from proton inflow.

Although thiomethylgalactoside produces partial inhibition of adenylate cyclase in a strain carrying wild-type lactose permease (Fig. 3A), the strain with permease uncoupled from proton transport is not inhibited by the galactoside (Fig. 3B).

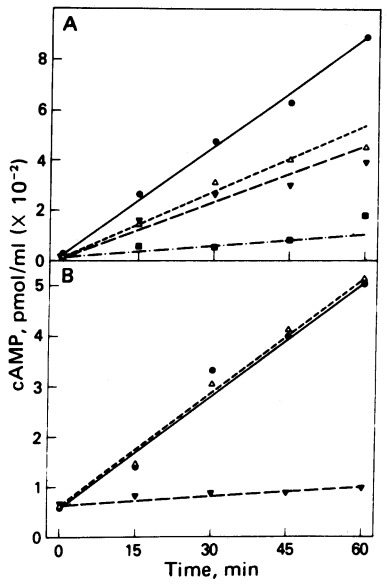

FIG. 3. Effect of thiomethylgalactoside, glucose, or lactose on adenylate cyclase in intact cells of strains carrying mutations in β-galactosidase or lactose permease. Strains ML308-225 ($i^-z^-y^+$) (*A*) or ML308-22 ($i^-z^+y^{unc}$) (*B*) were grown in nutrient-broth medium and assayed for adenylate cyclase activity in intact cells. Incubation mixtures contained thiomethylgalactoside (△), glucose (■), or lactose (▼) (1 mM). ●, Control. Data are expressed as pmol of cAMP per ml of incubation mixture.

Evidence that lactose carriers are functional in this strain is provided by the observation that lactose produces essentially complete inhibition of adenylate cyclase (Fig. 3*B*). A likely explanation for the inhibition by lactose in this strain is that the *process* of lactose transport does not affect adenylate cyclase in this strain; however, the action of β-galactosidase produces glucose which can then exit from the cells and be taken up again by the glucose-specific PTS. As indicated, the process of glucose transport is coupled to inhibition of adenylate cyclase.

Documentation for the notion that lactose uptake by strain ML308-22 produces glucose that is extruded from the cells is shown in Table 1. The data indicate that, during 1 hr, a steady-state concentration of ≈25 μM glucose is attained. We previously showed (8) that 10 μM glucose produces essentially complete inhibition of adenylate cyclase in toluene-treated cells.

As seen before (Fig. 3*A*), glucose inhibits adenylate cyclase. That the complete inhibition by lactose requires the presence of glucose phosphotransferase comes from a study using a mutant lacking glucose-specific PTS activity (19). When this strain is adapted to growth on lactose, exposure of cells to lactose produces a partial inhibition of adenylate cyclase while glucose elicits no inhibition (data not shown).

Adenylate Cyclase Is Inhibited by a Protonophore. The data of Fig. 3 suggest that inhibition of adenylate cyclase by thiomethylgalactoside is more directly related to lactose:proton symport than to accessibility of the galactoside to the inside of the cell. Further support for this idea comes from the observation that adenylate cyclase in intact cells is inhibited by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (20) (Fig. 4, *left*). Although lactose only produces partial inhibition of adenylate cyclase, CCCP leads to complete inhibition. The experiments of Kaback and coworkers (15, 16) indicate that lactose decreases PMF partially, but CCCP almost completely abolishes PMF (21).

The trivial explanation for the CCCP-dependent inhibition of adenylate cyclase, that ATP levels are lower in the presence of CCCP, is excluded by the experiment shown in Fig. 4, *right*.

Table 1. Production of extracellular glucose from lactose by strain ML308-22

Time, min	Extracellular glucose, μM
0	9
15	13
30	23
45	26
60	24

ML308-22 was grown as in the legend of Fig. 3. Washed cells were incubated with 1 mM [*glucose*-^{14}C]lactose. At the indicated times, 100-μl aliquots of the cell suspension were centrifuged for 2 min in a microfuge. Aliquots (50 μl) of the supernatant solution were spotted on Whatman 3 MM paper in 1-inch stripes. Chromatography (descending) in *n*-butanol/95% EtOH/H_2O, 52:32:16 (vol/vol) was for 18 hr. Radioactivity of the region of the paper corresponding to the position of glucose was determined in a scintillation counter. An aliquot (50 μl) of the medium applied to paper at zero time gave a recovery of 17,000 cpm, which corresponds to 1 mM lactose. Therefore, a specific activity of 17 cpm, equivalent to 1 μM, was used to calculate the glucose concentration.

Here it is seen that the rate of ATP utilization by cells is essentially unaffected by CCCP.

Evidence related to the mechanism by which CCCP inhibits adenylate cyclase comes from a comparison of the dose–response profile for the inhibition of thiomethylgalactoside uptake compared to the inhibition of cAMP accumulation (Fig. 5). There is a reasonably close correlation for the effect of CCCP concentration on inhibition of both processes in intact cells; approximately 50% inhibition of both thiomethylgalactoside uptake and cAMP formation is observed at 1 μM CCCP.

Inhibition of adenylate cyclase by possible generalized effects of CCCP unrelated to its action as an ionophore are excluded by the data in Fig. 5. Concentrations of CCCP that substantially inhibit both thiomethylgalactoside uptake and adenylate cyclase in intact cells do not inhibit the uptake of α-methylglucoside. α-Methylglucoside is transported via the PTS, which does not require the presence of an electrochemical proton gradient but does depend on the presence of some membrane-bound proteins. The highest concentration of CCCP tested (4 μM), which completely inhibits both thiomethylgalactoside uptake and cAMP accumulation, stimulates the uptake of α-methylglucoside (22). Therefore, a general destruction of membrane-dependent processes by CCCP is eliminated.

The data in Fig. 5 also eliminate the possibility that inhibition

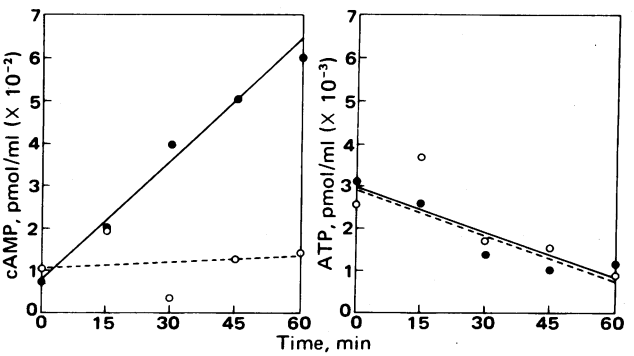

FIG. 4. Effect of CCCP on adenylate cyclase (*Left*) and ATP (*Right*) levels in intact cells of ML308-225. Bacteria were grown on nutrient-broth medium. ○, Incubation mixtures were supplemented with 4 μM CCCP dissolved in ethanol. The final concentration of ethanol in all incubation mixtures was 0.1%. Data are expressed as pmol of cAMP or ATP per ml of the incubation mixture. ●, Control.

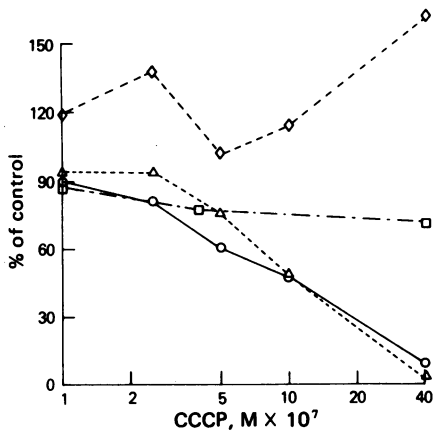

FIG. 5. Effect of CCCP concentration on uptake of thiomethylgalactoside (○) and α-methylglucoside (◇) and on adenylate cyclase in intact (△) and toluene-treated (□) cells of ML308-225. Bacteria were grown in nutrient broth medium. Thiomethylgalactoside uptake was assayed. At various times (15, 30, 45, and 60 min), 0.7-ml aliquots of the incubation mixtures were filtered and washed. The counts obtained were corrected for a boiled enzyme blank (620 cpm). Corrected radioactivity taken up in the control incubation (no CCCP added) was 5380, 4026, 4234, and 3594 cpm at 15, 30, 45, and 60 min, respectively. The plot represents the average fraction of the control values for the four time points at each concentration of CCCP. α-Methylglucoside uptake was assayed as described for thiomethylgalactoside uptake. The counts obtained were corrected for a boiled enzyme blank (922 cpm). Corrected radioactivity taken up in the control incubation (no CCCP added) was 2976, 1503, 2109, and 911 cpm at 15, 30, 45, and 60 min, respectively. The plot represents the average fraction of the control values for the four time points at each concentration of CCCP. Adenylate cyclase was determined in intact cells. A control incubation containing cells (0.2 mg of protein per ml) with 0.1% EtOH accumulated 610 pmol of cAMP per ml in 1 hr. The data are expressed as the fraction of this control value at the designated concentrations of CCCP. Adenylate cyclase in toluene-treated cells was determined as described (8). In a control incubation containing 1% EtOH but no CCCP, the adenylate cyclase activity was 2780 pmol/mg per hour. The data are expressed as the fraction of this control value at the designated concentrations of CCCP. CCCP was dissolved in EtOH. The final concentration of EtOH was 0.1% in all incubations except for adenylate cyclase in toluene-treated cells, where the concentration was 1%. These concentrations of EtOH had negligible effects on the activities tested.

of adenylate cyclase by CCCP reflects the sensitivity of the catalytic unit of the enzyme to this compound. It is clear that when adenylate cyclase is measured in cells permeabilized with toluene, the enzyme is much less sensitive to the action of CCCP than is the case in intact cells. Taken together, these data are compatible with the idea that CCCP-dependent inhibition of adenylate cyclase is due to the decrease in PMF caused by this proton ionophore.

DISCUSSION

In wild-type cells, inhibition of adenylate cyclase activity by lactose is complex, involving both lactose transport and metabolism to the PTS sugar glucose. As shown in Figs. 2 and 3, transport of IPTG or thiomethylgalactoside through the lactose permease leads to a partial inhibition of adenylate cyclase activity. Since a mutant (ML308-22) that has uncoupled proton inflow from the lactose permease shows no inhibition of adenylate cyclase activity by thiomethylgalactoside (Fig. 3*B*), we speculate that adenylate cyclase activity in intact cells is partly regulated by PMF.

Internalized lactose is converted by β-galactosidase to glucose, some of which is released into the medium (Table 1) (see Fig. 6). A similar exit reaction involving fructose formed from sorbitol has been demonstrated in *Bacillus subtilis* (23). Similarly, in *Staphylococcus aureus*, maltose enters the cells and is hydrolyzed to glucose, which leaks from the cells into the medium (24). Extracellular glucose is taken up into *E. coli* via the PTS by a mechanism that results in the inhibition of adenylate cyclase activity (1). Thus, a non-PTS sugar, such as lactose or maltose, which can be converted to a PTS sugar, inhibits adenylate cyclase by a combination of two mechanisms.

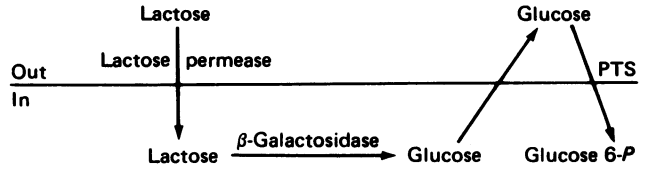

FIG. 6. Proposed scheme for the metabolism of lactose involving sequential transport of lactose and glucose.

The decreases in cAMP accumulation resulting from exposure of intact cells to lactose permease substrates or CCCP are actually effects on the rate of cAMP synthesis rather than an effect on cAMP phosphodiesterase. Similar effects have been observed in a mutant lacking cAMP phosphodiesterase (data not shown).

It might be speculated that the inhibition of adenylate cyclase activity by thiomethylgalactoside is due to the intracellular accumulation of the acetylated or phosphorylated derivatives of the galactoside (25, 26). This possibility was eliminated because Kashket and Wilson (26) showed that *E. coli* phosphorylated thiomethylgalactoside but not IPTG; the studies reported here indicate that both thiomethylgalactoside (Fig. 3) and IPTG (Fig. 2) inhibit adenylate cyclase activity in intact cells. Further, under the conditions (Fig. 3) where we observe inhibition of adenylate cyclase activity by thiomethylgalactoside, we have detected no accumulation of thiomethylgalactoside phosphate (data not shown). Since the galactoside inhibits adenylate cyclase activity in intact cells of strain ML308-831 (transacetylase negative, data not shown), we also conclude that the accumulation of acetylthiomethylgalactoside does not explain the thiomethylgalactoside-dependent inhibition of adenylate cyclase activity.

The experiments reported here provide a possible basis for understanding the mechanism by which non-PTS sugars inhibit adenylate cyclase activity. The hypothesis is presented that intact cells incubated under the conditions described here use some of their energy reserve to develop and maintain a proton gradient. When cells are suspended in medium devoid of a transportable sugar, the PMF is maintained at some steady-state level; in the presence of transportable sugars such as lactose, symport of protons reduces this steady-state PMF. It is postulated that some factor, possibly a protein, involved in the maintenance of the PMF can exist in two forms—energized and deenergized. We suggest that the state of energization of this factor influences the activity of adenylate cyclase. In the absence of a transportable sugar the factor is energized and adenylate cyclase is active; in the presence of a sugar that is rapidly symported with protons, the factor becomes deenergized and adenylate cyclase activity is inhibited. This model can serve as a unified mechanism to explain the inhibition of adenylate cyclase activity by either lactose permease substrates or the protonophore CCCP. It might also serve as the basis for understanding the mechanism by which many sugars that are cotransported with cations decrease cAMP levels. The degree of inhibition of adenylate cyclase activity by lactose permease substrates (partial) and CCCP (complete) correlates with the extent to which these compounds collapse PMF (15, 16).

The observation (see Figs. 4 and 5) that CCCP is a potent inhibitor of adenylate cyclase activity in intact cells provides

strong support for the notion that the electrochemical proton gradient influences the activity of adenylate cyclase. Other experiments (data not shown) indicate that effects on adenylate cyclase activity and thiomethylgalactoside uptake similar to those shown in Fig. 5 can be observed with other compounds, such as colicin E1 (27) and KCN (28), which decrease the PMF. Furthermore, glucose 6-phosphate, which is symported with protons (16), inhibits adenylate cyclase activity in cells in which the glucose 6-phosphate uptake system is active.

A comparison of adenylate cyclase activity in intact and toluene-treated cells reveals a fundamental difference in the two systems. Toluene treatment of strain ML308-225 cells has little effect on the specific activity. Fig. 5 shows that intact cells have an adenylate cyclase activity of 3050 pmol/mg per hr while toluene-treated cells have a specific activity of 2780 pmol/mg per hr. However, the sensitivity of the enzyme to regulation appears to be different in these two systems. While adenylate cyclase activity in intact cells is inhibited by either PTS or non-PTS sugars, adenylate cyclase activity in toluene-treated cells in inhibited by PTS sugars only. We have considered the likelihood that the activity of the adenylate cyclase catalytic unit may be sensitive to the PMF. While this possibility should not be eliminated from consideration, we favor the involvement of an additional regulatory factor. We suggest that the factor regulating adenylate cyclase activity can be deenergized in intact cells as a result of proton influx. However, toluene treatment of cells may uncouple the proton pump from the factor, thereby freezing the factor in the energized form. As a result, adenylate cyclase is in an active form which cannot be inhibited by compounds that stimulate proton influx, while it can still be inhibited by the alternate mechanism coupled to the PTS.

While the experiments reported here suggest that some factor associated with maintenance of the cellular PMF is important in regulating adenylate cyclase activity, the identity of this protein or other factor remains to be established. It might be a component of the ATPase complex (29), a portion of the respiratory chain, or some "coupling factor" (30). While non-PTS sugars do not affect the rate of uptake of PTS sugars, the transport of PTS sugars markedly inhibits the uptake of non-PTS sugars (31). This interrelation of the two transport systems suggests that the unidentified factor that is involved in that process is the adenylate cyclase regulatory factor.

Emerging studies lend credence to the notion that the adenylate cyclase in eukaryotic systems may also be affected by the electrochemical proton gradient. In *Neurospora crassa*, treatments that depolarize the plasma membrane increase the levels of cAMP (32). Studies with cultured thyroid cells suggest that not only does thyrotropin increase cAMP concentrations, but it also hyperpolarizes the cell membrane (33). The possibility that the PMF may regulate adenylate cyclase activity may therefore be widespread.

We thank our colleagues in the field of microbial transport (Drs. W. Epstein, H. R. Kaback, and T. H. Wilson) for providing both the extensive literature which guided these studies and the well-characterized mutant strains used here. We also appreciate the critical comments on the manuscript from Drs. R. Hendler, H. R. Kaback, and S. Ramos.

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