Involvement of the Glucose Enzymes II of the Sugar Phosphotransferase System in the Regulation of Adenylate Cycilase by Glucose in Escherichia coli (Received for publication, November 7, 1975)

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The nature of the interaction of glucose with toluene-treated cells of Escherichia coli leading to inhibition of adenylate cyclase was examined by the use of analogues. Those analogues with variations of the substituents about carbon atoms 1 or 2 (e.g. α-methylglucoside or 2-deoxyglucose) are inhibitory, and they are also substrates of the phosphoenolpyruvate-dependent sugar phosphotransferase system. Analogues with changes in other parts of the molecule (e.g. 3-O-methylglucose or galactose), L-glucose and several disaccharides and pentoses, do not inhibit adenylate cyclase and are not substrates of the phosphotransferase system. This correlation suggests some functional relationship between the adenylate cyclase and phosphotransferase systems.

Further studies were done with mutants defective in glucose enzymes II of the phosphotransferase system (designated GPT and MPT); these two activities are measured by phosphorylation of α-methylglucoside and 2-deoxyglucose, respectively. The wild-type parent phosphorylates both analogues, and both inhibit adenylate cyclase. In the GPT− mutant, α-methylglucoside does not inhibit adenylate cyclase and is not phosphorylated, while 2-deoxyglucose is inhibitory and phosphorylated. In the GPT−MPT− double mutant, adenylate cyclase activity is present, but neither α-methylglucoside nor 2-deoxyglucose inhibits adenylate cyclase, and neither sugar is phosphorylated. These studies demonstrate that glucose inhibition of adenylate cyclase in toluene-treated cells requires an interaction of this sugar with either the GPT or MPT enzyme II of the phosphotransferase system.

The mechanism by which bacteria adapt to an altered environment via the induction of enzyme synthesis involves changes in the intracellular level of cyclic adenosine 3′:5′ monophosphate (1). Recent studies have attempted to define the mechanism by which cyclic AMP levels are regulated. In whole cells pulse-labeled with [3H]adenosine (2) or toluene-treated cells using [α-32P]ATP (3), it has been shown that adenylate cyclase activity is inhibited in the presence of glucose. The correlation of intracellular cyclic AMP with the rate of cyclic AMP excretion has provided a further basis for concluding that catabolite repression is primarily due to lowered intracellular cyclic AMP resulting from the glucose-mediated inhibition of adenylate cyclase (4).

It was previously shown that the spectrum of sugars which inhibited adenylate cyclase in Escherichia coli B was dependent on the sugar on which the bacteria were grown (5). Inhibition by glucose was constitutive whereas inhibition by the other sugars was inducible. The coincident development of inhibitory activity by sugars other than glucose with the development of transport systems for these sugars suggested some relationship between transport and the regulation of adenylate cyclase. We have extended these studies by determining the structure activity requirements of the glucose molecule necessary for inhibition of adenylate cyclase activity for glucose-grown cells. These findings were correlated with the effects of the sugars on transport. Only those sugars which inhibited adenylate cyclase were active in the transport assay.

Glucose transport in E. coli, defined by Roseman (6), can be described by the following scheme:

\[ \text{enzyme I} \]

\[ \text{HP} + \text{P-enolpyruvate} \underset{\text{enzyme I}}{\rightleftharpoons} \text{P-HP} + \text{pyruvate} \] (1)
Enzyme I and HPβ are soluble components and not sugar-specific, whereas there is a family of sugar-specific enzymes II which are membrane-bound. For glucose there are two enyzes II, designated GPT and MPT (7); both transport glucose but they can be distinguished by their activity toward α-methylglucoside and 2-deoxyglucose, respectively. A study of E. coli GPT- and MPT- mutants revealed that the absence of both GPT and MPT led to a loss of the regulation (inhibition) by glucose, although adenylate cyclase activity was present. Loss of one of the enzymes II (GPT) led to a loss of regulation by α-methylglucoside, while 2-deoxyglucose still gave full inhibition of adenylate cyclase activity. Thus, interaction of glucose with either of the glucose enzymes II causes inhibition of adenylate cyclase activity.

**EXPERIMENTAL PROCEDURE**

**Materials**—The [α-32P]ATP (7 to 10 Ci/mmol) used for the assay of adenylate cyclase was purchased from International Chemical and Nuclear Corp.; other components used in this assay were as described previously (3). 2-Deoxy[1-14C]glucose (45 to 55 mCi/mmol) was from New England Nuclear Corp. and α-[2-14C]methylglucoside (3 mCi/ 

m
eq mol) was from Amersham/Searle Corp. Phosphoenolpyruvate, potassium chloride, ethanol, and toluene for both adenylate cyclase and phosphotransferase assays was prepared as described previously (3). The sugars were checked for purity by descending chromatography on Whatman No. 1 paper for 60 

m

hours in a solvent system containing t-amyl alcohol/l-butyl alcohol/water, 5/4/1. After drying, the paper was sprayed with p-anisidine reagent, and heated in an oven at 100° for 10 min. Reducing sugars gave brown spots. Applying 360 μg of the sugar readily enabled detection of contamination at the 1% level. Furthermore, the adenylate cyclase assays were run kinetically such that low levels of an active agent contaminating an inactive agent, e.g. glucose in a maltose sample, could be detected as a change in rate during the course of the assay (see adenylate cyclase assay).

**Assay of Phosphotransferase Activity**—Enzyme assays were performed in toluene-treated cells under conditions as close as possible to those used for the assay of adenylate cyclase activity in toluene-treated cells. This procedure was adopted in order to minimize the possibility that any differences in the activity of sugars tested in the two systems would be due to changes in the conditions. The changes in the assay procedure, modified from that of Fox and Wilson (10), were checked for their effects in the assay.

**Regulation of Adenylate Cyclase and Phosphotransferase E II s**

**Effect of threshold concentrations of glucose, mannose, and 2-deoxyglucose on adenylate cyclase activity**

Fig. 1. Effect of threshold concentrations of glucose, mannose, and 2-deoxyglucose on adenylate cyclase activity in toluene-treated cells of *Escherichia coli* B. Cells were grown on minimal salts medium (8) supplemented with glucose, harvested, washed, and treated with tolune for assay of adenylate cyclase activity as described under "Experimental Procedure." The specific activity of ATP (1 mM) was 44 cpm/pmol and adenyl cyclase activity is expressed as picomoles of cyclic AMP formed/mg of protein/min at 30°.
FIG. 2. Effect of 5-thioglucose (TG) on adenylate cyclase activity in toluene-treated cells of Escherichia coli B. Toluene-treated cells were prepared and assayed for adenylate cyclase as in Fig. 1. 5-Thioglucose was present at 0.01, 0.02, 0.05, 0.10, and 1.00 mM. Complete inhibition was maintained for the duration of the assay for the three higher concentrations. Some of these points are omitted for clarity. Glucose (G) was present at 0.01 and 1.00 mM. ATP was at a concentration of 1 mM (specific activity, 50 cpm/pmol) and adenylate cyclase activity is expressed as picomoles of cyclic AMP formed/mg of protein.

Table I. Increasing the pH from 7.5 to 8.5 resulted in a 50% increase in activity. Fluoride at 10 mM, normally added to phosphotransferase assay, did not appear to be needed in the toluene-treated cells, in the presence of either 1 or 5 mM P-enolpyruvate; since fluoride inhibits adenylate cyclase (3), it was omitted from the phosphotransferase assay. The presence of phosphate (35 mM) produced a 50% increase in activity; phosphate appears to be essential for the measurement of adenylate cyclase activity (3). The table also shows that activity is much lower when cells are grown on nutrient broth compared to glucose, a change which is also reflected in adenylate cyclase activity (data not shown). ATP which is present in the adenylate cyclase assay was added to the phosphotransferase assay without effect (data not shown). In summary, the standard assay conditions were as follows: 25 mM Tris, pH 8.5, 10 mM MgCl₂, 1 mM dithiothreitol, 1 mM ATP, 15 mM phosphate, 5 mM P-enolpyruvate, and no fluoride. Protein was 0.84 μg/assay point for glucose-grown cells and 1.84 for nutrient broth-grown cells. The rate was calculated from four points taken at 4-min intervals.

\[ \text{Results} \]

Effect of Glucose, Glucose Analogues, and Other Sugars on Adenylate Cyclase Activity in Toluene-treated Cells of Escherichia coli B—Table II (second column) shows the inhibition of adenylate cyclase produced by a variety of sugars when E. coli B was grown on glucose as sole carbon source. α-Glucose, the reference compound, produced essentially complete inhibition. β-Glucose, in which the —OH on carbon 1 is inverted, was also inhibitory. Substitution by a methyl group in either position α or β of carbon 1, as in α- or β-methylglucoside, did not affect the inhibition of adenylate cyclase. Variation of substituents about carbon 2, as in mannose, where the —OH is inverted, or about carbon 3, galactose (—OH of carbon 4 inverted), 2-deoxygalactose, and 6-deoxyglucose, led to a loss of inhibitory activity. L-Glucose, the enantiomer of glucose, was inactive. A group of disaccharides (sucrose, maltose, lactose, and cellulbiose) and pentoses (fucose and xylose) had no activity. In contrast to glucose and mannose which were active, GDP-glucose and GDP-mannose (which are linked through position 1 of the sugar) were inactive. 5-Thioglucose, first reported by Feather and Whistler (11), is the nearest structural analogue of glucose and has the ring oxygen substituted by sulfur. Recent studies have shown that it interferes with sugar uptake mechanisms in rabbit kidney cortex slices (12). In the present studies 5-thioglucose caused marked inhibition of adenylate cyclase (Fig. 2); the inhibition was reversible, and temporary inhibition at threshold concentrations was similar to that seen with glucose.

Some of the compounds which were not active in inhibiting adenylate cyclase were tested to see whether they would interfere with the inhibitory effect of glucose. Galactose, sucrose, maltose, and L-glucose, at concentrations 10- to 100-fold in excess of the concentration of glucose used, did not interfere with the inhibitory effect of glucose.
Sugar specificity for inhibition of adenylate cyclase compared to phosphoenolpyruvate-dependent sugar phosphotransferase activity in toluene-treated cells of Escherichia coli B

Cells were grown on minimal salts medium supplemented with glucose, harvested, treated with toluene, and assayed for adenylate cyclase and phosphotransferase activities as described under "Experimental Procedure." For adenylate cyclase assays, all experiments were run kinetically and the standard concentration of sugar used in the assay was 1 mM. Glucose was run as a reference control in every experiment, and where possible at least two samples of a sugar were examined. The average specific activity of adenylate cyclase in 27 experiments conducted for the screening of the sugars was 212 ± 25 (S.E.M.) pmol of cyclic AMP formed/mg of protein/min, and the average activity seen in the presence of glucose for the same experiments, expressed as a percentage, was 3.08 ± 0.28 (S.E.M.).

For measurement of the effect of the indicated unlabeled sugars on the phosphorylation of α-methyl[14C]glucoside or 2-deoxy[14C]glucose, the assays were conducted in the presence and absence of P-enolpyruvate, and the P-enolpyruvate values were subtracted from experimental values. All experiments were conducted kinetically and the rate calculated from a line drawn through four points taken at 5-min intervals. The activities in the presence of unlabeled sugars are expressed as a percentage of the rate in the absence of added unlabeled sugar. The average specific activity for 12 experiments performed with 0.1 mM α-methyl[14C]glucoside was 101 ± 7 (S.E.M.) nmol of α-methylglucoside-6-P formed/mg of protein/min at 30°, and for 9 experiments using 2-deoxy[14C]glucose, the specific activity was 55 ± 4 (S.E.M.) nmol of 2-deoxyglucose-6-P formed/mg of protein/min at 30°. Unlabeled sugars were present at concentrations 10-fold in excess of the labeled substrate concentration used, i.e. 1 mM for α-methylglucoside and 10 mM for 2-deoxyglucose, except where indicated by * where 1 mM was used.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Adenylate cyclase activity</th>
<th>Phosphotransferase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-Methyl[14C]glucose (0.1 mM)</td>
<td>2-Deoxy[14C]glucose (1.0 mM)</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>α-Glucose</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>β-Glucose</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>α-Methylfructose</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>β-Methylfructose</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Mannose</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>6</td>
<td>84</td>
</tr>
<tr>
<td>2-Fluoro-2-deoxyglucose</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>β-Thioglucose</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>l-Glucose</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>3-O-Methylglucose</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Galactose</td>
<td>89*</td>
<td>98*</td>
</tr>
</tbody>
</table>

The following sugars were also found not to inhibit adenylate cyclase: 2-deoxygalactose, D-glucose, GDP glucose, GDP mannose, maltose, sucrose, lactose, cellobiose, dulcitol, xylose, and fructose.

* Maltose and lactose were also found not to inhibit the phosphorylation of either α-methylglucoside or 2-deoxyglucose.

Sugar Specificity for Phosphotransferase Activity in Toluene-treated Cells of E. coli B—A previous study relating the development of a transport system for a sugar to inhibition of adenylate cyclase suggested a relationship between these two systems (5). This relationship was further explored by determining whether the spectrum of sugars which inhibited adenylate cyclase corresponded to those sugars transported by the phosphotransferase system (Table II). These studies were carried out with cells grown under the same conditions, i.e. on glucose. The assays were performed on toluene-treated cells under conditions closely comparable to those used for adenylate cyclase assays, as described under "Experimental Procedure." Phosphotransferase activity in toluene-treated cells was measured by the formation of the phosphate derivative of the sugar and this reaction has been shown to be a valid measure of the transport process (13). There are two pathways for glucose transport which can be distinguished by their specificities toward certain sugars: α-methylglucoside is specific for the GPT pathway and 2-deoxyglucose is specific for the MPT pathway (7).

Using α-methyl[14C]glucoside as substrate (Table II, column 3), α- or β-glucose caused a large decrease in activity and β-methylglucoside was also active. Compounds with changes about carbon atom 2 (e.g. mannose, 2-deoxyglucose, and 2-fluoro-2-deoxyglucose) had little or no effect thereby confirming the specificity of the GPT pathway. Glucose derivatives with alterations about any of the other carbon atoms, as well as the disaccharides, did not inhibit α-methyl[14C]glucoside phosphorylation. Thus only α- and β-glucose, and α- and β-methylglucoside are transported via the GPT pathway.

With 2-deoxy[14C]glucose as substrate (Table II, column 4), the activity was markedly reduced in the presence of both α- and β-glucose and 5-thioglucose. Mannose at an equivalent and a 10-fold excess concentration produced 44 and 10% of control activity, respectively, α- and β-Methylglucoside were of interest in that a 10-fold excess of these compounds caused about a 50% fall in the activity, suggesting either that α-methylglucoside was not specific for the GPT pathway or that some 2-deoxyglucose may be phosphorylated by GPT in toluene-treated cells. The experiments with the phosphotransferase mutants lacking GPT or MPT support the latter interpretation (see later). Other analogues of glucose, and the disaccharides, did not interfere with 2-deoxy[14C]glucose phosphorylation. In summary, only α- and β-glucose, 5-thioglucose, mannose, and 2-deoxyglucose were transported via the MPT pathway. By combining the data from both pathways, a comparison of those sugars active in the phosphotransferase assay (Table II, columns 3 + 4) with the survey of those sugars which inhibit adenylate cyclase (Table II, column 2) indicates that the sugar specificity for both reactions is identical.

Effect of Mutations of Glucose Enzymes II of Phosphotransferase System on Adenylate Cyclase Activity and Sensitivity to Inhibition by Sugars—Recently a series of mutants have been described which are defective in one or both of the glucose
enzymes II of the phosphotransferase system (7). The parent and mutants were grown on nutrient broth and examined for phosphorylation of both α-methylglucoside and 2-deoxyglucose (Fig. 3A), and assayed for adenylate cyclase activity and susceptibility to inhibition by glucose, α-methylglucoside, and 2-deoxyglucose (Fig. 3B). The wild-type parent (ZSC17) has P-enolpyruvate-dependent phosphotransferase activity toward both substrates (Fig. 3A, first panel). In the GPT− MPT− double mutant (ZSC112) there was no detectable activity toward either substrate (Fig. 3A, second panel), and in the GPT− mutant (ZSC103) there was only activity toward 2-deoxyglucose (Fig. 3A, third panel). In this mutant no activity could be detected toward α-methylglucoside when assayed at substrate levels of 0.1, 1.0, and 10.0 mM. The phosphotransferase studies on the MPT− strain ZSC114 indicated that, while there was substantial phosphorylation of α-methylglucoside, there was some residual activity for phosphorylation of 2-deoxyglucose (Fig. 3A, fourth panel). This is compatible with the observation in Table II that unlabeled α-methylglucoside partially inhibited the phosphorylation of labeled 2-deoxyglucose, but that unlabeled 2-deoxyglucose did not inhibit the phosphorylation of labeled α-methylglucoside. This data is consistent with the notion that toluene-treated cells can phosphorylate 2-deoxyglucose through both GPT and MPT, but can phosphorylate α-methylglucoside only through GPT.

The adenylate cyclase data on these mutants is shown in Fig. 3B. In the wild-type parent (ZSC17), adenylate cyclase activity was inhibited by glucose, α-methylglucoside, and 2-deoxyglucose (Fig. 3B, first panel). In the GPT− MPT− double mutant (ZSC112), adenylate cyclase activity was detected, but none of the sugars inhibited activity (Fig. 3B, second panel). Thus the absence of both of the enzymes II does not affect the activity of the catalytic moiety, but their absence does lead to a complete loss of regulation by the sugars. Inhibition by α-methylglucoside is specifically associated with GPT as shown by the GPT− strain (ZSC103) in which inhibition by α-methylglucoside was lost in parallel with the loss of phosphotransferase activity for this sugar, whereas glucose and 2-deoxyglucose still caused complete inhibition of adenylate cyclase (Fig. 3B, third panel). The 2-deoxyglucose inhibition is there-

![Fig. 3](http://www.jbc.org/)
fore associated with the presence of MPT activity for that sugar.

The adenylate cyclase studies on the MPT- strain ZSC114 (Fig. 3B, fourth panel) indicated that glucose and α-methylglucoside gave nearly complete inhibition, consistent with the presence of GPT activity. It was also observed that 2-deoxyglucose (1 mM) produced approximately 40% inhibition. The partial inhibition by 2-deoxyglucose is compatible with the observation that the MPT- mutant showed a low level of 2-deoxyglucose phosphorylation (Fig. 3A, fourth panel). This suggests that, in toluene-treated cells, 2-deoxyglucose is a poor substrate for the GPT (see also Table II).

**DISCUSSION**

The purpose of this study was to explore the relationship between adenylate cyclase and the phosphotransferase system. Glucose both inhibits adenylate cyclase activity in toluene-treated cells (3) and is phosphorylated via the phosphotransferase system (6). Analogues of glucose as well as some disaccharides and pentoses were examined to see whether only those sugars which inhibited adenylate cyclase (Table II) were either substrates or inhibitors of the phosphotransferase system.

An examination of Table II reveals a precise correlation between the two systems. For adenylate cyclase activity, inhibition was observed with glucose and only those glucose analogues with changes about carbon atoms 1 or 2 (α-methylglucoside, mannose, and 2-deoxyglucose) or when the ring oxygen was replaced by sulfur, as in 5-thioglucose. All other sugars listed were inactive, suggesting that the substituents about carbon atoms 3 through 6 were critical for inhibition of adenylate cyclase. This pattern of activity was reflected in those sugars active in the phosphotransferase assay, although the situation is more complex because of the existence of two pathways (GPT and MPT) for glucose transport. Fortunately, however, the two pathways can be monitored independently by using α-methyl[14C]glucoside as a measure of the GPT pathway, and 2-deoxy[14C]glucose as a measure mainly of the MPT pathway. Only the glucose (α and β) and the methylglucosides (α and β) were active in the GPT pathway, while the glucose (α and β), 2-deoxyglucose, and mannose were active in the MPT pathway. Thus, irrespective of which channel is used to transport the sugar, only those sugars which were phosphorylated were active in inhibiting adenylate cyclase.

In the experiments designed to correlate those sugars active in the adenylate cyclase and phosphotransferase systems, the conditions for the two assays were made as similar as possible to reduce the chance that any differences would be due to varying conditions. Thus both assays were conducted on toluene-treated cells, grown under the same conditions, and the parameters for the phosphotransferase assay with respect to pH, magnesium, dithiothreitol, phosphate, ATP, fluoride, etc., were evaluated and made similar to the conditions used for adenylate cyclase assays (see “Experimental Procedure” and Table I). Both assays were run kinetically over similar time periods and all the data is based on rates calculated from the kinetic experiments. Kinetic assays enabled a clear distinction to be made between those compounds which were active in inhibiting adenylate cyclase and those which were inactive.

The temporary inhibition seen with threshold doses of glucose was also seen with 5-thioglucose, mannose, and 2-deoxyglucose (Figs. 1 and 2) suggesting that these sugars might act in a manner analogous to glucose. These studies also show that the inhibition seen with these compounds was not likely to be due to contamination by, for example, glucose. Paper chromatography revealed that the sugars were at least 98% pure and since the compounds were active at concentrations comparable to glucose it strongly suggests that the inhibition seen with these compounds was not due to contamination. The arguments against α-methylglucoside being hydrolyzed to glucose (not active in the GPT- mutant) were made earlier under “Experimental Procedure.” From these considerations it was concluded that α- and β-glucose, α- and β-methylglucoside, mannose, 2-deoxyglucose, and 5-thioglucose were bona fide inhibitors of adenylate cyclase activity. The possibility that “receptors” might be generated during the course of the assay seems unlikely since the kinetic assays showed that active compounds were inhibiting at the earliest time points. Furthermore, it has been shown that compounds, e.g. galactose, normally inactive on adenylate cyclase activity of glucose-grown cells will only inhibit the enzyme following growth on the compound and the development of the corresponding transport system (5).

The correlation of those sugars which were active in the two systems did not establish a functional relationship between phosphotransferase and adenylate cyclase. The studies with the enzyme II mutants, however, clearly indicate some interaction between the two systems. In the GPT- MPT- double mutant in which there was a complete loss of phosphotransferase activity through both pathways, there was a complete loss of regulation by the sugars. Adenylate cyclase activity was still present as in the wild type parent (in the wild type, the activity was inhibited by glucose, α-methylglucoside, and 2-deoxyglucose) indicating that the catalytic moiety did not require either of the enzyme II components, but that they are necessary for regulation by the sugars. The studies with the GPT- mutant supports this conclusion, and also shows that both of the enzymes II can regulate adenylate cyclase. In this mutant, loss of the GPT component led to a loss of transport via the GPT pathway and a loss of regulation by α-methylglucoside; at the same time, the mutant was normal with respect to 2-deoxyglucose transport and 2-deoxyglucose inhibition of adenylate cyclase, indicating that, in this strain, inhibition by this sugar proceeded via the MPT component.

Another question resolved by these studies concerns the lack of specificity of α-methylglucoside seen in Table II in which this sugar caused about a 60% decrease of 2-deoxy[14C]glucose phosphorylation. These data suggested either that α-methylglucoside is not specific for the GPT pathway, or that 2-deoxyglucose is not specific for the MPT pathway. The latter model that 2-deoxyglucose can be phosphorylated strongly via MPT, but weakly through GPT is supported by studies with the phosphotransferase mutants. While a GPT mutant showed substantial 2-deoxyglucose phosphorylation activity (Fig. 3A, third panel), an MPT mutant showed a low level of 2-deoxyglucose phosphorylation (Fig. 3A, fourth panel). Experiments on sugar inhibition of adenylate cyclase in the phosphotransferase mutants showed the same pattern as sugar phosphorylation. Thus, the low level of 2-deoxyglucose phosphorylation through the GPT is reflected in a low level of 2-deoxyglucose inhibition of adenylate cyclase.

Fig. 2B shows that, provided the respective enzyme II is present, both α-methylglucoside and 2-deoxyglucose produce essentially complete inhibition, as seen with glucose. This is also seen for E. coli B grown on glucose, where sugar inhibition
The wild type showed chemotaxis toward α-methylglucoside and another property of cellular behavior, namely chemotaxis. The same series of enzyme II mutants examined in this study have one or the other of the glucose enzyme II components (15). The glucose enzymes related to glucose was dependent upon the presence of a specific sugar. Chemotaxis toward certain sugars related to glucose was dependent upon the presence of one or the other of the glucose enzyme II components (15). The glucose 6-phosphate does not inhibit adenylate cyclase but which was not active in the phosphotransferase system would support the receptor hypothesis (3) does not rule out the possibility that some consequence of the mechanism by which glucose phosphorylation occurs is responsible for adenylate cyclase regulation by glucose.

The glucose enzyme II components appear to be related to another property of cellular behavior, namely chemotaxis. The same series of enzyme II mutants examined in this study have recently been used to show that chemotaxis toward certain sugars related to glucose was dependent upon the presence of one or the other of the glucose enzyme II components (15). The wild type showed chemotaxis toward α-methylglucoside and 2-deoxyglucose, the GPT− MPT− mutant showed no chemotaxis, and the GPT− mutant showed chemotaxis toward only mannose and glucose, but not α-methylglucoside. The data gave strong support to the concept that these components were critical for the chemotactic response. As in the present studies, it could not be established whether phosphorylation was an integral part of the chemotactic response. The enzymes II of the sugar phosphotransferase system appear to be involved in catabolite repression via adenylate cyclase, transport, and chemotaxis. Sugar interaction with the enzymes II could result in the enzyme II protein interacting uniquely with other proteins in the membrane to bring about the biological response. On the other hand, sugar interaction with the enzyme II could produce several intermediate stages, with a different or a common intermediate being responsible for the biological effect. In either case, the enzyme II proteins appear to behave as cell surface receptors, and this system could provide a useful model for the development of concepts of receptor mechanism.

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Involvement of the glucose enzymes II of the sugar phosphotransferase system in the regulation of adenylate cyclase by glucose in Escherichia coli.


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