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Phosphotransferase-System Enzymes as Chemoreceptors for Certain Sugars in *Escherichia coli* Chemotaxis

(bacterial behavior/sugar transport)

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ABSTRACT **For D-glucose and analogs there are two distinct phosphotransferase enzymes II with different specificities. Transport and chemotaxis were studied in *E. coli* mutants having only one or the other of these two enzymes. It was found that the transport specificity of a given enzyme II correlates with taxis specificity, and mutational loss of an enzyme II abolishes taxis toward only those sugars which it alone transports. Although enzyme I and the phosphate-carrier protein are required for full D-glucose taxis, it could not be determined if phosphorylation and transport are also required.**

Bacteria detect chemicals for chemotaxis by means of "chemoreceptors"—sensing devices that tell the flagella about changes in the extracellular concentration of a chemical without requiring extensive metabolism of that chemical (1). For certain chemoreceptors, the "recognition component"—the part that recognizes the chemical—has been identified; soluble binding proteins, which are released by osmotic shock (2) and bind D-galactose (3, 4), D-ribose (3, 5), or maltose (3), serve the corresponding chemoreceptors. For other sugars, binding proteins released by osmotic shock were not found (3). This report presents evidence that for some of those sugars the phosphotransferase system (6–8) serves as the chemoreceptor recognition component in *Escherichia coli*.

Phosphorylation is coupled to transport of certain sugars by the phosphotransferase system (6–8). Enzyme I catalyzes the phosphorylation of the HPr protein (a phosphate-carrier protein with phosphate linked to histidine) by phosphoenol pyruvate; then enzyme II catalyzes the phosphorylation of the sugar by the phosphorylated HPr protein (6–8). Enzyme II, really two proteins (8), is specific for the sugars. Thus, separate enzymes II are known for D-glucose (8–10), D-fructose (8, 11–13), D-mannitol (14), D-sorbitol (15), *N*-acetyl-D-glucosamine (16), and others.

For D-glucose, two distinct enzymes II with different specificities have been recognized in *E. coli* (8–10). One, here referred to as glucose enzyme II, phosphorylates D-glucose and methyl-α-D-glucoside. The other, here referred to as mannose enzyme II, phosphorylates D-glucose, D-mannose, D-glucosamine, and 2-deoxy-D-glucose‡.

Abbreviations: HPr protein, a phosphate-carrier protein with phosphate linked to histidine.

‡ These two activities were referred to as glucosephosphotransferase A (GPT-A) and glucosephosphotransferase B (GPT-B), respectively, in earlier work (9, 10).

For chemotaxis by *E. coli*, D-glucose is detected not only by a "glucose chemoreceptor" (1, 17) but also by the galactose chemoreceptor (1, 17), whose recognition component, the galactose binding protein (18), binds both D-galactose and D-glucose (18, 19). To study only the "glucose chemoreceptor," we used mutants lacking the galactose binding protein. It was then found that mutants having glucose enzyme II but not mannose enzyme II carry out taxis with the specificity of glucose enzyme II; mutants having mannose enzyme II but not glucose enzyme II carry out taxis with the specificity of mannose enzyme II; and mutants lacking both enzymes II fail to carry out D-glucose taxis. A preliminary account of some of this work has appeared (17).

MATERIALS AND METHODS

Chemicals. The sugars and analogs were obtained from various commercial sources, except that D-rhamnose was synthesized (20) from methyl 2,3,4-tribenzoyl-α-D-rhamnopyranoside, a gift of Drs. L. Anderson and H. A. Lardy. D-Mannose, 2-deoxy-D-glucose, D-xylose, D-lyxose, methyl-α-D-glucoside, and methyl-β-thio-D-galactoside were purified before use (1, 17). D-rhamnose (for which we thank Marc A. Muskavitch) was purified by paper chromatography in a *n*-butanol–ethanol–water (5:1:4) system (17) for 16 hr. D-Galactose (Sigma Chemical Co., St. Louis, "glucose-free") was used without purification. Purity of the following chemicals was studied by descending chromatography on Whatman no. 1 paper in a *tert*-amyl alcohol-*n*-propanol-water (4:1:1.5; v/v/v) system. Sugars were detected by spraying with alkaline silver nitrate. Up to 300 μg of sugar can be run without much streaking, and as little as 0.1 μg of D-glucose or D-mannose was readily detected. There was no detectable (<0.05%) contamination by D-glucose or D-mannose in *N*-acetyl-D-mannosamine, 6-deoxy-D-glucose, D-glucosamine, D-mannosamine, phenyl-α-D-glucoside, and phenyl-β-D-glucoside; <0.1% in methyl-β-D-glucoside; and approximately 0.25% in methyl-α-D-mannoside.

[1-^{14}C]D-Mannose, [1-^{14}C]2-deoxy-D-glucose, and [U-^{14}C]D-glucose, obtained from New England Nuclear Corp., and [U-^{14}C]methyl-α-D-glucopyranoside, obtained from Calatomic Division of Calbiochem, were purified by electrophoresis on Whatman 3 MM paper at pH 9.5 in 50 mM Na borate. [1-^{14}C]D-Glucosamine, obtained from New England Nuclear Corp., was purified by electrophoresis on 3 MM paper in 50 mM formic acid brought to pH 3.5 with methylamine.

TABLE 1. *Properties of bacterial strains*

Strain	Parent and source	Glucose enzyme II	Mannose enzyme II	Enzyme I	HPr	Gluco-kinase
AW579	ZSC71t (10)	+	+	+	+	−
AW582	ZSC103agl (10)	+	−	+	+	−
AW590	ZSC103 (9, 10)	−	+	+	+	−
AW581	ZSC103a (9, 10)	−	−	+	+	−
AE29	AW581	−	−	+	+	+
AW586	MO [C. F. Fox; (1)]	+	+	+	+	+
AW583	X17 [C. F. Fox; (1)]	+	+	−	+	+
AW589	1100 (22)	+	+	+	+	+
AW588	1101 (22)	+	+	+	−	+

MO is the parent of X17. 1100 is the parent of 1101. Other strains are closely related if not isogenic (9, 10). Strains at left have galactose chemoreceptor activity missing by virtue of possessing the AW543 mutation (3) for galactose binding protein.

Bacteria. A galactose chemoreceptor mutation—the galactose binding protein mutation of AW543 (3)—was introduced into various strains by F^+-mediated transfer of chromosomal genes. The donor, AW576, an AW543 derivative that is F^+, resistant to nalidixic acid, and requires L-histidine, L-leucine, L-methionine, and L-threonine for growth, was mated for 2 hr in Penassay medium (Difco Laboratories) with the nalidixic acid sensitive recipient. Then growth on glycerol minimal medium that contained nalidixic acid (0.2 mM) selected against the donor by omission of the required amino acids and for those recipients carrying nalidixic acid resistance, a marker that maps close to the galactose binding protein (E. N. Kort, R. W. Reader, and J. Adler, in preparation). Among the latter, those that failed to make a ring on a galactose swarm plate (21) but would do so on a tryptone swarm plate (21) were considered galactose chemoreceptor mutants. (Strains 1100 and 1101 are Hfr strains, rather than F^-; therefore to effect mating they were grown into late stationary phase.) The resulting nalidixic acid resistant, galactose taxis mutants are listed in Table 1, together with relevant properties.

Chemotaxis Assays. Strains AW582 and AW590 were grown according to a published procedure (23) in minimal medium (23) containing 30 mM D-glucose as sole energy source. D-Glucose was used to make certain that the enzyme II was actually present; the enzyme is required for growth on D-glucose by each of the two strains. (These strains are not sensitive to glucose repression of flagella synthesis.) Growth on D-glucose induces the glucose enzyme II level two- to three-fold (10) while growth on D-glucose rather than on glycerol actually lowers levels of mannose enzyme II apparently because of catabolite repression. Taxis responses by cells grown in glycerol (50 mM) were identical to those of D-glucose-grown cells except that AW590 gave more reproducible responses to 2-deoxy-D-glucose when grown in glycerol. Strain AW581, which is unable to grow on D-glucose, was grown in glycerol (50 mM) minimal medium. AW586, AW583, AW589, and AW588 were grown in D-galactose (30 mM) minimal medium.

After growth, the bacteria were washed three times by centrifugation at room temperature in 10 mM potassium phosphate and 0.1 mM ethylenediaminetetraacetate both at pH 7.0 ("chemotaxis medium"), and resuspended in that medium to 6×10^7 bacteria per ml. The number of bacteria accumulating in 1 hr at 30° inside a capillary tube containing the test chemical in chemotaxis medium was determined according to published procedure (23). Experimental points

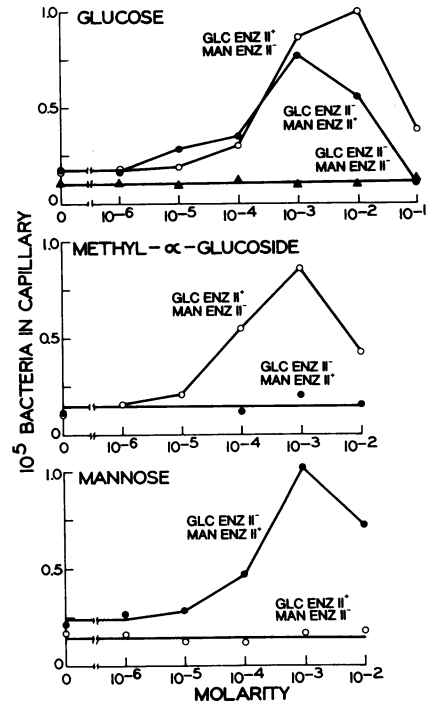

FIG. 1. Role of enzymes II in sugar taxis. Chemotaxis toward sugars by *E. coli* strain AW582 having glucose enzyme II but lacking mannose enzyme II (O——O), AW590 having mannose enzyme II but lacking glucose enzyme II (●——●), and AW581 having neither (▲——▲); all strains lack the galactose binding protein. AW581 does not respond to methyl-α-D-glucoside or D-mannose (data not shown). For studies with methyl-α-D-glucoside (*center panel*), 1 mM pyruvate was present in both capillary and bacterial suspensions. In each case, 10 mM L-aspartate was used as a positive control; the values were: *top panel*, 405,000, 448,000, and 270,000 bacteria for AW582, AW590, and AW581, respectively; *center panel*, 675,000 for AW582 and 427,000 for AW590; *bottom panel*, 467,000 for AW582 and 396,000 for AW590. (Plotted data are not normalized to the aspartate response.) Taxis toward substrates detected by only one of the two enzymes II (such as methyl-α-D-glucoside or D-mannose) was the same in the strain having only that one enzyme II as in the strain (AW579) having both (data not shown).

were in duplicate and averages are reported. Some typical results are presented in Fig. 1. "Threshold" is the lowest concentration that gives a detectable response; "peak response" is the number of bacteria accumulating in the capillary at the optimum concentration. Nonmetabolizable chemicals were tested with and without 1 mM pyruvate which was added as energy source to the bacterial suspension and the capillary medium. In the case of methyl-α-D-glucoside pyruvate gave a large increase in response (up to 5-fold); pyruvate gave a smaller stimulation for the other nonmetabolizable attractants and had no effect on taxis toward metabolizable attractants, such as D-glucose or D-mannose.

At 1/20 the usual AW590 concentration, a similar fraction of the added cells entered the capillary in response to D-glucose and D-mannose, indicating that the results are independent of the cell concentration used.

Transport Assays. Cells were grown to mid-logarithmic phase in K115 minimal medium (24) containing 10 mM D-glucose as carbon source, collected and washed in substrate-

TABLE 2. *Chemotaxis and transport in mutant having glucose enzyme II but lacking mannose enzyme II*

	Chemotaxis			Transport		
		Peak response				
	Threshold (mM)	(mM)	Bacteria in capillary	K_m (mM)	K_i (mM)	V_{max} [μmoles/ (g dry wt × min)]
Active						
D-Glucose	0.003	1–10	80,000	0.02		82
Methyl-α-D-glucoside	0.003	1	45,000	0.4	0.4	110
Methyl-β-D-glucoside	0.01	10	90,000		0.1	
D-Glucosamine	10	100	15,000	>1	>10	
Phenyl-β-D-glucoside	>100[d]				5	
Inactive[a]						

D-Mannose, D-galactose, D-mannosamine, *N*-acetyl-D-mannosamine, 2-deoxy-D-glucose, 6-deoxy-D-glucose, 6-deoxy-D-mannose (D-rhamnose), D-xylose[b], D-lyxose[c], methyl-α-D-mannoside, methyl-β-thio-D-galactoside, phenyl-α-D-glucoside.

The strain used, AW582, has glucose enzyme II but lacks mannose enzyme II and the galactose binding protein. For procedures of chemotaxis and transport experiments, see *Materials and Methods*. The error in thresholds is ± 3-fold. The thresholds for chemotaxis toward the metabolizable compounds (D-glucose and possibly methyl-β-D-glucoside) must be lower than indicated here since the bacteria rapidly destroy low concentration gradients by metabolism. After subtracting the background response (the number of bacteria in a capillary containing no attractant), the peak chemotaxic response was normalized to the response to 10 mM L-aspartate always included in each experiment (standard L-aspartate response = 400,000 bacteria). Results for *N*-acetyl-D-glucosamine are not reported because *E. coli* has a separate chemoreceptor for it (17). The highest concentration of the chemotactically active agents tried was 100 mM.

[a] No chemotactic response at highest concentrations tried (D-mannose and D-xylose 10 mM, all others 100 mM) and no transport (K_i > 10 mM).

[b] D-Xylose is equivalent to D-glucose without carbon 6.

[c] D-Lyxose is equivalent to D-mannose without carbon 6.

[d] There was less than the background response above 1 mM, indicating inhibition or negative chemotaxis (35).

> Indicates highest concentration tested in chemotaxis, or minimal value for transport K_m or K_i.

free K115 medium by filtration, suspended at from 200 to 500 μg dry wt per ml in substrate-free K115 medium, and aerated for approximately 30 min at 30°. Measurements of radioactive uptake were initiated by adding cell suspensions to tubes containing the desired amounts of radioactive sugars and inhibitors. Approximately twelve and 24 sec later, the radioactive samples were pipetted into a filtration apparatus in which a 25-mm membrane filter (0.45 μm pore size, Millipore type HA) was covered with 2 ml of ice-cold 0.4 M glucose solution. Suction was applied as the sample was added, and the filters were washed twice with 2 ml of cold 0.4 M glucose, then dried and counted in a liquid scintillation spectrometer. In most work the 12 sec values were used to compute initial rates of uptake, but for the metabolizable sugars very similar results were obtained when data from later time points were used. Pyruvate (1 mM) was added as energy source when uptake of a nonmetabolizable sugar was tested. Inhibitors were tested at at least two concentrations, with the higher concentration chosen to be close to 10 mM, in the presence of a radioactive substrate (concentration equal to its K_m for transport) of the system present. For strain AW-582 the radioactive substrate was D-glucose at 18 μM; for strain AW590, D-mannose at 26 μM was used. Values for K_i were estimated from plots of 1/initial rate versus inhibitor concentration. A calibration curve allowed determination of dry weight from measures of the turbidity of the cell suspensions at 610 nm.

TABLE 3. *Chemotaxis and transport in mutant having mannose enzyme II but lacking glucose enzyme II*

	Chemotaxis			Transport		
		Peak response				
	Threshold (mM)	(mM)	Bacteria in capillary	K_m (mM)	K_i (mM)	V_{max} [μmoles/ (g dry wt × min)]
Active						
D-Glucose	0.003	1	55,000	0.007		23
D-Mannose	0.003	1	80,000	0.03		43
2-Deoxy-D-glucose	0.01	1	50,000	0.2	0.2	51
D-Glucosamine	0.03	10–100	190,000	0.3	0.5	37
D-Mannosamine	1	100	65,000		5	
N-Acetyl-D-mannosamine	1	10	20,000		2	
D-Lyxose[c]	1	100	20,000		>30	
Inactive[a]						

D-Galactose, 6-deoxy-D-glucose, 6-deoxy-D-mannose (D-rhamnose), D-xylose[b], methyl-α-D-glucoside, methyl-α-D-mannoside, methyl-β-D-glucoside, methyl-β-thio-D-galactoside, phenyl-α-D-glucoside, phenyl-β-D-glucoside.

The strain used, AW590, has mannose enzyme II but lacks glucose enzyme II and the galactose binding protein. Otherwise as in *Table 2*.

[a] No chemotactic response at highest concentration tried (D-xylose 10 mM, all others 100 mM) and no transport (K_i > 20 mM). Footnotes [b] and [c] and *symbol* > as in *Table 2*.

RESULTS

Specificity for Transport by Enzyme II Compared to the Specificity for Taxis. Table 2 reports transport of, and chemotaxis toward, certain sugars in a mutant having glucose enzyme II but lacking mannose enzyme II and also the galactose binding protein in order to eliminate taxis toward D-glucose and analogs mediated by the galactose chemoreceptor (17). For both transport and chemotaxis there is good activity for D-glucose, methyl-α-D-glucoside, and methyl-β-D-glucoside, but not for the other sugars. See Fig. 1 for examples of complete data. Phenyl-β-D-glucoside is a weak substrate for transport but it is not an attractant (see however footnote[d], Table 2). This specificity is similar to that reported for the system that accumulates methyl-α-D-glucoside in *E. coli* and *Salmonella typhimurium* (25, 26).

Table 3 reports such experiments for a mutant having mannose enzyme II but lacking glucose enzyme II and the galactose binding protein. Four sugars, D-glucose, D-mannose, 2-deoxy-D-glucose, and D-glucosamine, are good attractants in this strain (see also Fig. 1), and all are good substrates for transport with K_m's of 0.3 mM or lower. Three others, D-mannosamine, *N*-acetyl-D-mannosamine, and D-lyxose, are weak attractants. Two of these (D-mannosamine and *N*-acetyl-D-mannosamine) are substrates for transport but with only modest affinity as reflected in K_i values above 1 mM. D-Lyxose was not detectably a substrate for transport by mannose enzyme II.

When glucose enzyme II is missing (Table 3), there is no detectable transport of, or taxis toward, methyl-α-D-glucoside (Fig. 1) and methyl-β-D-glucoside, the substrates it alone recognizes. When mannose enzyme II is missing (Table 2), there is no transport or taxis activity for D-mannose (Fig. 1) and the several other sugars it alone recognizes.

Chemotaxis for all the attractants of Tables 2 and 3 is absent in a mutant (AW581) lacking both the glucose and mannose enzymes II and also the galactose binding protein (Fig. 1; Table III of ref. 17; other data not shown), except for

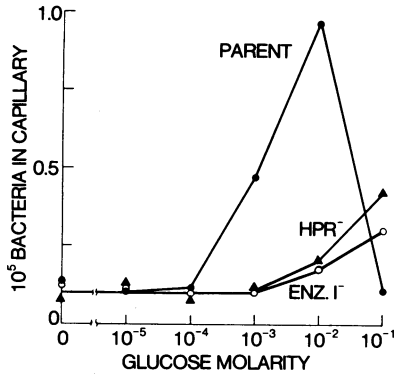

FIG. 2. Role of enzyme I and HPr in glucose taxis. Chemotaxis toward D-glucose by parental strain AW586 (●——●), mutant AW583 lacking enzyme I (○——○), and mutant AW588 lacking HPr protein (▲——▲). AW586 is the parent of AW583. The response to D-glucose by AW589, the parent of AW588, is not shown but is practically identical to that shown for AW586. All four strains lack the galactose binding protein. In each case 10 mM L-aspartate was used as a positive control; the values were: 386,000, 212,000, 439,000, and 446,000 for AW586, AW583, AW589, and AW588, respectively. (Plotted data are not normalized to the aspartate response.)

a small residual response to D-glucosamine and D-mannosamine, which might be due to interaction with the *N*-acetylglucosamine receptor (17). (This could also explain the small response to D-glucosamine reported in Table 2.) This mutant AW581 fails to show taxis toward D-glucose (Fig. 1), the only sugar which is recognized by both enzymes II. Transport of D-glucose, the only sugar tested for transport in this strain, is very defective (K_m about 1 mM, V_{max} about 3.5 μmoles/(g dry weight × min)).

In summary, the sugars that can be transported by a particular enzyme II are also attractants, and mutational loss of an enzyme II results in loss of taxis toward the sugars it alone transports.

Role of Enzyme I and HPr Protein in Chemotaxis. Mutants lacking enzyme I or the HPr protein, and also the galactose binding protein, are defective in D-glucose taxis and show a response only at high concentrations (Fig. 2). Chemotaxis toward L-aspartate (a positive control) was the same in mutants and parents. Mutants lacking enzyme I or the HPr protein but having normal galactose binding protein show normal taxis toward D-glucose and D-galactose, a result of the galactose chemoreceptor (1).

It is concluded that enzyme I and the HPr protein are required for the "glucose chemoreceptor" to function normally, but they are not required for chemotaxis mediated by other chemoreceptors, such as the aspartate or galactose chemoreceptors.

The residual taxis toward D-glucose in enzyme I and HPr mutants (Fig. 2) probably involves an interaction of sugars with the enzymes II, since strain AW581 which lacks both enzymes II shows no taxis response to D-glucose whatsoever (Fig. 1). Taxis toward D-glucose was not altered by introduction of a glucokinase mutation into the enzyme I mutant or the HPr mutant (results indistinguishable from those of Fig. 2). Thus, the residual taxis by the mutants, shown in Fig. 2, is not due to glucokinase.

Basis of the Requirement of the Phosphotransferase System. The requirement for the phosphotransferase system is not due simply to a need that sugars be transported, phosphorylated, or metabolized. Rather there is some specific interaction of the sugars with the phosphotransferase system that in an unknown way leads to chemotaxis. The evidence follows.

(*1*). An alternate route of D-glucose transport is a permease that can recognize D-galactose and D-glucose (27). (This is not the methyl-β-galactoside permease that utilizes the galactose binding protein.) Once inside the cell, the D-glucose can be phosphorylated by an alternate route—by glucokinase. These alternate routes, however, do not lead to chemotaxis since a strain (AE29) that has a functional glucokinase gene added to the strain (AW581) which lacks glucose enzyme II, mannose enzyme II, and galactose binding protein, showed taxis toward D-glucose that was at most twice the accumulation found in a capillary lacking attractant. The bacteria were grown in D-galactose (30 mM) for this chemotaxis assay. Subsequent growth in D-glucose (30 mM) occurred with a doubling time of 2–3 hr for several hours, showing the effectiveness of this route to D-glucose-6-phosphate.

(*2*). Transporting the *phosphorylated* sugars to the inside of the cell is not adequate for bringing about chemotaxis toward them. *E. coli* are attracted very poorly if at all to phosphorylated hexoses, the products of the phosphotransferase system, even in cells that have been induced (28) for the hexose phosphate transport system by growth for one generation in the presence of D-glucose-6-phosphate or 2-deoxy-D-glucose-6-phosphate, or in cells that are constitutive (28) for this system. For example, D-glucose-6-phosphate, D-mannose-6-phosphate, and 2-deoxy-D-glucose-6-phosphate attract extremely poorly or not at all (17). This fact, that phosphorylated sugars are transported but are not attractants, eliminates the idea that the phosphotransferase system is required simply to transport and phosphorylate the sugars so that they will be available to an *internal* chemoreceptor.

(*3*). Metabolism of the phosphotransferase product is not required for sugar taxis. Taxis toward D-glucose is normal (Fig. 9 of ref. 1) in a mutant, DF2000 (29), that is 97% blocked in the oxidation of D-glucose (1) owing to a lack of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase (29). This mutant consequently accumulates D-glucose-6-phosphate (28, 29). Furthermore, various of the phosphotransferase products are nonmetabolizable in wild-type bacteria, for example, the phosphorylated 2-deoxy-D-glucose or methyl-α-D-glucoside (ref. 28 and references cited there).

DISCUSSION

These results provide strong evidence that enzymes II of the phosphotransferase system serve as the recognition component for certain chemoreceptors; there is good correlation between the ability of a cell to respond chemotactically to a chemical and its ability to transport the substance by means of an enzyme II. This has been shown in detail for two different enzymes II for D-glucose, the "glucose enzyme II" and the "manose enzyme II." The "glucose chemoreceptor" thus can have two different recognition components; or better, one should now speak of two different receptors, which we shall call the "glucose chemoreceptor" and the "mannose chemoreceptor."

Likewise, preliminary results with mutants (11, 12, 14) lacking the enzyme II for D-fructose (8, 11–13) or D-mannitol (14) indicate that they fail to carry out the respective taxis (data not shown). A direct role for these enzymes II in chemo-

taxis is more difficult to demonstrate than for the glucose enzymes II because the enzymes II (11, 12, 14) and chemoreceptors (17) for D-fructose and D-mannitol are highly inducible, unlike those for D-glucose (10, 17). Since enzyme II mutants are impaired in the entry of the inducer, and lack of taxis could result from lack of induction of the chemoreceptors, special efforts to try to induce the chemoreceptors were required. Enzymes II are known also for D-sorbitol (15), *N*-acetyl-D-glucosamine (16), and aryl-β-glucosides (22), and these might be expected to serve the corresponding chemoreceptors (ref. 17 and unpublished data for the aryl-β-glucosides, arbutin, and salicin). Thus all sugars for which there is an enzyme II are attractants, suggesting that all enzymes II in *E. coli* can serve as receptors for chemotaxis; this is not the case for all the known binding proteins released by osmotic shock (17).

It is not clear just what function of the phosphotransferase system is crucial in allowing a sugar to elicit taxis upon interaction with an enzyme II. It is natural to assume that transport and phosphorylation of the sugar are required because of the markedly impaired taxis in enzyme I and HPr mutants. However, it is possible that mutational defects in enzyme I or HPr result in a configuration of the enzyme II with low affinity for substrate. According to this view, supported by several studies (6, 30, 31), enzymes II have good substrate affinity only when they have first bound the phosphorylated HPr or are actually phosphorylated. Poor taxis in these mutants is not due to absence of the enzymes II active on D-glucose and related sugars, since both *E. coli* and *Salmonella typhimurium* mutants defective in enzyme I or HPr have normal or elevated levels of these enzymes II (10, 32, 33).

We examined two sugars, 6-deoxy-D-glucose and 6-deoxy-D-mannose, which cannot be phosphorylated by the phosphotransferase system due to absence of a hydroxyl at carbon 6. However, neither showed significant affinity for the enzymes II as judged by lack of inhibition of transport of D-glucose or D-mannose, nor did they act either as attractants or as inhibitors for taxis toward D-glucose or D-mannose. Another approach to this problem is to examine enzyme II mutants to see if the taxis function can be separated from phosphorylation and transport.

It cannot be maintained that phosphorylation is essential for sugar taxis mediated by *all* chemoreceptors: The galactose chemoreceptor allows D-galactose taxis in mutants lacking galactokinase and hence unable to grow on D-galactose (1), and it allows D-glucose taxis in a strain (ZSC103a) lacking glucose and mannose enzymes II as well as glucokinase and hence unable to grow on D-glucose (data not shown).

It should be emphasized that the phosphotransferase system plays no function in chemoreception of sugars that can be recognized by other means; for example, D-galactose, recognized by the galactose binding protein, elicits taxis that is normal in phosphotransferase mutants lacking enzyme I or HPr, and even D-glucose taxis is good in these mutants as long as the galactose chemoreceptor is present (1).

This and previous studies (3, 4, 17) show that there is a very close relationship between chemotaxis and the transport process, although transport itself is not necessarily required, at least in certain cases (1, 3, 17, 34). It is clear, however, that not all transport systems are linked to chemotaxis since many chemicals that are transported are not attractants (17) or repellents (35).

The present work provides direct evidence that chemotaxis must involve the cytoplasmic membrane, since that is where the enzymes II reside (6–8).

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