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Chemotaxis of *Salmonella typhimurium* to Amino Acids and Some Sugars†

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Patterns of chemotaxis by *Salmonella typhimurium* strain LT-2 to L-amino acids and to several sugars were quantitated by the Adler capillary procedure. Competition experiments indicated that LT-2 possesses three predominant receptors, or interacting sets of receptors, for amino acids. These were termed the aspartate, serine, and alanine classes, respectively. Studies with strains carrying point and deletion mutations affecting components of the phosphoenolpyruvate: glycose phosphotransferase system (PTS) made unlikely a role in primary reception of D-glucose by the three soluble PTS components, namely HPr, enzyme I, and factor III. A *ptsG* mutant defective in membrane-bound enzyme IIB′ of the high-affinity glucose transport system was shown to exhibit normal chemotaxis providing pleiotropic effects of the mutation were eliminated by its genotypic combination with other *pts* mutations or, phenotypically, by addition of cyclic AMP and substrate. A correlation was demonstrated between chemotaxis to glucose and activity of the low-affinity glucose transport complex, membrane-bound enzymes IIB:IIA, and an enzyme IIB:IIA mutant was shown to have a preponderant defect in chemotaxis to glucose and mannose. Of four systems capable of galactose transport, only the β-methylgalactoside transport system was implicated in chemotaxis to galactose. Some properties of a mutant possibly defective in processing of signals for chemotaxis to sugars is described.

The Adler capillary assay (1, 2) has made it possible to measure semiquantitatively the chemotaxis responses of *Escherichia coli* (cf. 1, 4, 22), *Bacillus subtilis* (12, 31), and a *Streptococcus* (32) to a variety of compounds. Here we summarize some observations on the chemotaxis of *Salmonella typhimurium* to some amino acids and sugars. We diagnose three primary receptors or receptor sets for amino acid chemotaxis in *S. typhimurium*. In concordance with studies of *E. coli* (1, 15, 23, 24), our physiological experiments and studies on an *mgl* (β-methylgalactoside [MGL] permease) and a *galP melB* (galactose permease, methyl-β-D-thiogalactoside [TMG] permease) double mutant have specifically implicated components of the MGL transport system in chemotaxis of *S. typhimurium* to galactose. Studies utilizing a series of mutants (8–10, 21, 29) defective in components of the phosphoenolpyruvate:glycose phosphotransferase system (PTS) implicate the membrane-bound enzyme IIB:IIA system as one system important in chemotaxis to D-glucose, D-mannose, and D-fructose. Finally, we describe some properties of a mutant possibly defective in integration of receptor signals for chemotaxis to galactose and to various PTS sugars.

MATERIALS AND METHODS

Bacterial strains. *S. typhimurium* LT-2 strain SB3507 (*ara-9 trpB223*) served as prototype strain in all experiments. Genetic nomenclature (8, 28) includes usage of *ptsG* (enzyme IIB′) and *ptsM* (enzyme IIB:IIA), in accord with Bachmann et al. (6). Details are given elsewhere on the isolation and characterization of *pts* mutants (9, 10, 21). Strain SB1687 (*ptsM225*) is the strain originally designated *manA12* (29). Strain PP165, carrying a galactose permease mutation, *galP1903* (27), also carries an additional mutation, *melB118*, and lacks TMG permease activity (unpublished data; P. Postma, personal communication). An MGL transport-deficient strain, SB3549 (*trpB223 mgl-1*), was isolated as a galactose-negative colony on eosin-methylene blue plates containing 1% galactose after treatment of strain SB3507 with diethyl sulfate and then penicillin selection in the presence

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of galactose. Strain SB3777 (*trpB223 nal-241*) was isolated as a mutant resistant to 40 μg of nalidixic acid per ml in medium A containing lactate which, upon transfer, grew but failed to show a positive chemotaxis ring in tetrazolium semisolid plates (21) containing galactose as sole carbon source.

Media and CHE buffer. Medium A (11) with citrate omitted and supplemented with 20 μg of L-tryptophan per ml and 0.2% lactate was used for growth of bacteria unless otherwise specified. Nutrient agar (Difco) was used for enumeration of viable bacteria. During assays for chemotaxis, bacteria were suspended in CHE buffer, which contained per liter of distilled water, 1.74 g of K_2HPO_4 and 1.36 g of KH_2PO_4 (pH 7.0) to which ethylenediaminetetraacetic acid was added to a final concentration of 0.1 mM.

Chemotaxis assay. Samples of 10 ml from exponentially growing cultures in medium A at 35°C were harvested at an optical density at 590 nm (OD_{590}) of 0.5 (measured on a Gilford 2000 spectrophotometer) by gentle sedimentation in a tabletop Sorvall centrifuge at room temperature. The bacterial pellet was resuspended in residual liquid by gentle shaking, and then 5 ml CHE buffer was added. This process was repeated twice before resuspension in CHE buffer to a final OD_{590} of 0.1 (about 7×10^7 bacteria/ml). Exponentially growing bacteria exhibited optimal chemotaxis (Fig. 1), although microscopic examination revealed that bacteria were highly motile at all growth phases.

The capillary assay was a modification of that described by Adler (2). A glass plate (60.4 cm by 15.2 cm by 2 mm) was placed over a slide warmer, which was maintained at 30°C. At lower temperatures, chemotaxis was drastically curtailed. Each chamber was constructed of a U-shaped glass tube made by bending Kimax-51 capillary tubing and placement over this of a 22-mm^2 cover slip (insert at top, Fig. 2), forming a chamber with a capacity of about 0.5 ml. Capillaries containing test compounds were filled to about half-capacity by sealing one end, flaming, and inserting into a filled 0.25-dram (9 by 30 mm) vial before insertion into the filled test chamber. After 45 min was allowed for chemotaxis, test capillaries were removed and connected to the needles of sterile disposable plastic syringes by 5-mm lengths of plastic tubing, and the capillary contents were discharged into tubes containing 0.9 ml of 0.9% NaCl solution. Bacteria were assayed on nutrient agar plates after serial dilution. Each capillary test was run in duplicate, and the results were averaged.

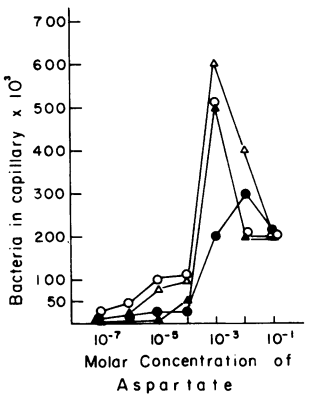

FIG. 1. *Chemotaxis of strain SB3507 to L-aspartate after bacteria were grown at 35°C in 0.2% lactate medium and harvested at different optical densities (OD_{590}): (▲) 0.29; (△) 0.50; (○) 1.0; (●) 2.4. Background accumulations in the absence of aspartate have been subtracted; they were 14,000, 16,700, 15,600, and 25,800, respectively.*

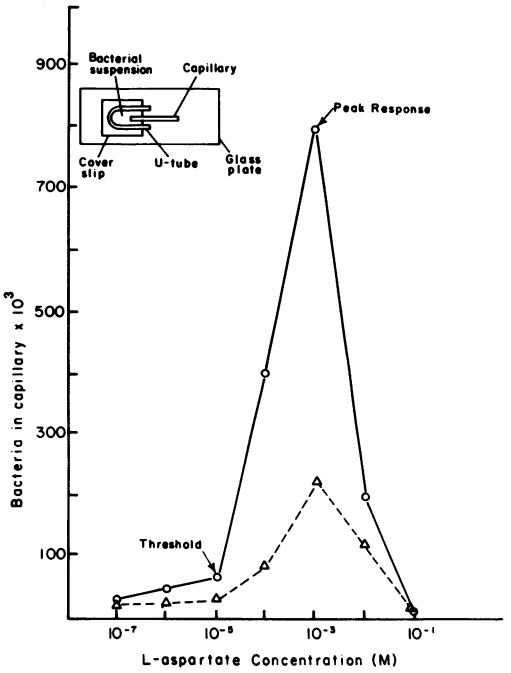

FIG. 2. *Concentration response curve for chemotaxis to L-aspartate by strain SB3507 previously grown on lactate A medium. Symbols: (○) chemotaxis to L-aspartate alone; (△) chemotaxis to L-aspartate when 0.1 mM L-methionine is present both in the capillary and in the chamber. The insert depicts the arrangement of the test system. Parameters of the response curve are indicated as the maximum or peak response at 10^{-3} M (the 800,000 bacteria attracted is termed the peak concentration) and the threshold concentration at 10^{-5} M, the lowest concentration of attractant that gave an accumulation of bacteria in the capillary significantly greater than that obtained in the absence of attractant (background).*

Enzyme IIB:IIA assays. Bacteria were grown and extracts were prepared and assayed as described previously (21).

Transport studies. Bacteria were grown in 1-liter flasks containing 500 ml of the appropriate induction medium specified in individual experiments (see Results). When the bacteria reached an OD_{590} of 0.5, they were harvested, and transport measurements were performed as previously described (21). A final concentration of 1 mM labeled sugar was present in

the stop-flow apparatus: D-[U-^{14}C]MGL (specific activity, 5×10^5 cpm/μmol) or [U-^{14}C]TMG (specific activity, 1.8×10^5 cpm/μmol).

RESULTS

Amino acid receptor classes. Figure 2 shows one representative experiment in which L-aspartate in test capillaries was used as attractant. The arrangement of the test chamber is depicted at the top of the diagram, and the threshold (10^{-5} M), peak concentration (10^{-3} M), and peak response (800,000 bacteria in the capillary) are defined in the legend. Table 1 lists these three parameters in analogous experiments with the L-isomers of amino acids. Methionine had a general inhibitory effect on chemotaxis to amino acids (Fig. 2 and Table 2) and to a lesser degree also inhibited chemotaxis to sugars (data not shown).

Table 2 summarizes competition experiments in which aspartate, alanine, serine, and asparagine were placed in test capillaries and 14 amino acids were present both in the capillary and in the chamber. The data are interpreted at the right-hand side of Table 2 and in the Venn diagram (Fig. 3). There appeared to be three predominant receptor classes for chemotaxis to free L-amino acids. This interpretation is based on the assumption that most observed inhibitions are due to direct competition at the level of the primary receptors or at some early stage in processing (cf. 16, 30). However, some additional inhibitions, perhaps reflecting other stages in processing, also appeared to take place. For example, serine inhibited chemotaxis to alanine and aspartate, alanine inhibited chemotaxis to aspartate and serine, valine inhibited chemotaxis to alanine and serine, and leucine inhibited chemotaxis to aspartate and alanine.

TABLE 1. *Chemotaxis toward L-amino acids by strain SB3507 previously grown in minimal lactate medium. Headings are defined in the legend to Fig. 2*

Amino acid	Threshold (M)	Peak (M)	Peak response
Alanine	2×10^{-6}	10^{-1}	600,000
Arginine	$\sim 10^{-3}$	10^{-2}	20,000
Asparagine	10^{-5}	10^{-1}	1,000,000
Aspartate	2×10^{-6}	10^{-3}	1,600,000
Cysteine	6×10^{-6}	10^{-3}	160,000
Glutamate	4×10^{-6}	10^{-2}	400,000
Glutamine	7×10^{-5}	10^{-1}	1,200,000
Glycine	$\sim 10^{-2}$	10^{-1}	250,000
Histidine	$\sim 10^{-2}$	10^{-1}	22,000
Isoleucine	3×10^{-4}	10^{-3}	50,000
Leucine	10^{-3}	10^{-1}	10,000
Lysine	$\sim 10^{-2}$	10^{-1}	35,000
Methionine	10^{-5}	10^{-2}	100,000
Phenylalanine	$\sim 10^{-3}$	10^{-2}	100,000
Proline	$\sim 10^{-4}$	10^{-2}	200,000
Serine	2×10^{-6}	10^{-2}	500,000
Threonine	$\sim 10^{-3}$	10^{-1}	80,000
Tryptophan		No response	
Tyrosine	$\sim 10^{-3}$	10^{-2}	100,000
Valine		No response	

S. typhimurium exhibited chemotaxis to fumarate and L-malate, and these two dicarboxylic acids did not serve as significant competitors for chemotaxis to aspartate even though present in 100-fold molar excess (Table 3).

Galactose receptor. Although *S. typhimurium* has four transport systems for galactose (26, 27), the following observations demonstrate that the MGL permease system furnishes the predominant receptor for galactose chemotaxis. In confirmation of data of Postma (27), fucose induced ability to transport MGL (Fig. 4A), and melibiose induced ability to transport TMG (Fig. 5B). Besides the MGL and TMG permeases, galactose can enter by the galactose-specific system (27) and the PTS (26). Strain PP165, doubly mutant in the gene for the galactose-specific permease (27) and in gene *melB*, affecting transport of TMG, was a mutant that grew slowly on galactose and failed to grow on melibiose or to ferment these two sugars on eosin-methylene blue plates. However, strain PP165 still exhibited fucose-induced chemotaxis to galactose, entirely comparable to the parent strain (Table 4). In contrast, strain SB3549, defective in the MGL permease system, simultaneously lost ability to be induced by fucose for MGL uptake (Fig. 4C), lost ability for chemotaxis to galactose (Fig. 5), but retained inducibility of TMG uptake (Fig. 4D). Since some residual MGL uptake was observed in melibiose-grown bacteria (Fig. 4B), we concluded that the MGL system was present at a low level in bacteria not exposed to exogenous fucose (cf. 29). The MGL cannot have entered MGL$^+$ bacteria by the PTS system, since no MGL uptake was observed in the MGL$^-$ mutant grown on fucose medium (Fig. 4C). The PTS system could be responsible, however, for the low residual ability of the MGL$^-$ mutant in galactose chemotaxis. While the MGL system appeared predominantly responsible for reception in chemotaxis to galactose, MGL itself was a much less effective attractant than was galactose (Fig. 5).

Receptors for PTS sugars. Concentration-response curves for chemotaxis by a PTS$^+$ strain, SB3507, are shown for four PTS sugars (Fig. 6). Strain SB1687, defective in a component of the enzyme IIB:IIA system (29), showed greatly lowered chemotaxis to glucose and failed to exhibit chemotaxis to mannose (Fig. 6A and B), whereas chemotaxis to aspartate was normal (data not shown). The residual chemotaxis to glucose could be due to an ability of glucose to bind to the galactose receptor (34), eliciting glu-

TABLE 2. *Competition for chemotaxis to amino acids by strain SB3507 previously grown in minimal lactate medium*[a]

Competitor	Concn[b] (M)	Competitor response ($\times 10^4$)[c]	Percent inhibition of chemotaxis to amino acids used as attractants				Receptor classes[d]
			L-Aspartate, 10^{-3} M	L-Alanine, 10^{-1} M	L-Serine, 10^{-2} M	L-Asparagine, 10^{-2} M	
None			0	0	0	0	
L-Aspartate	10^{-3}	160	100	0	0	80	Aspartate
L-Asparagine	10^{-2}	100	80	0	0	100	
L-Glutamine	10^{-1}	120	20	0	0	100	
L-Phenylalanine	10^{-2}	10	24	0	0	25	
L-Alanine	10^{-1}	60	75	100	58	100	Alanine
L-Glycine	10^{-2}	20	0	70	0	25	
L-Lysine	10^{-2}	3	0	30	0	13	
L-Proline	10^{-2}	20	0	93	10	0	
L-Serine	10^{-2}	50	43	100	100	73	Serine
L-Threonine	10^{-1}	8	0	0	22	35	
L-Tryptophan	10^{-2}	None	0	20	27	0	Alanine and serine
L-Valine	10^{-2}	None	0	62	50	17	
L-Leucine	10^{-1}	1	24	70	0	80	Aspartate and alanine
L-Methionine	10^{-2}	10	60	66	52	69	General[e]

[a] Results are presented as percent inhibition of chemotaxis in the presence of inhibitor compared with chemotaxis at the same molar concentration in the absence of inhibitor.

[b] Concentration of competitor used to make a uniform concentration in capillaries and chambers.

[c] Competitor response indicates the number of cells attracted by each competitor when used alone as an attractant at the given concentration.

[d] Receptors are defined by the amino acid with the lowest threshold value specific for the given receptor class.

[e] Methionine inhibits chemotaxis; see text and Fig. 2.

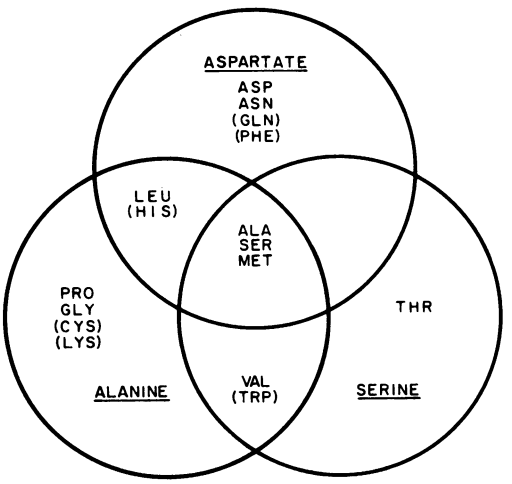

FIG. 3. *Venn diagram of possible amino acid receptor classes defined by competition experiments (Table 2).*

TABLE 3. *Failure of two dicarboxylic acids to compete for chemotaxis to L-aspartate by strain SB3507 previously grown on minimal lactate medium*

Competitor and concn	Chemotaxis to compound used as competitor[a]	Effect of competitor on chemotaxis to L-aspartate at[a]:	
		10^{-3} M	10^{-4} M
Expt I			
None		549	265
L-Malate			
10^{-2} M	148	569	369
10^{-3} M	30	606	330
Expt II			
None		528	296
Fumarate			
10^{-2} M	15	526	387
10^{-3} M	7	636	359

[a] Results are expressed as bacteria in capillary above background $\times 10^3$.

cose chemotaxis (3), and to the factor III:enzyme IIB' PTS system. The observation that methyl-α-D-glucopyranoside (αMG) was an attractant (Fig. 6D) illustrates the point that extensive metabolism is not necessary for chemoattraction, for αMG is merely accumulated as the phosphate ester (28).

Bacteria grown in glucose or mannose exhibited increased chemotaxis to these two sugars, and chemotaxis to fructose was decreased (Table 5). In contrast, prior growth on

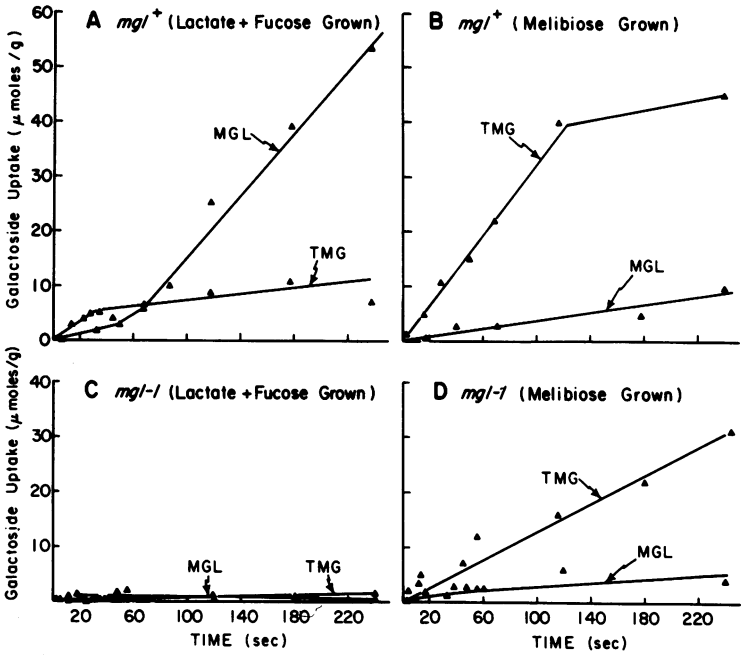

FIG. 4. *Transport of MGL and TMG in strain SB3507 (mgl+) (A and B) and in strain SB3549 (mgl-1) (C and D). Prior growth in 0.2% lactate medium A containing 1 mM D-fucose was used to induce uptake of MGL (A and C), and growth in 0.2% melibiose medium A was used to induce TMG uptake (B and D). Symbols: (▲) MGL uptake; (△) TMG uptake. Sugar concentration was 1 mM.*

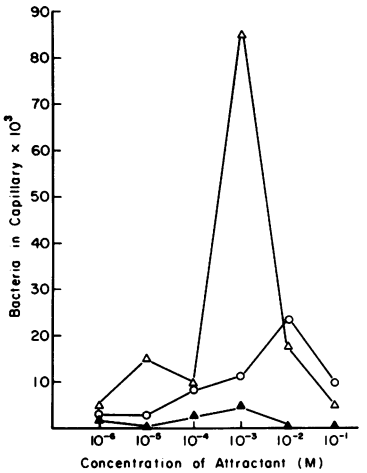

FIG. 5. *Chemotaxis to galactose by strains SB3507 (mgl+, △) and SB3549 (mgl-1, ▲) and chemotaxis to MGL by strain SB3507 (mgl+, ○). Bacteria were pregrown in 0.2% lactate medium containing 1 mM D-fucose and assayed as described in Materials and Methods.*

fructose as sole carbon source enhanced chemotaxis to fructose. Bacteria grown on each of the five carbon sources responded equally well in chemotaxis tests to aspartate (last column, Table 5), and direct microscopic examination of the cultures did not reveal detectable differences in motility. The data indicated that there were at least two major modes of chemoreception for PTS sugars, at least one mode for glucose and mannose (cf. 3), and a supplementary mode for fructose (4, 8, 12, 29). The relative chemotaxis responses correlated well with data on relative membrane-bound enzyme IIB:IIA levels in *S. typhimurium* grown on the various carbon sources (28; C. Cordaro, personal communication).

TABLE 4. *Comparable chemotaxis to D-galactose by strains SB3507 (trpB223) and PP165 (trpB223 galP1903 melB118) previously grown on medium containing 0.2% lactate plus 1 mM D-fucose*

Strain	Chemotaxis[a]					
	Galactose concn (M)				Aspartate concn (M)	
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}
SB3507	30	48	78	15	12	600
PP165	26	38	75	40	19	680

[a] Results expressed as in Table 3.

In examination of further mutants deficient in various components of the PTS, we detected variations in motility between strains and, consequently, in bacterial yields in chemotaxis tests. Yokota and Gots (33) showed that flagellum

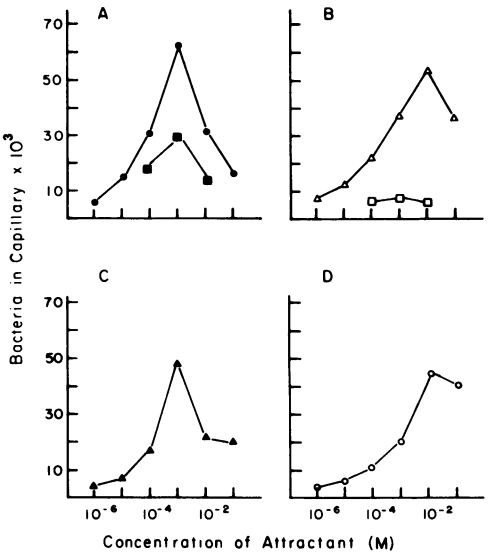

FIG. 6. *Concentration response curves of lactate-grown strains SB3507 (pts⁺) and SB1687 (ptsM225) for chemotaxis to: (A) D-glucose (●, SB3507; ■, SB1687); (B) D-mannose (△, SB3507; □, SB1687); (C) D-fructose (▲, SB3507); and (D) αMG (○, SB3507).*

TABLE 5. *Relative chemotaxis to D-glucose, D-mannose, and D-fructose of pts⁺ S. typhimurium previously grown on different carbon sources*

Growth medium[a]	Relative chemotaxis with given attractant[b]			
	D-Glucose	D-Mannose	D-Fructose	L-Aspartate
Lactate[c]	1.0	1.0	1.0	1.0
D-Glucose	1.5	1.5	0.23	1.0
D-Mannose	2.3	1.8	0.5	1.0
D-Fructose	1.8	1.5	2.0	1.0
D-Galactose	0.7	0.6	ND[d]	1.0

[a] Growth medium contained 0.2% of the respective carbon source.

[b] Attractants were tested at peak concentrations: 10^{-3} M for glucose and fructose, 10^{-2} M for mannose (Fig. 6), and 10^{-3} M for aspartate (Fig. 2).

[c] Values obtained using lactate-grown bacteria were set at unity: for glucose and mannose, 40,000 bacteria per capillary; for fructose, 30,000 bacteria per capillary; and for aspartate, 750,000 bacteria per capillary.

[d] ND, Not determined.

formation in *S. typhimurium* requires cAMP, and we have observed that "tight" enzyme I mutants exhibit decreased motility, as also observed in *E. coli* (3). This decrease is possibly due to an influence of enzyme I deficiency on adenyl cyclase activity (25). Since the variations often appeared to involve effects on chemotaxis to all agents tested, we decided to express quantitative aspects of chemotaxis in terms of a chemotaxis index (CI), utilizing chemotaxis by the reasonably constitutive and highly active aspartate system as a standard. Thus, the CI was calculated as:

$$CI = \frac{\text{sugar response of mutant}}{\text{sugar response of wild type}} \times \frac{\text{L-aspartate response of wild type}}{\text{L-aspartate response of mutant}}$$

Table 6 presents the CI, actual cell yields, and peak concentrations for chemotaxis to glucose of lactate-grown parental *pts⁺* strain SB3507 and for twelve *pts* mutant strains, including five carrying deletion mutations (9, 10, 21). We conclude that soluble PTS components HPr (structural gene *ptsH*), enzyme I (structural gene *ptsI*), and factor III (structural or regulatory gene *crr*) are not essential parts of the primary chemotaxis receptors for glucose; in fact, the CI in these strains often was enhanced over that observed for the *pts⁺* parent (see Discussion). We also conclude that phosphorylation and transport by the PTS are not essential for glucose-stimulated taxis since, for example, deletion mutant strains SB2950, SB3686, and SB3749 (Table 4) lack all three soluble components of the PTS (9, 10). On the other hand, the CI for glucose was decreased in the *ptsG* mutant (strain SB3730) defective in membrane-bound enzyme IIB′ (23). However, this reduction in the CI was counterbalanced when the *ptsG* mutation was combined with *ptsI* or *ptsH* mutations (strains SB3770 and SB3774 in last two lines, Table 6). These data indicated that the *ptsG* mutation exerted its effect on chemotaxis by indirect means. Previously we showed that these two double mu-

TABLE 6. *CI of various lactate-grown PTS mutants to D-glucose*

Strain	PTS defect	CI[a]	Peak concn (M)
SB3507	*pts⁺*	1.0 (80,000)[b]	10^{-3}
SB3737	*ptsH196*	1.24 (89,000)	10^{-3}
SB3769	*ptsH197*	2.70 (59,000)	10^{-3}
SB3798	*ptsH198*	1.50 (71,000)	10^{-3}
SB3802	*ptsIΔ194*	0.85 (42,000)	10^{-4}
SB3680	*ptsIΔ163*	1.60 (20,000)	10^{-3}
SB3799	*ptsI199*	1.12 (70,000)	10^{-3}
SB2950	*cysK ptsPHI crrΔ49*	1.30 (40,000)	10^{-2}
SB3686	*ptsPHI crrΔ166*	5.80 (7,000)	10^{-1}
SB3749	*ptsHI crrΔ180*	3.40 (40,000)	10^{-3}
SB3730[c]	*ptsG217*	0.40 (27,000)	10^{-3}
SB3770[c]	*ptsG217 ptsI184*	1.30 (31,000)	10^{-3}
SB3774[c]	*ptsG217 ptsH196*	1.90 (84,000)	10^{-3}

[a] Defined in the text.

[b] The peak response (maximum number of bacteria accumulated per capillary in tests from 10^{-1} through 10^{-5} M glucose) is included in parentheses.

[c] Strains carrying *ptsG217* also contain an independent and separable marker, *mal-78*, not involved in chemotaxis to PTS sugars.

tants had the phenotypes (Table 5 in reference 21) and combination of enzyme deficiencies (Table 6 in reference 21) expected of double mutants and thus retained the *ptsG* mutation. Our conclusion is that *ptsG* mutations (enzyme IIB' deficiencies) exert pleiotropic effects and that the membrane-bound enzyme IIB' component of the high-affinity glucose transport system is not a critical primary chemoreceptor for taxis to glucose.

ptsG mutations also affected chemotaxis to mannose and fructose (Table 7). Assays for membrane-bound enzyme IIB:IIA activity (Table 8) demonstrated that mutation in the *ptsG* gene decreased function of the IIB:IIA system and that the IIB:IIA defect was partially rectified in double mutants also carrying *ptsH* or *ptsI* mutations (Table 8), just as chemotaxis was restored (last three lines, Table 6).

Relative chemotaxis to glucose (CI) also was restored in mutant *ptsG217* pregrown in medium containing cAMP and glucose (Table 9). The collective data indicate that bacteria deficient in enzyme IIB' activity also have lowered enzyme IIB:IIA activity and lowered chemotaxis to PTS sugars. These pleiotropic effects of the enzyme IIB' deficiency can be reversed genotypically in *ptsH* or *ptsI* double mutants or merely by growth in substrate plus cAMP.

Possible sugar taxis mutant. Strain SB3777 was isolated as a nalidixic acid-resistant mutant that was unable to exhibit chemotaxis toward galactose in semisolid agar (Materials and Methods). Strain SB3777 was phenotypically wild type with respect to utilization and fermentation of galactose, glucose, mannose, mannitol, fructose, and maltose. However, the strain was defective in chemotaxis to galactose and also to varying degrees defective in chemotaxis to various PTS sugars, while retaining normal chemotaxis to aspartate (Table 10). Thus, strain SB3777 may be competent in reception of signals for chemotaxis but may be unable to process and transmit those signals involving reception of sugars (cf. M. Fahnestock, cited in reference 30).

TABLE 7. *CI of lactate-grown pts⁺ and ptsG bacteria for 10^{-3} M glucose, mannose, and fructose*

Strain	CI[a]		
	D-Glucose	D-Mannose	D-Fructose
SB3507 (*pts⁺*)[b]	1.0	1.0	1.0
SB3730 (*ptsG217*)	0.40	0.50	0.60

[a] Defined in the text.

[b] Values obtained with strain SB3507 are set at unity. This corresponds to 72,000 bacteria per capillary for glucose, 55,000 for mannose, and 32,000 for fructose.

DISCUSSION

Data obtained in chemotaxis and chemotaxis competition experiments (Fig. 2; Tables 1 and 2) indicate that *S. typhimurium* possesses three primary receptors or sets of receptors for free L-amino acids. These are termed the aspartate, serine, and alanine classes, respectively (Fig. 3). In *S. typhimurium*, the most highly attractive amino acids are those that can serve as sole carbon and nitrogen sources or as sole carbon sources (7, 14; we find that aspartate can serve either as sole nitrogen or as sole carbon source). Further studies, particularly with mutants lacking specific receptors, are needed to sort out true competition for primary receptors from competition for components of the signalling system, as noted elsewhere (16, 22, 24, 30).

We do not find significant competition by fumarate or by L-malate for chemotaxis to aspartate (Table 3). The results with L-malate are in contrast to a strong competition reported where a 1,000-fold molar excess of competitor was used (5). Comparisons of the strains and methods used (for example, aspartate concentrations) and examination of mutants (cf. 20) will be required to unravel this apparent discrepancy. *S. typhimurium* exhibits chemotaxis to dicarboxylic acids (Table 3), and recently a fu-

TABLE 8. *Relative levels of enzyme IIB:IIA for α-MG, glucose, mannose, and fructose in extracts of lactate-grown bacteria*[a]

Strain	PTS genotype	αMG	D-Glucose	D-Mannose	D-Fructose
SB3507[b]	*pts⁺*	1.0	1.0	1.0	1.0
SB3730	*ptsG217*	0.15	0.36	0.29	0.13
SB3774	*ptsG217 ptsH194*	0.21	0.64	0.54	0.18
SB3770	*ptsG217 ptsI184*	0.22	0.66	0.60	0.34

[a] Substrates were: [^{14}C]αMG (specific activity, 3.1 × 10^5 cpm/μmol), D-[^{14}C]glucose (specific activity, 3.1 × 10^5 cpm/μmol), D-[^{14}C]mannose (specific activity, 1.5 × 10^5 cpm/μmol), D-[^{14}C]fructose (specific activity, 1.5 × 10^5 cpm/μmol), and D-[^{14}C]fructose (specific activity, 1.3 × 10^5 cpm/μmol). [^{14}C]αMG was obtained from Amersham/Searle; the other isotopes were from New England Nuclear Corp.

[b] Specific activities for strain SB3507 (micromoles of sugar phosphate formed in 30 min at 37°C per milligram of protein) were: αMG, 1.68; glucose, 2.1; mannose, 4.2; and fructose, 1.82.

TABLE 9. *Effects of cAMP upon levels of enzyme IIB:IIA activity and the glucose CI in pts⁺ and ptsG strains*

Strain	Additions[a]	Relative enzyme IIB:IIA activities[b]		Glucose CI[c]
		Lactate-grown	Glucose-grown	
SB3507 (*pts⁺*)	−cAMP	1.0	1.0	1.0
	+cAMP	1.11	2.68	1.78
SB3730 (*ptsG217*)	−cAMP	0.34	0.24	0.42
	+cAMP	0.36	0.65	1.69

[a] 5 mM cAMP (Sigma) added to growth medium.

[b] D-[^{14}C]glucose (specific activity, 3.1×10^5 cpm/μmol) was used in the enzyme IIB:IIA assays. For strain SB3507, the specific activity (micromoles of sugar phosphate formed in 30 min at 37°C per milligram of protein) for bacteria grown in lactate without cAMP addition was 2.89; for bacteria grown in glucose without cAMP addition, it was 2.32.

[c] Defined in the text. The value of 1.0 corresponds to a peak response for strain SB3507 of 81,000 for D-glucose chemotaxis and 670,000 for L-aspartate chemotaxis.

TABLE 10. *Peak responses of strain SB3777 relative to its parent, strain SB3507, in chemotaxis to seven attractants*

Attractant	CI[a]
L-Aspartate	0.94 (680,000)[b]
Galactose	<0.05 (58,000)
MGL	<0.1 (23,000)
D-Glucose	0.54 (56,000)
αMG	0.41 (43,000)
D-Mannitol	0.09 (56,000)
D-Fructose	0.13 (43,000)

[a] Number of bacteria attracted relative to chemotaxis by strain SB3507 at the peak response for each strain on each compound.

[b] Average number of bacteria accumulated per capillary by strain SB3507 is shown in parentheses.

marate receptor has been identified with an enzyme(s) involved in electron transport (B. L. Taylor, J. Miller, H. M. Warrick, and D. E. Koshland, Jr., cited in reference 16).

Galactose can enter *S. typhimurium* through four permease systems, and fucose induces two of these, the galactose-specific and the MGL permeases (26, 27). Our data (Fig. 4 and 5; Table 4) directly implicate the MGL permease in chemotaxis to galactose, as assumed for *Salmonella* (30, 34) and previously demonstrated for *E. coli* (1, 15, 23, 24). The MGL system also is one of three permease systems involved in chemotaxis to D-glucose in *E. coli* (3).

Adler and Epstein (3) showed that a membrane-bound enzyme IIB:IIA component of the PTS is one of several components involved in reception in chemotaxis to glucose. Our data (Tables 5–9) are fully in accord with this conclusion and make improbable an important role of the three soluble PTS components, HPr, enzyme I, and factor III (Table 6). Mutations affecting enzyme IIB′ activity have little effect on chemotaxis to glucose provided pleiotropic effects are circumvented (Tables 8 and 9). Our data do not rule out the possibility that enzyme IIB′ serves as a secondary receptor for glucose and as the primary receptor for αMG, as it does in *E. coli* (3, 8). Enzyme IIB:IIA activities are correlated with chemotaxis to mannose (Fig. 6B; Table 5) (cf. 3) and to fructose (Table 5) (cf. 8). Sugar-specific enzymes IIA have been implicated in chemotaxis to *E. coli* to D-mannitol, D-glucitol (and D-sorbitol), and galactitol (18, 19).

Mutants defective in the soluble components of the PTS often show an elevated chemotaxis towards D-glucose (Table 6). We envision that the enzyme IIB component, which may comprise 10% of the protein in the inner cell membrane (17), interacts with phospho-HPr and, in turn, influences interaction of enzyme IIB with enzyme IIA. In this view, enzyme IIA molecules on the outer membrane surface not active in transport (no phospho-HPr present) may gain enhanced accessibility or affinity for exogenous sugars and, thence, show enhanced chemotaxis. Similar interactions between phospho-factor III and enzyme IIB′ could influence the relative role of enzyme IIB′ in chemotaxis to glucose. The IIB:IIA system is accessible to the outer surface of the membrane, since D. A. Beneski (Ph.D. thesis, The Johns Hopkins University, Baltimore, 1977) has shown, using membrane vesicles, that phosphorylation of sugar can take place on the outer surface in the presence of external HPr, enzyme I, and phosphoenolpyruvate.

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