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Adenylate Cyclase Is Required for Chemotaxis to Phosphotransferase System Sugars by *Escherichia coli*

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We report that in *Escherichia coli*, chemotaxis to sugars transported by the phosphotransferase system is mediated by adenylate cyclase, the nucleotide cyclase linked to the phosphotransferase system. We conclude that adenylate cyclase is required in this chemotaxis pathway because mutations in the cyclase gene (*cya*) eliminate or impair the response to phosphotransferase system sugars, even though other components of the phosphotransferase system known to be required for the detection of these sugars are relatively unaffected by such mutations. Moreover, merely supplying the mutant bacteria with the products of this enzyme, cyclic AMP and cyclic GMP, does not restore the chemotactic response. Because a residual chemotactic response is observed in certain strains with residual cyclic GMP synthesis but no cyclic AMP synthesis, it appears that the guanylate cyclase activity rather than the adenylate cyclase activity of the enzyme may be required for chemotaxis to sugars transported by the phosphotransferase system. Mutations in the cyclic nucleotide phosphodiesterase gene, which increase the level of both cyclic AMP and cyclic GMP, also reduce chemotaxis to these sugars. Therefore, it appears that control of the level of a cyclic nucleotide is critical for the chemotactic response to phosphotransferase system sugars.

Most chemotactic responses of *Escherichia coli* are mediated by one of three methyl-accepting chemotaxis proteins (39), but one apparent exception is the response to sugars transported by the phosphotransferase system (PTS sugars) (25). We report here that chemotaxis to these sugars (PTS chemotaxis) appears to be mediated by adenylate cyclase, the nucleotide cyclase linked to the phosphotransferase system.

The phosphotransferase system concomitantly transports and phosphorylates a variety of sugars (11, 30). Although the components vary depending on the sugar, most commonly phosphate is transferred from phosphoenolpyruvate to a soluble component designated enzyme I and from enzyme I to another soluble component called HPr. Finally, the phosphate is transferred from HPr to the sugar being transported in a process catalyzed by one of a number of membrane-bound enzymes II, each of which is relatively specific for one sugar.

The enzymes II also serve as receptors for chemotaxis to PTS sugars; binding of a given sugar to its enzyme II is required for chemotaxis toward that sugar via the phosphotransferase system (3, 21). Metabolism of the compound (beyond the phosphorylation step) is not required for chemotaxis toward it (3, 21). In addition to an appropriate enzyme II, enzyme I and HPr are required for all PTS chemotactic responses (3, 21), and PTS chemotaxis appears to be obligatorily coupled to transport (21). Therefore, it has been suggested that an alteration in the level of phosphorylation of some component of the phosphotransferase system during translocation of a sugar triggers the chemotactic signal (21).

The adenylate cyclase of *E. coli*, which converts ATP to cyclic AMP (cAMP) (27), is also coupled to the phosphotransferase system. PTS sugars inhibit the synthesis of cAMP by this enzyme (16, 28, 34) under conditions similar to the conditions required for chemotaxis to these compounds; the appropriate enzyme II is required for a sugar to affect adenylate cyclase activity (15, 35), metabolism of the compound is not required (28), and mutations in enzyme I and HPr lower adenylate cyclase activity or result in abnormal regulation of the activity by PTS sugars (10, 14, 29, 34). Just as for chemotaxis, it has been suggested that the level of phosphorylation of a component of the phosphotransferase sys-

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tem is the essential variable in determining adenylate cyclase activity (29, 34).

There is evidence that the adenylate cyclase synthesizes cyclic GMP (cGMP) as well as cAMP (36, 40). In view of our previous studies implicating a role for cGMP in chemotaxis (7, 8, 17), we considered the possibility that an alteration in the guanylate cyclase activity of the adenylate cyclase might generate the chemotaxis signal for PTS sugars. Our results suggest that indeed a cyclic nucleotide is involved in the chemotactic response to these attractants.

MATERIALS AND METHODS

Bacteria. All of the strains used are *E. coli* K-12 derivatives and are described in Table 1.

The deletion mutation in the adenylate cyclase gene (Δ*cya*) was transferred from strain CA-8306 to strains CA-8000, AW723, and RP487 as follows: an isoleucine- and valine-requiring derivative of the recipient strain was obtained by ampicillin enrichment (24) after transduction with P1 *kc* phage (24) propagated on an HfrH Ilv$^-$ strain selected by E. N. Kort (unpublished data); this derivative was then infected with P1 *kc* phage grown on strain CA-8306 (Δ*cya*), and Ilv$^+$ transductants were screened for the *cya* marker. (*ilv* is 67% cotransducible with *cya* [9, 36].) Transductants which failed to form rings on a standard tryptone swarm plate (1) but did form rings if the plate was supplemented with 5 mM cAMP were considered probable Δ*cya* derivatives; cAMP is required for the synthesis of flagella (43) and chemotaxis proteins (37, 38). Such transductants were further tested for the ability to grow on minimal agar plates containing maltose as the sole carbon source and the required amino acids; cAMP is required for the utilization of maltose (27). Those transductants which neither swarmed without cAMP nor grew on the maltose-containing plates were considered to be Δ*cya*.

The cAMP phosphodiesterase activity in *E. coli* also hydrolyzes cGMP, and the gene coding for this activity is cotransducible with *metC* (unpublished data), as it is in *Salmonella typhimurium* (6). We refer to the gene coding for this activity as *cpd*, the designation used in *S. typhimurium* (6). Strains AW723 and AW724 are spontaneous *cpd* mutants that were selected by the procedure of Alper and Ames (6). Briefly, this procedure is based on the fast growth rate of such mutants on minimal agar plates containing succinate and ammonia; colonies of these mutants appear on such plates before a lawn arises. Cpd$^-$ strains grow poorly on minimal agar plates containing glycerol and cAMP (6), and this test was used to screen putative mutants obtained from the selection procedure. Those strains which also appeared to be Cpd$^-$ on the basis of this test were assayed for cGMP phosphodiesterase activity (41). For strain AW723, this assay was carried out with bacteria permeabilized by osmotic shock (32) and also with crude extracts prepared by ultrasonic disruption and by use of a French pressure cell; for

TABLE 1. Bacterial strains

Strain	Genotype	Source	Reference	Comments
CA-8000	HfrH *thi*	J. Beckwith	9	Parent of CA-8306, CA-8404, 20-2, AW723, and AW724
CA-8306	HfrH *thi* Δ*cya*	J. Beckwith	9	This strain carries a deletion in *cya*, the gene for adenylate cyclase
CA-8404	HfrH *thi* Δ*cya crp** Sm^r	J. Beckwith	33	*crp** designates a cAMP-independent cAMP receptor protein
20-2	HfrH *thi cya*	J. Beckwith		
AW723	HfrH *thi cpd*		This paper	Independent spontaneous cyclic nucleotide phosphodiesterase mutant derived from CA-8000 (see text)
AW724	HfrH *thi cpd*		This paper	Independent spontaneous cyclic nucleotide phosphodiesterase mutant derived from CA-8000 (see text)
AW725	HfrH *thi cpd* Δ*cya*		This paper	AW723 made Δ*cya* (see text)
RP487	*thr his leu met lac xyl ara* Δ*gal eda rpsL tonB*	This laboratory	26	A strain commonly used in chemotaxis studies
AW729	RP487 Δ*cya* (see RP487)		This paper	See text
ZSC103agl	*glk ptsM*	W. Epstein	Curtis[a]	

[a] S.J. Curtis, Ph. D. thesis, University of Chicago, Chicago, Ill., 1973.

strain AW724, only a crude extract prepared by ultrasonic disruption was tested. No activity was detected in either strain.

The *cpd* mutation from strain AW723 was transduced into strain CA-8000 as follows: strain CA-8000 was made *metC* by ampicillin enrichment (24) after transduction with P1 *kc* phage (24) propagated on an HfrH *metC* strain selected by E. N. Kort (unpublished data); this derivative was then infected with P1 *kc* phage grown on strain AW723, and Met$^+$ transductants were selected. *cpd* transductants were identified by fast growth on plates containing succinate and ammonia and slow growth on plates containing glycerol and cAMP (6), and the cGMP phosphodiesterase defect was confirmed by an enzyme assay (41).

Growth of bacteria. Except where otherwise indicated, the bacteria that were used for behavioral or biochemical assays were grown in tryptone broth (1% tryptone [Difco Laboratories], 0.5% NaCl) at 35°C with rotary shaking to an optical density at 590 nm of 0.50 to 0.60 (3.5×10^8 to 4.2×10^8 cells per ml). Minimal agar plates for genetic procedures (other than selection and screening for *cpd* mutants) contained Vogel-Bonner salts (42), the required amino acids at concentrations of 1 mM, a carbon source at a concentration of 0.2%, and 0.001% thiamine (when required).

Assays for cAMP and cGMP production. Assays for cAMP and cGMP production were performed essentially as described by Black et al. (7). Briefly, the method used involves suspending freshly washed bacteria in buffer, treating samples with trichloroacetic acid at different times, removing the trichloroacetic acid, by ether extraction, and assaying for the cyclic nucleotide by a radioimmunoassay. Our procedures were different from the previously described procedures in the following ways: (i) in the experiment with strain AW725, 2-ml samples rather than 1-ml samples were withdrawn for each time point; (ii) in all assays for cAMP, 20 μl of the ether-extracted sample was diluted to 665 μl, 100 μl of which was used for the assay; and (iii) in the experiment comparing production by strains CA-8000 and AW723, lactate was omitted from the buffer.

Assay for guanylate cyclase. Bacteria were harvested by centrifugation, washed three times at room temperature by suspension in 50 mM Tris-hydrochloride (pH 7.8)–10 mM sodium DL-lactate–0.1 mM L-methionine and centrifugation, and finally resuspended in the same medium to an optical density at 590 nm of 30 (2.1×10^{10} cells per ml). The resulting suspensions were subjected to two 10-s bursts of ultrasonic disruption with a Branson model W185 Sonifier Cell Disruptor fitted with a microtip. The resulting crude extracts were used immediately for the assay of guanylate cyclase activity. This assay measured the conversion of [α-^{32}P]GTP to [^{32}P]cGMP and was essentially the assay described by Macchia et al. (22), except that the GTP concentration in the reaction mixture was 15 μM, 1.5×10^6 to 1.8×10^6 cpm of [α-^{32}P]GTP was used, and 1 mM cAMP was added (in addition to the 3 mM cGMP) to reduce hydrolysis of the [^{32}P]cGMP by cyclic nucleotide phosphodiesterase. For each experiment the assay mixture contained 0.27 ml of crude extract in a final volume of 0.30 ml; 0.07-ml samples were removed immediately after the reaction was started (zero time) and at 6-min intervals thereafter.

Chemotaxis assay. Bacteria were grown as described above, except that in all experiments comparing *cya*$^+$ and *cya* strains the tryptone broth was supplemented with the sugar to be used as attractant at a concentration of 10 mM and 5 mM cAMP. Growth with the sugar induced components required for chemotaxis (4, 21, 31). The cAMP was required for transcription of flagellar proteins (43) and chemotaxis-specific proteins (37, 38) in *cya* strains, and it prevented catabolite repression by the inducing sugar in *cya*$^+$ strains (12). In most cases preinduction is not actually required for this chemotaxis assay because of either partial constitutivity or the occurrence of induction during the assay (3, 4). However, we chose to preinduce in experiments with *cya* strains, because induction of at least some phosphotransferase system components requires cAMP (31), which is not normally included in the chemotaxis assay.

After growth, bacteria were harvested by centrifugation at 4,000 × *g* for 2 to 5 min at room temperature and washed two times at room temperature by suspension in 10 mM potassium phosphate (pH 7.0)–0.1 mM EDTA–0.1 mM L-methionine–50 mM D-galactose and centrifugation. The pellet was then resuspended in the same medium to an optical density at 590 nm of 0.005 (3.5×10^6 cells per ml). The number of bacteria accumulated in 1 h at 30°C inside a capillary tube containing the attractant compound in the same medium was determined by a previously described procedure (2). Each point in the figures represents the average of duplicates; the separate values are indicated by error bars. All experiments were performed at least twice, and all results were similar to those reported below. D-Galactose was included in the medium (both in the capillary and in the bacterial suspension) to block the chemotaxis which is known to occur to some PTS sugars via the galactose-binding protein (4). That this procedure successfully blocked the alternative pathway for D-mannose chemotaxis was demonstrated with strain ZSC103agl; in the absence of D-galactose, this strain was attracted to D-mannose even though it is missing mannose enzyme II, but inclusion of D-galactose totally prevented accumulation.

Sugar transport assay. Bacteria were grown, harvested, and washed as described above for the chemotaxis assay, except that the wash medium contained 10 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, 0.1 mM L-methionine, and 10 mM sodium DL-lactate. After the final wash the bacteria were suspended to a density of about 5×10^9 cells per ml and placed on ice. For each assay, a sample of the suspension was diluted to a density of 3.5×10^8 cells per ml in 1 ml of the wash medium supplemented with 50 mM D-galactose (see below), and the resulting suspension was incubated for 5 min at 30°C. Then the radioactively labeled sugar (in 10 μl) was added to a final concentration of 18 μM for D-mannose (2.7 mCi/mmol), 15 μM for D-glucose (7 mCi/mmol), or 5 μM for D-mannitol (20 mCi/mmol), and 0.2-ml samples were withdrawn for filtering at 15, 30, 45, and 60 s after the addition. Each sample was pipetted into a filtration apparatus in which a 25-mm membrane filter (pore size, 0.6 μm; polycarbonate; Nuclepore Corp.) was covered with 2 ml of ice-cold wash medium containing the sugar being tested at a concentration of 0.4 M. Suction was applied as the sample was added, and the filters were washed twice with 2 ml of the same ice-cold solution, dried, and counted by liquid scintillation spectrometry. In all

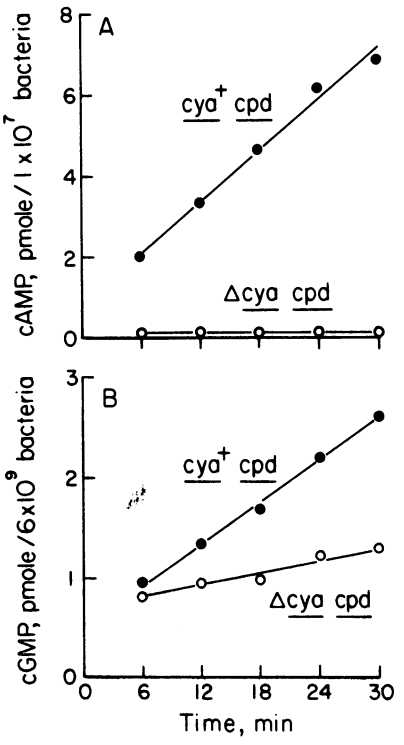

FIG. 1. Rates of production of cAMP (A) and cGMP (B) by strains AW723 (*cya*$^+$ *cpd*) (●) and AW725 (Δ*cya cpd*) (○). The bacteria excrete both cAMP and cGMP into the medium, and the assay (7) measured the combined intra- and extracellular cAMP or cGMP level. The bacteria were grown in tryptone broth supplemented with 0.2% glucose (*cya* mutants grow poorly on tryptone broth alone), and then they were washed and suspended in incubation medium (zero time). Samples of the suspension were withdrawn at different times and treated with trichloroacetic acid. cAMP and cGMP levels in the acid-soluble portion were determined by radioimmunoassays. The cAMP values for strain AW725 were less than 0.1 pmol/10^7 cells for all time points. The residual production by strain AW725 shown in (B) was still observed if only the cGMP fraction from an AG1-X8 column (19) was assayed.

cases, linear rates of uptake were observed for at least 60 s. The figures reported below are based on the average of two time courses. As with the chemotaxis assay, D-galactose was included to block uptake by systems other than the phosphotransferase system. The success of this procedure for D-mannose was demonstrated with strain ZSC103agl; in the absence of D-galactose this strain accumulated radioactivity from labeled D-mannose at a significant rate even though it is missing the mannose enzyme II, but inclusion of D-galactose virtually eliminated accumulation. Previous work has indicated that this procedure should also block D-glucose uptake by non-phosphotransferase systems (3). There are no alternative D-mannitol transport systems (20).

Chemicals. Cyclic nucleotide radioimmunoassay kits and [α-^{32}P]GTP (10 to 50 Ci/mmol) were obtained from New England Nuclear Corp. [8-^{3}H]cGMP was obtained from ICN. D-[*U*-^{14}C]glucose and D-[*U*-^{14}C]mannose were obtained from Amersham/Searle; D-[1-^{14}C]mannitol was from New England Nuclear Corp. D-Galactose and lactose were from Sigma Chemical Co. L-Arabinose, maltose, and D-mannose were from Calbiochem. D-Glucose was from MCB (associate of E. Merck AG, Darmstadt, Germany). D-Mannitol was from Eastman Kodak.

RESULTS

Role of adenylate cyclase in both cAMP synthesis and cGMP synthesis. We first confirmed the cAMP deficiency caused by the deletion (Δ*cya*) in the adenylate cyclase gene in strain CA-8306 (9). We found that strain CA-8306 is totally nonmotile when it is grown without cAMP and is unable to grow on L-arabinose, lactose, or maltose. Furthermore, a strain carrying the Δ*cya* mutation, as well as a mutation in the cyclic nucleotide phosphodiesterase gene (*cpd*), was totally deficient in cAMP production in vivo (Fig. 1A). The *cpd* strain was used for the production assay to eliminate variations due to differences in the rate of degradation rather than differences in the rate of synthesis of cAMP.

In agreement with Shibuya et al. (36), we found that the Δ*cya* mutation also significantly reduced the production of cGMP in vivo, to 31% of the level in the isogenic *cya*$^+$ strain under our conditions (Fig. 1B); values of 22 and 28% were obtained in two additional experiments. To determine more definitively whether adenylate cyclase is involved in the synthesis of cGMP, we tested crude extracts of strains CA-8000 (wild type) and CA-8404 (Δ*cya crp**) for the ability to convert [α-^{32}P]GTP to [^{32}P]cGMP. (Because of the *crp** mutation, strain CA-8404 has a cAMP receptor protein which activates transcription in the absence of cAMP [33]. Therefore, the use of this strain takes account of the possibility that

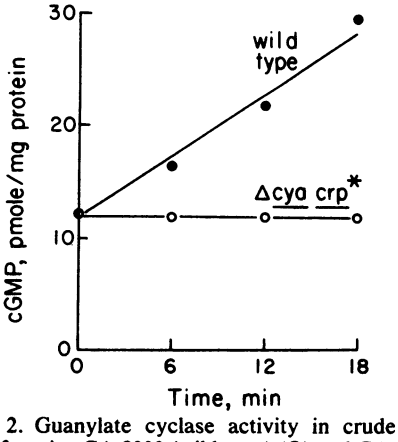

FIG. 2. Guanylate cyclase activity in crude extracts of strains CA-8000 (wild type) (●) and CA-8404 (Δ*cya crp**) (○). Essentially the same results were obtained in two additional experiments.

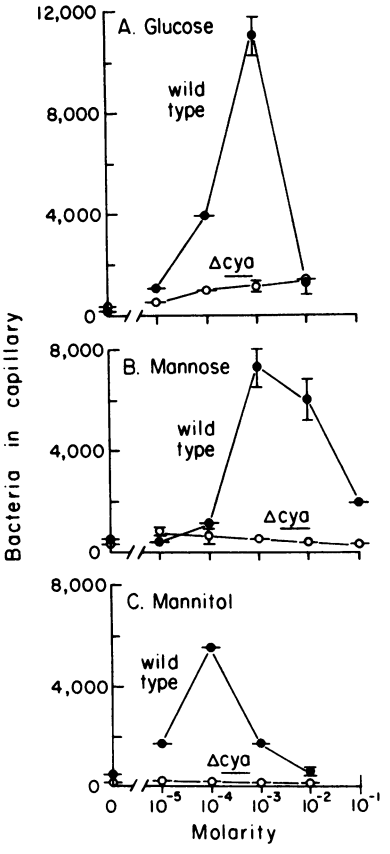

FIG. 3. Chemotaxis toward PTS sugars by strains CA-8000 (wild type) (●) and CA-8306 (Δ*cya*) (○). (A) D-Glucose chemotaxis. (B) D-Mannose chemotaxis. (C) D-Mannitol chemotaxis. In each experiment, 1 mM L-aspartate was used as a positive control. The levels of accumulation with aspartate were 66,165 cells for strain CA-8000 and 131,670 cells for strain CA-8306 (A), 87,780 cells for strain CA-8000 and 184,140 cells for strain CA-8306 (B), and 90,530 cells for strain CA-8000 and 31,130 cells for strain CA-8306 (C). (The data plotted are not normalized to the aspartate response in this figure or Fig. 4 through 7. For all of the chemotaxis assays in this experiment and the experiments shown in Fig. 4 through 7, 50 mM D-galactose was included in both the bacterial suspensions and the capillary tubes to block chemotaxis via systems other than the phosphotransferase system; galactose was also included in both the bacterial suspensions and the capillary tubes for the aspartate controls. The inclusion of galactose slightly increased the levels of accumulation with aspartate, perhaps because galactose serves as an energy source (2).

cGMP may be synthesized by a protein which requires cAMP for transcription and is absent in a *cya* mutant for this reason.) No activity was detected in the strain CA-8404 extract (Fig. 2). It was previously reported (40) that a partially purified adenylate cyclase from *E. coli* converted GTP to cGMP, but there was no genetic identification of this enzyme. The residual in vivo cGMP production by the Δ*cya* strain described above (and also observed with strain CA-8404) could have been due to an impaired *cya* gene product that was able to function partially in vivo but not at all in vitro, or it could have been due to another enzyme which was not detected in the in vitro assay. However, it is clear from our evidence and previous work (36, 40) that mutations in *cya* affect cGMP synthesis as well as cAMP synthesis.

Requirement of adenylate cyclase for chemotaxis to PTS sugars. To test whether adenylate cyclase might be involved in chemotaxis to PTS sugars, we compared the levels of accumulation of strains CA-8000 (wild type) and CA-8306 (Δ*cya*) in capillary assays with D-glucose, D-mannose, or D-mannitol as the attractant (Fig. 3). In all three cases, strain CA-8306 showed virtually no accumulation above the background value (no PTS sugar in the capillary tube), whereas with strain CA-8000 there was an accumulation above the background level of several thousand bacteria. This defect was specific for PTS sugars; L-aspartate was used as a positive control in each experiment, and strain CA-8306 clearly responded to this amino acid in all cases (see the legend to Fig. 3). Strains CA-8000 and CA-8306 were also comparable in their responses to L-serine (112,970 cells for strain CA-8000 and 133,320 cells for strain CA-8306 at 10^{-2} M L-serine in one experiment) and to D-galactose (6,270 cells for strain CA-8000 and 6,025 cells for strain CA-8306 at 10^{-4} M D-galactose in one experiment).

To ensure that the PTS chemotaxis deficiency was a result of the *cya* mutation, this marker was transduced from strain CA-8306 into strain CA-8000, and the resulting Δ*cya* transductant was tested for chemotaxis to D-mannose. This strain showed no chemotactic response to the sugar. To test the possibility that the chemotaxis deficiency in the Δ*cya* strains was due not to the cyclase defect but to a defect in another protein affected by the deletion, we determined the effect of a *cya* point mutation in strain 20-2 on chemotaxis to D-mannose. Even though this mutation resulted in only a leaky Cya^- phenotype (some utilization of L-arabinose, lactose, and maltose and only partial dependence on cAMP for formation of rings on tryptone swarm plates), it too markedly reduced the chemotactic response (Fig. 4). Thus, there is a correlation between a defective adenylate cyclase and impaired chemotaxis to PTS sugars.

We then considered whether it was the cyclase defect per se that was impeding PTS chemotaxis or whether this defect might be causing a deficiency in some other component of the phosphotransferase system that is essential for chemotaxis. To test the latter possibility, we assayed the sugar uptake abilities of cya^+ and

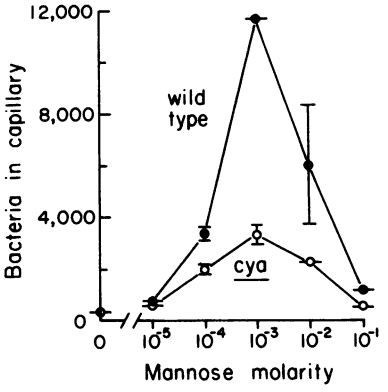

FIG. 4. Chemotaxis toward D-mannose by strains CA-8000 (wild type) (●) and 20-2 (*cya*) (○). The levels of accumulation in the aspartate controls were 109,065 cells for strain CA-8000 and 81,345 cells for strain 20-2. D-Galactose at a concentration of 50 mM was included in both the bacterial suspensions and the capillary tubes (see the legend to Fig. 3).

cya strains grown under the conditions described above for the chemotaxis assays. We found no substantial deficiency in the sugar transport systems of *cya* strains (Table 2), indicating that the required enzymes II and other components necessary for transport were present and functional. (A similar result has been obtained with *cya* mutants of *S. typhimurium* [31].) The extent to which the *cya* strains are reduced in transport cannot account for their defectiveness in chemotaxis, since substantial PTS chemotactic responses are observed even in cases where transport is greatly reduced (21; unpublished data).

We also considered the possibility that it is not the cyclase itself that is required for PTS chemotaxis but merely the presence of one of its products, cAMP or cGMP. This possibility was ruled out by the finding that inclusion of either 5 mM cAMP or 5 mM cGMP in the chemotaxis assay (in both bacterial suspensions and capillary tubes) did not restore PTS chemotaxis to strain CA-8306 (Δ*cya*). The addition of exogenous cyclic nucleotides does raise the intracellular concentrations of these compounds; exogenous cAMP at a concentration of 5 mM supports a normal level of transcription of cAMP-dependent operons in *cya* mutants (27; unpublished data with strain CA-8306), and 5 mM cGMP similarly stimulates transcription in Δ*cya* strains with mutant cAMP receptor proteins which are activated by cGMP (5; unpublished data).

The data presented above do not indicate whether it is the cAMP synthetic activity or the cGMP synthetic activity of the adenylate cyclase that is required for PTS chemotaxis. (There could, of course, be a third synthetic activity of the enzyme.) An analysis of two additional strains carrying the same Δ*cya* mutation as strain CA-8306 allowed us to rule out the possibility that cAMP synthesis is required. These strains were strains CA-8404 (Δ*cya crp**) and AW729 (a Δ*cya* transductant of strain RP487, a strain unrelated to strain CA-8000). We found that both of these strains were totally deficient in cAMP production, as expected, but showed substantial chemotaxis to D-mannose (Fig. 5). These strains did have at least as much residual cGMP production as shown in Fig. 1B. Possible explanations for the difference in PTS chemotaxis between these two strains and strain CA-8306 (Fig. 3) are discussed below.

TABLE 2. Uptake of sugars by *cya* strains relative to an isogenic *cya*$^+$ strain[a]

Strain	% Uptake of:		
	D-Glucose	D-Mannose	D-Mannitol
CA-8000 (wild type)	100	100	100
CA-8306 (Δ*cya*)	77 ± 3	83 ± 16	58 ± 1
20-2 (*cya*)	ND[b]	73 ± 2	ND

[a] Assays were carried out with 50 mM D-galactose present to block uptake by systems other than the phosphotransferase system (see text).
[b] ND, Not determined.

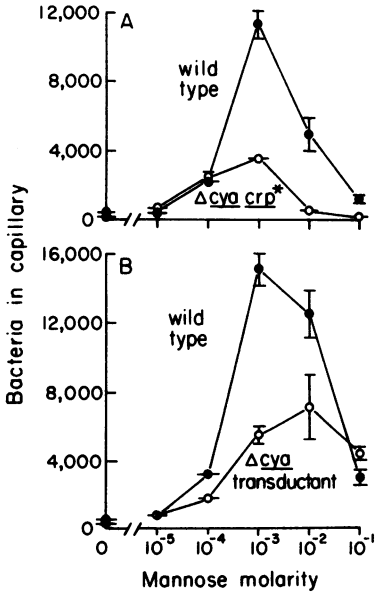

FIG. 5. Chemotaxis toward D-mannose by strains CA-8000 (wild type) (●) and CA-8404 (Δ*cya crp**) (○) (A) and by strains RP487 (wild type) (●) and AW729 (Δ*cya*) (○) (B). The levels of accumulation in the aspartate controls were 108,570 cells for strain CA-8000 and 79,090 cells for strain CA-8404 (A) and 155,760 cells for strain RP487 and 149,930 cells for strain AW729 (B). For all of the experiments, D-galactose at a concentration of 50 mM was included in both the bacterial suspensions and the capillary tubes (see the legend to Fig. 3).

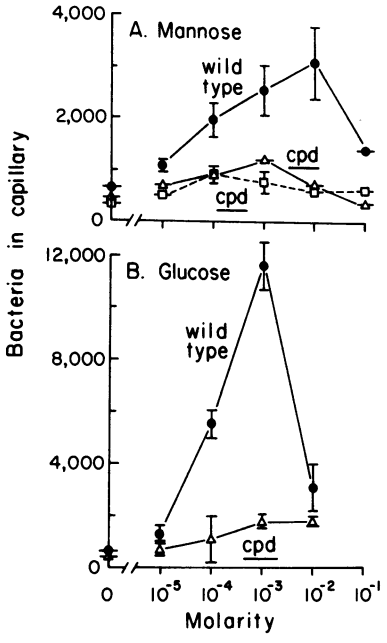

FIG. 6. (A) Chemotaxis toward D-mannose by strains CA-8000 (wild type) (●), AW723 (*cpd*) (△), and AW724 (*cpd*) (□). (B) Chemotaxis toward D-glucose by strains CA-8000 (wild type) (●) and AW723 (*cpd*) (△). The levels of accumulation in the aspartate controls were 152,900 cells for strain CA-8000, 102,630 cells for strain AW723, and 92,180 cells for strain AW724 (A) and 217,910 cells for strain CA-8000 and 117,590 cells for strain AW723 (B). For all of the experiments, D-galactose at a concentration of 50 mM was included in both the bacterial suspensions and the capillary tubes (see the legend to Fig. 3). The response of strain CA-8000 to D-mannose (A) was lower than the response in Fig. 3B because for the experiment shown in this figure, the bacteria were not grown with D-mannose.

Effect of cyclic nucleotide phosphodiesterase mutations (*cpd*) on PTS chemotaxis. *cpd* mutants of *E. coli* are defective in the hydrolysis of both cAMP and cGMP (unpublished data). If the function of adenylate cyclase during the chemotactic response is to alter the level of cGMP or a related compound, a *cpd* mutation might be expected to inhibit PTS chemotaxis by impairing the regulation of cyclic nucleotide levels. Therefore, we tested two independently isolated *cpd* mutants, strains AW723 and AW724, for chemotaxis toward D-mannose; both of these mutants were found to be defective (Fig. 6A). Strain AW723 was also tested for its response to D-glucose, and a similar result was obtained (Fig. 6B). A strain constructed by transducing the *cpd* mutation from strain AW723 into strain CA-8000 (wild type) (see above) was found to be as defective in chemotaxis to D-mannose as the original strain; the transductant showed an accumulation of 665 cells compared with 2,330 cells for the wild type at 10^{-3} M D-mannose. This result indicates that the PTS chemotaxis defect can indeed be attributed to the *cpd* mutation. Chemotaxis to L-aspartate was relatively unaffected by the *cpd* mutations (see the legend to Fig. 6).

To determine whether the PTS chemotaxis defect of *cpd* mutants could in fact be due to the elevated level of a cyclic nucleotide, we measured the rates of production of both cAMP and cGMP by strain AW723. We found that the rate of cAMP production was 345% of the rate in the parent, strain CA-8000, and that the rate of cGMP production was 205% of the rate in the parent (Fig. 7).

DISCUSSION

The data reported here indicate that PTS chemotaxis requires adenylate cyclase and cyclic nucleotide phosphodiesterase in addition to the phosphotransferase system. Our principal findings are as follows: (i) that either a deletion or a point mutation in the gene coding for adenylate cyclase (*cya*) impairs PTS chemotaxis, whereas transport of PTS sugars is relatively unaffected by such a mutation; (ii) that merely

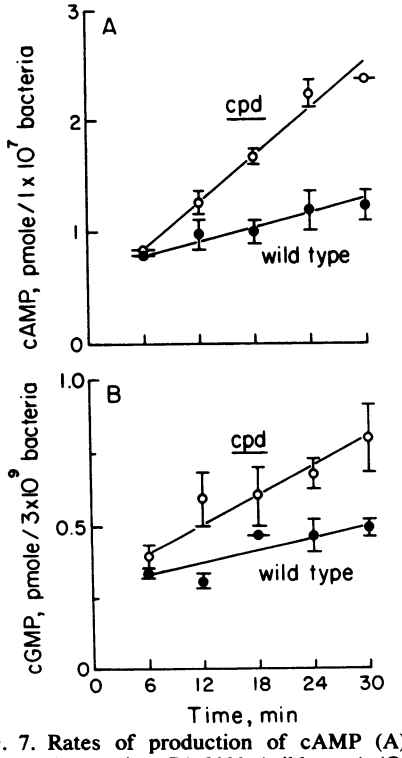

FIG. 7. Rates of production of cAMP (A) and cGMP (B) by strains CA-8000 (wild type) (●) and AW723 (*cpd*) (○). The points represent the averages of two experiments, and the error bars indicate the ranges.

supplying the bacteria with the products of this enzyme, cAMP and cGMP, does not restore the chemotactic response; (iii) that certain Δ*cya* strains with residual cGMP synthesis, but no cAMP synthesis, have a residual chemotactic response to PTS sugars; and (iv) that mutations in the cyclic nucleotide phosphodiesterase gene (*cpd*) severely reduce PTS chemotaxis and increase the rate of production of both cAMP and cGMP.

Previous work (36, 40) indicated that adenylate cyclase synthesizes cGMP as well as cAMP, and our findings confirm this conclusion. We extended the previous studies by using a *cpd* background (to eliminate apparent differences in synthesis due to differences in cyclic nucleotide phosphodiesterase activity) and by measuring the in vitro conversion of GTP to cGMP by extracts from *cya*$^+$ and *cya* strains. We do not know whether the in vivo cGMP production which remains in Δ*cya* strains is due to residual activity of the *cya* gene product or to another cyclase. Although the mutation was determined to be a deletion on the basis of non-revertibility and an inability to give recombinants with other mutations (9), the extent of the deletion is not known.

We are not certain why the Δ*cya* mutation eliminates PTS chemotaxis in strain CA-8306 whereas a residual response remains in strains CA-8404 (Δ*cya crp**) and AW729 (a Δ*cya* transductant of strain RP487). If the deletion mutation does not abolish all guanylate cyclase activity of the *cya* gene product, one possibility is that there is more residual guanylate cyclase activity in strains CA-8404 and AW729. In the case of strain CA-8404, another possibility is that the altered *crp* gene product interacts with the defective adenylate cyclase and partially restores its function in chemotaxis; this suggestion arises from a report that the wild-type *crp* gene product does interact with adenylate cyclase (18). Another possible explanation for the presence of a residual response in strains CA-8404 and AW729 but not in strain CA-8306 is that there is an alternative guanylate cyclase activity or an alternative chemotaxis pathway which is expressed to a greater extent under the influence of *crp** and in the strain RP487 background than it is in strain CA-8306.

The observation that not only *cya* mutations but also mutations in the gene for cyclic nucleotide phosphodiesterase (*cpd*) impair chemotaxis to PTS sugars suggests that modulation of the level of a cyclic nucleotide is critical in PTS chemotaxis. In fact, both *cya* and *cpd* mutants have normal motility and respond to non-PTS chemotaxis attractants, indicating that the mutations do not significantly affect the basal level of any central mediator in chemotaxis.

Which cyclic nucleotide plays a role in PTS chemotaxis? It appears more likely that cGMP or a related compound, rather than cAMP, is involved since certain strains with residual cGMP production but no cAMP production have a residual PTS chemotactic response. Our previous studies on amino acid chemotaxis also indicated no role for cAMP but a central role for cGMP or a related compound (7, 8, 17).

Thus far we have been unable to demonstrate an increase in cGMP level during stimulation of bacteria by a PTS sugar. This result may mean that cGMP is not the critical compound, or it may simply reflect the brevity and minor extent of the change expected for the PTS chemotactic signal. (PTS sugars elicit a very weak response compared with amino acid attractants [4, 23].) Indeed, even after stimulation of the bacteria with amino acid attractants, we no longer consistently observed the increase in intracellular cGMP level reported previously (7). To measure the intracellular concentration of cGMP, it is necessary to remove extracellular cGMP by filtering the bacteria and then washing them on the filter, and much of the chemotactic signal may be lost during this procedure. Furthermore, the experiment is complicated by irreproducible filtering of the dense bacterial suspensions which are required to collect enough cGMP to measure. Clearly an improved technique for measuring intracellular cyclic nucleotides will be required to detect any alteration in the levels of such compounds during PTS chemotaxis.

What are the implications of our findings concerning PTS chemotaxis for other chemotaxis pathways? *cya* mutants retain responses to sugars detected by periplasmic binding proteins and to amino acids, so it is clear that adenylate cyclase is not involved in producing the intracellular signal in these cases. If the non-PTS chemotaxis pathways also use cGMP or a related compound as an intracellular regulator, they must be linked to their own cyclases.

In conclusion, our model for chemotaxis to PTS sugars is that concomitant with transport and phosphorylation of a sugar, there is an increase in the guanylate cyclase activity (or in a third, as-yet-unidentified activity) of the adenylate cyclase, leading to a change in swimming behavior. The control of this activity could be mediated by the level of phosphorylation of a component of the phosphotransferase system, as has been suggested for the modulation of adenylate cyclase activity (29, 34).

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