Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11583–11587, December 1995
Biochemistry

Coupling the phosphotransferase system and the methyl-accepting chemotaxis protein-dependent chemotaxis signaling pathways of *Escherichia coli*

(enzyme I/histidine kinase/autophosphorylation)

RENATE LUX*, KNUT JAHREIS*†, KATJA BETTENBROCK*, JOHN S. PARKINSON†, AND JOSEPH W. LENGELER*‡

*Fachbereich Biologie/Chemie, Universität Osnabrück, D-49069 Osnabrück, Germany; and †Biology Department, University of Utah, Salt Lake City, UT 84112

Communicated by Hans Kornberg, University of Cambridge, Cambridge, England, June 28, 1995

ABSTRACT **Chemotactic responses in *Escherichia coli* are typically mediated by transmembrane receptors that monitor chemoeffector levels with periplasmic binding domains and communicate with the flagellar motors through two cytoplasmic proteins, CheA and CheY. CheA autophosphorylates and then donates its phosphate to CheY, which in turn controls flagellar rotation. *E. coli* also exhibits chemotactic responses to substrates that are transported by the phospho*enol*pyruvate (PEP)-dependent carbohydrate phosphotransferase system (PTS). Unlike conventional chemoreception, PTS substrates are sensed during their uptake and concomitant phosphorylation by the cell. The phosphoryl groups are transferred from PEP to the carbohydrates through two common intermediates, enzyme I (EI) and phosphohistidine carrier protein (HPr), and then to sugar-specific enzymes II. We found that in mutant strains HPr-like proteins could substitute for HPr in transport but did not mediate chemotactic signaling. In *in vitro* assays, these proteins exhibited reduced phosphotransfer rates from EI, indicating that the phosphorylation state of EI might link the PTS phospho-relay to the flagellar signaling pathway. Tests with purified proteins revealed that unphosphorylated EI inhibited CheA autophosphorylation, whereas phosphorylated EI did not. These findings suggest the following model for signal transduction in PTS-dependent chemotaxis. During uptake of a PTS carbohydrate, EI is dephosphorylated more rapidly by HPr than it is phosphorylated at the expense of PEP. Consequently, unphosphorylated EI builds up and inhibits CheA autophosphorylation. This slows the flow of phosphates to CheY, eliciting an up-gradient swimming response by the cell.**

Escherichia coli and other motile bacteria perceive many carbohydrates as chemoattractants. Some, such as maltose, galactose, and ribose, are sensed by transmembrane receptors known as methyl-accepting chemotaxis proteins (MCPs) (for review, see refs. 1 and 2). MCP molecules do not transport carbohydrates into the cell but, rather, measure their external levels through interactions with a periplasmic binding domain. Stimulus information is conveyed across the membrane to the cytoplasmic domain, which in turn communicates with rotational switches at the flagellar motors to control the cell's swimming movements. Several cytoplasmic proteins, principally CheA and CheY, relay MCP signals to the flagella (Fig. 1) (for review, see refs. 2 and 3). CheA autophosphorylates at a His residue by using ATP as the phosphodonor and, subsequently, donates the phosphate group to an Asp residue in CheY. Phosphorylation of CheY induces a conformational change that enables it to interact with the flagellar switch and trigger clockwise rotation (tumbles or random turns), counterclockwise (forward runs) being the default state. Phospho-CheY is short-lived, decomposing through self-catalyzed hydrolysis and through a reaction augmented by another protein, CheZ. MCPs control the flux of phosphate groups through this signaling pathway by modulating CheA autophosphorylation rate in response to changes in ligand occupancy. An increase in attractant concentration causes inhibition of CheA and consequent smooth swimming, whereas a drop in attractant level stimulates CheA and initiates a tumbling episode.

Carbohydrate attractants such as mannitol, mannose, and glucitol are sensed by a very different mechanism. These compounds are transported into the cell by phospho*enol*pyruvate (PEP)-dependent carbohydrate phosphotransferase systems (PTSs) (4) and somehow sensed as chemoeffectors during the uptake process (5, 6). PTSs consist of membrane-associated substrate-specific enzymes II (EIIs) and a common cytoplasmic phosphodonor relay (Fig. 1). EIIs are phosphorylated at the expense of PEP through enzyme I (EI), a histidine kinase, and a phosphohistidine carrier protein (HPr). During transport of PTS carbohydrates, phosphate groups are transferred through EI and HPr to the appropriate EII and finally to the substrate molecule as it enters the cell (for review, see ref. 7). This phospho-relay activity generates a signal that suppresses clockwise flagellar rotation, thereby extending swimming runs that carry the organism toward higher substrate concentrations (8).

The signaling connection between the PTS and MCP chemotactic pathways has long been a mystery. MCPs are not required for PTS chemotaxis, but CheA and CheY are required (9–11), suggesting that PTS signals elicit flagellar responses by modulating phospho-CheY levels, possibly through control of CheA activity (12). *E. coli* has at least 15 EIIs, each of which serves as the "chemoreceptor" for its transport substrates (7). However, neither the binding of substrate to an EII nor the generation of intracellular carbohydrate-phosphate nor its subsequent degradation is sufficient to trigger a chemotactic response (5, 6, 10, 13, 14). In contrast, the common phospho-relay components EI and HPr are necessary for uptake of all PTS carbohydrates and for chemotactic responses to them. Conceivably, the flagellar signal derives from an uptake-driven change in phosphate flux through these shared PTS components (6, 15). This article describes *in vivo* and *in vitro* studies that indicate that the unphosphorylated form of EI may be the long-sought missing link between the PTS and MCP phospho-relay circuits. Its signaling target appears to be the CheA kinase.

MATERIALS AND METHODS

Bacteria and Plasmids. Bacteria used were derivatives of *E. coli* K12 JWL184-1 (6) (for PTS transport and taxis assays),

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MCP, methyl-accepting chemotaxis protein; PEP, phospho*enol*pyruvate; PTS, phosphotransferase system; EI, enzyme I; EII, enzyme II; HPr, phosphohistidine carrier protein; PHPr, pseudo-HPr domain.

‡To whom reprint requests should be addressed.

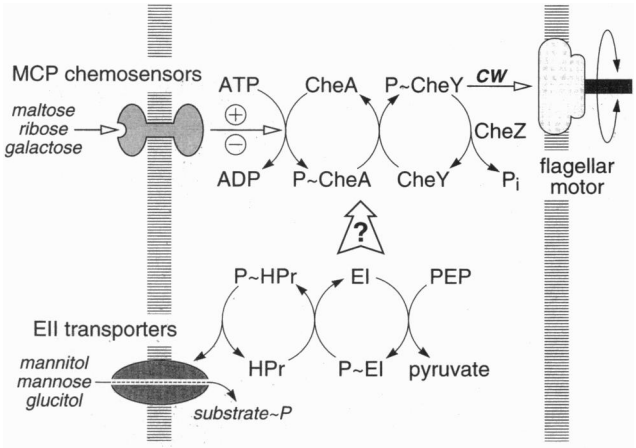

FIG. 1. Principal components of the MCP and PTS phospho-relays. (*Upper*) Protein phosphorylation reactions modulated by MCP molecules to elicit flagellar responses and chemotaxis. (*Lower*) Reactions involved in the uptake and phosphorylation of carbohydrates by the PTS system. Chemotactic responses to PTS substrates require a cross-circuit connection (open arrow) between the two pathways.

BL21(λDE3) (16) (host for *ptsH* and *fruF* plasmids), and RP3098 (17) (host for *cheA* plasmid). Parent vectors for plasmid constructs were pT7-5, pT7-6, and pT7-7 (18) (cloned genes expressed from the T7p promoter) and pTM30 (19) and pBCP342 (45) (*pts* genes expressed from the p_{tac} promoter).

Chemotaxis Assays. Soft agar plates were used for qualitative tests of chemotaxis and capillary tube assays were used for quantitative determinations (6, 20).

Protein Purifications. CheA was prepared from strain RP3098 carrying plasmid pKJP9 (pTM30 *cheA*). The cells were grown, harvested, and lysed as described (21). Subsequent purification of CheA closely followed a published procedure (22).

EI was prepared from strain LLR101 (JWL184-1 Δ*pts*) carrying plasmid pBCP342*ptsI*. Cells were grown in L broth to midlogarithmic phase, induced with 1 mM isopropyl β-D-thiogalactopyranoside for 90 min, harvested by centrifugation, resuspended in 10 mM potassium phosphate (pH 7.5), and broken by sonication. The EI-containing cytoplasm was clarified by ultracentrifugation and EI was purified essentially as described (23). Active enzyme fractions were dialyzed against 10 mM potassium phosphate (pH 7.5) at 4°C for at least 5 h to eliminate PEP, then lyophilized, and stored at room temperature.

HPr was prepared from strain BL21(λDE3) carrying plasmid pHPR-2 (pT7-6 *ptsH*). Cells were grown and harvested as in the EI purification above, with subsequent purification of HPr essentially as described (24). Purified fractions were dialyzed, lyophilized, and stored at room temperature. FPr was similarly prepared from strain BL21(λDE3) carrying plasmid pFPR-2 (pT7-5 with the *fruF* gene of *Salmonella typhimurium*). In this case, all solutions contained 1 mM *p*-methyltoluenesulfonyl fluoride because FPr is very sensitive to proteases. Purification followed a published procedure (25). The HPr-like proteins pseudo-HPr (PHPr) and FHPr-1 (see Fig. 2) were purified in a similar manner through the gel-filtration step. PHPr was prepared from strain LLR20 [BL21(λDE3) Δ*pts*] carrying plasmid pPHPR-7 (pT7-7′*fruF*); FHPr-1 was prepared from LLR20 carrying plasmid pFHPR1-2 (pT7-6 *fruF′–ptsH* fusion).

Enzyme Assays. EI activity was assayed by measuring the ability of EI to stimulate mannitol phosphorylation by mannitol-specific EII (EII^{Mtl})-containing membranes (26) by using extracts from strain JWL191 (*ptsI*) (26) as the source of HPr and EII^{Mtl}.

The ability of HPr, FPr, and HPr-like proteins to accept phosphate from purified EI and PEP was measured by following PEP consumption with a lactate dehydrogenase test (23). The ability of these proteins to donate phosphate to an EII was measured with *in vivo* transport assays (27) or with the mannitol phosphorylation assay, using extracts from JLV92 (*ptsH*) (15) as the source of EI and EII^{Mtl}.

CheA autophosphorylation was measured essentially as described (22).

RESULTS

HPr-Like Proteins and HPr Mutations Uncouple PTS Transport from Chemotaxis. Despite intensive research efforts, few genetic alterations have been found capable of uncoupling PTS transport from chemotaxis. The dearth of uncoupled mutants implies that there are no signaling elements solely dedicated to cross-circuiting the PTS and MCP pathways. However, several HPr alterations can uncouple the two phospho-relays and provide important clues about the nature of the signaling connection between them (12, 15).

Mutations in the structural genes for EI (*ptsI*) or HPr (*ptsH*), the shared PTS phospho-relay components, cause a pleiotropic transport-negative phenotype (5, 6). Degradation of fructose, however, is not affected by *ptsH* mutations because a protein, FPr, inducibly expressed from the *fru* operon, contains an HPr-like or PHPr domain that acts in its stead (cf. ref. 7). When expressed constitutively, FPr substituted for HPr in PTS carbohydrate transport but not in chemotaxis (Fig. 2) (15). This implies a functional difference between FPr and HPr that is specifically related to production of the chemotaxis signal. To identify that difference, several other genetic constructs were examined. The C-terminal PHPr domain of FPr, when freed from its N-terminal EIIA domain, also complemented a *ptsH* mutant for transport but not for chemotaxis (Fig. 2). High-level expression of PHPr, however, alleviated the chemotaxis defect as well, demonstrating that PHPr can generate a chemotaxis signal, but does so less efficiently than HPr. HPr

Protein	Domains: EIIA	Domains: HPr-like	Chemotaxis to PTS Substrates	Phosphotransfer Activity: from EI	Phosphotransfer Activity: to EII
(1) HPr			+	1.0	1.0
(2) FPr			–	0.4	0.8-1.1
(3) PHPr			-/+	0.4-0.6	1.0-1.2
(4) FHPr-1			+/-	0.4	0.7-0.8
(5) HPr-P11E			-/+	0.5	1.0
(6) HPr-F48M/K49G			+	1.0	0.5
(7) HPr-E85Δ			+	1.1	0.6

FIG. 2. Chemotaxis and phosphotransfer activities of HPr-like proteins. Plasmids expressing various HPr-like proteins were tested for ability to support chemotaxis in strain JLV92, which lacks HPr due to a chromosomal *ptsH* mutation (15). Results of capillary tests with the attractant D-mannitol are shown: +, response comparable to HPr control; +/−, weak response that is impaired further upon an increase in expression level of the HPr-like protein; −, response <10% of control; −/+, negative response that is improved upon an increase in expression level of the HPr-like protein. Phosphotransfer activities of purified proteins were measured and normalized to those of HPr. Proteins (and corresponding plasmids) were HPr (pHPR-2), FPr (from *S. typhimurium*) (pFPR-2), PHPr (PHPr domain of FPr), (pPHPr-7), FHPr-1 (IIA^{Fru} domain of FPr fused to HPr) (pFHPR1-2), HPr-P11E (pHPr11PE) (28), HPr-F48M/K49G (pHPr48/49) (28), and HPr-E85Δ (pHPrdel85) (29).

exhibited similarly attenuated signaling behavior when linked to the EIIA domain of FPr (Fig. 2), suggesting that HPr and PHPr differ mainly in amount of an activity needed for chemotactic signaling. A mutant HPr protein with a Pro → Glu replacement (HPr-P11E) was also specifically defective in chemotactic ability (Fig. 2), whereas two other HPr mutants with partially impaired uptake of PTS substrates remained proficient in chemotactic signaling.

HPr has two phosphotransfer functions, either of which might be related to production of the chemotactic signal: (*i*) removal of phosphate groups from phospho-EI and (*ii*) donation of those phosphates to EII molecules engaged in transport. We compared these two phospho-relay activities of HPr to those of the chemotaxis-uncoupled HPr-like constructs and mutant proteins listed in Fig. 2. Phosphotransfer from EI to HPr was evaluated by measuring the rate of conversion of PEP to pyruvate in assays containing PEP, EI, and a stoichiometric excess of HPr. Phosphotransfer between HPr and an EII was evaluated by measuring initial rates of mannitol phosphorylation by EII^{Mtl}-containing membrane vesicles. HPr-like proteins competent for chemotaxis exhibited normal rates of phosphotransfer from EI (Fig. 2), whereas those with partial or complete chemotaxis defects had reduced abilities to dephosphorylate EI. The phosphotransfer rates of the uncoupled proteins ranged from 40% to 60% of the HPr control. In contrast, phosphotransfer rates from the HPr-like proteins to EII^{Mtl} were normal or above in three of the four uncoupled constructs and as low as 50% in the chemotaxis-positive controls.

These findings indicate that the ability of HPr-like proteins to generate a chemotaxis signal during uptake of PTS substrates is correlated with the rate at which they dephosphorylate EI. Even a 2-fold reduction in that activity blocks production of a meaningful chemotaxis signal. If autophosphorylation of EI from PEP is slower than the subsequent phosphotransfer step from EI to HPr, the signal could stem from an increase in the proportion of unphosphorylated EI molecules triggered by carbohydrate transport. A reduction in the EI–HPr phosphotransfer rate, as in the chemotaxis-defective HPr constructs, might prevent accumulation of enough unphosphorylated EI molecules to elicit a chemotactic response. Because chemotactic ability in the HPr-like constructs was not correlated with their rate of phosphotransfer to EII^{Mtl}, it seems unlikely that a transport-driven change in the proportion of unphosphorylated HPr molecules is the chemotaxis signal. Accordingly, we looked for direct interactions between EI and components of the MCP phospho-relay that might form a cross-circuit signaling mechanism. Previous studies had established that chemoreceptors of the MCP class were not essential for PTS chemotaxis (9, 10), so we focused our attention on the CheA kinase of the MCP pathway as a logical target for cross-circuiting signals.

EI Inhibits CheA Autophosphorylation. Positive gradients of PTS substrates elicit smooth-swimming (counterclockwise flagellar rotation) responses (6), presumably by lowering the phosphorylation state of CheY. Thus, if CheA is the target of EI control, two PTS signaling strategies are possible: (*i*) EI might inhibit the autophosphorylation activity of CheA or (*ii*) EI might remove phosphate groups from CheA, either through phosphotransfer or hydrolysis. Both control mechanisms predict that EI should slow the accumulation of phosphate in CheA during the autophosphorylation reaction. We tested this prediction by measuring the initial rate of CheA autophosphorylation in the presence of various amounts of unphosphorylated EI, under assay conditions that approximated the *in vivo* concentrations of the reactants (Fig. 3). The apparent rate of CheA autophosphorylation began to decline at a roughly 3-fold molar excess of EI to CheA. At a 6- to 10-fold molar excess, EI reduced the rate to a minimum of 10–20% of normal. As controls, we tested bovine serum albumin and HPr.

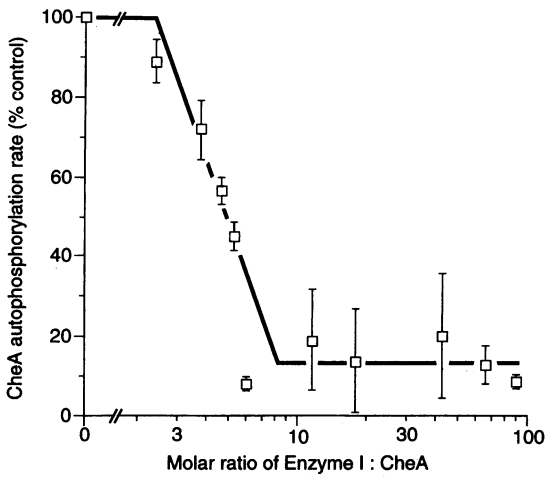

FIG. 3. Inhibition of CheA autophosphorylation by EI. CheA (2.8 μM) was mixed with [γ-^{32}P]ATP (0.1 mM) at 24°C and samples were taken at 15, 30, and 45 sec to determine initial reaction rates. Autophosphorylation rates at each EI concentration were normalized to control reactions containing the same molar ratio of bovine serum albumin. Error bars indicate the SD. The line connecting the data points was drawn by hand.

Neither protein caused significant inhibition of CheA activity at molar ratios comparable to those that yielded the maximal EI effect (data not shown).

Several results discount the possibility that phosphates are shunted from CheA to EI in these experiments. In the CheA autophosphorylation assays, there was no detectable transfer of ^{32}P to either EI or HPr (data not shown), consistent with a previous report (30). In *in vitro* phosphorylation assays containing EI, HPr, and EII^{Mtl}-containing membranes, neither ATP nor ATP plus CheA yielded any detectable phosphorylation of the mannitol substrate (data not shown). We conclude that EI and HPr do not accept phosphates from CheA, despite the fact that all three proteins use similar phosphohistidine chemistry. Although these experiments cannot exclude the possibility that EI slows CheA phosphorylation through dephosphorylation, it seems likely that EI inhibits the autophosphorylation reaction directly, in a manner analogous to the MCP signaling strategy.

Two experiments were done to verify that the unphosphorylated form of EI was, in fact, responsible for this inhibitory effect. (*i*) Pretreatment of EI with a 5-fold molar excess of HPr, to ensure that it was fully dephosphorylated, did not change its extent of CheA inhibition (data not shown). (*ii*) Pretreatment of EI with PEP, converting it to the phosphorylated form, alleviated its inhibitory effect on CheA autophosphorylation (data not shown).

PEP Stimulates CheA Autophosphorylation. As a control for the EI phosphorylation experiment just described, we also examined the effect of PEP alone on CheA autophosphorylation. Unexpectedly, 5 mM PEP consistently yielded 2- to 3-fold higher CheA autophosphorylation rates. A more detailed analysis of this effect is shown in Fig. 4. The enhancement of CheA activity by PEP follows saturation kinetics, with half-maximal stimulation at ≈1 mM PEP. This concentration value falls within the range of intracellular PEP levels (31), suggesting that the stimulatory effect could have physiological significance.

DISCUSSION

A Model for Chemotactic Signaling by the PTS Phospho-Relay. Uncoupled HPr mutants, able to transport PTS substrates but chemotactically unresponsive toward them, exhibited reduced phosphotransfer rates from EI, indicating that the

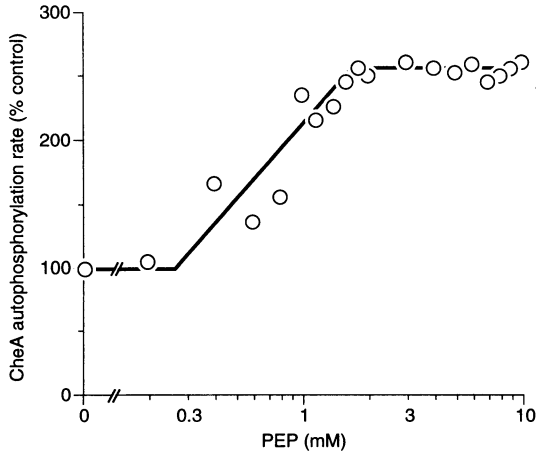

FIG. 4. Stimulation of CheA autophosphorylation by PEP. CheA activity was measured as described in Fig. 3. The line connecting the data points was drawn by hand.

unphosphorylated form of EI could be the signaling link between the PTS and MCP phospho-relay pathways. Tests with purified proteins revealed that unphosphorylated EI inhibited CheA autophosphorylation, whereas phosphorylated EI did not. These findings suggest the following model for signal transduction in PTS-dependent chemotaxis (Fig. 5). We propose that during uptake of a PTS carbohydrate through an EII, EI is dephosphorylated more rapidly by HPr than it is phosphorylated at the expense of PEP. Consequently, unphosphorylated EI builds up and in turn inhibits the autophosphorylation of CheA. This slows the flow of phosphates to CheY, eliciting an up-gradient swimming response by the cell.

The unusual nature of the EI autophosphorylation reaction may be largely responsible for the proportional increase in unphosphorylated EI molecules during PTS transport (Fig. 5). Before using PEP for autophosphorylation, EI subunits must dimerize. After phosphorylation, the dimers dissociate and

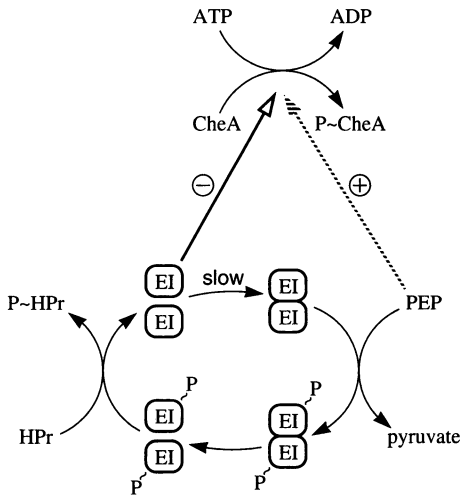

FIG. 5. Model of chemotactic signaling by the PTS phospho-relay. Uptake of PTS carbohydrates causes a rapid dephosphorylation of phospho-EI monomers through HPr. These monomers must dimerize in a slow (rate-limiting) process before they can be rephosphorylated at the expense of PEP. Rapid transport also causes a transient decrease in the PEP pool, further slowing the rephosphorylation of EI. The consequent buildup of unphosphorylated EI molecules inhibits the autophosphorylation activity of CheA, leading to a change in flagellar rotation. The stimulation of CheA activity by high levels of PEP could be a second cross-circuiting signal (hatched arrow). An uptake-dependent drop in intracellular PEP level would reduce CheA activity, augmenting the inhibitory signaling effect of unphosphorylated EI.

transfer phosphate to HPr as monomers. The obligate dimerization of EI subunits prior to autophosphorylation appears to be the rate-limiting step in the EI phosphorylation cycle (32, 33). Thus, rapid dephosphorylation by HPr would create within seconds a pool of unphosphorylated EI monomers that are slow to rephosphorylate. The size of this pool, the PTS cross-circuiting signal, should be sensitive to changes in the EI to HPr phosphotransfer rate. Slower dephosphorylation of EI would lead to fewer unphosphorylated molecules available for CheA control and could account for the inability of some HPr mutants and HPr-like proteins to generate a chemotaxis signal.

Cells presented with a PTS carbohydrate also experience a rapid decrease in PEP levels (31). These transport-related changes in the intracellular PEP pool might contribute to the PTS signaling process in two ways (see Fig. 5). (*i*) A drop in PEP concentration would further slow the rephosphorylation of EI, conceivably augmenting the strength and duration of CheA inhibition. (*ii*) We found that CheA was severalfold more active in the presence of PEP at ≥1 mM, so depletion of the PEP pool could directly slow the rate of CheA autophosphorylation by negating this stimulatory effect.

Behavioral Considerations. The MCP pathway shows high-gain signaling, with concentration changes of <1% eliciting readily detectable flagellar responses (34). PTS responses are 10- to 20-fold less efficient (5), but even so, is the observed inhibition of CheA by EI sufficient for chemotactic signaling? The *in vivo* concentrations of CheA and EI are reportedly comparable (CheA = 2–4 μM; EI = 2–6 μM) (35, 36), whereas a molar excess of EI was needed to inhibit CheA autophosphorylation *in vitro*. Maximal inhibition required a roughly 10-fold excess of EI, a concentration ratio difficult to reconcile with *in vivo* measurements. The discrepancy between *in vivo* and *in vitro* conditions may be offset to some extent by molecular crowding inside the cell (33). Nevertheless, the maximum possible effect of EI *in vivo* may be less than the 5-fold inhibition of CheA activity seen *in vitro*. Our model proposes that the proportion of EI molecules in the unphosphorylated state, the CheA-inhibitory form, will vary with the concentration of transport substrate. The threshold concentration for chemotactic responses to PTS substrates is roughly 1 μM (5, 6); detection capability saturates at about 1 mM. If essentially all EI molecules are in the phosphorylated form at threshold and in the unphosphorylated form at saturation, the cell would have at most a 5-fold range of control over CheA activity (for reasons discussed above). However, the cells can respond chemotactically to roughly 10% differences in PTS substrate concentrations, so much smaller changes in CheA activity would need to produce significant changes in rotational bias of the flagellar motors. The computer program of Bray *et al.* (37), which simulates the signaling reactions of the MCP pathway, predicts a steep dependence of rotational bias on CheA activity, largely because of the highly cooperative nature of the interaction between phospho-CheY and the flagellar switch. For example, a 5% deviation from the unstimulated CheA activity corresponds to a 4% change in bias, ample for chemotactic migration but less efficient than the bias changes produced by comparable MCP stimuli, which modulate CheA activity over a 100-fold range or more.

To track chemical gradients, *E. coli* must make temporal comparisons of chemoeffector levels as it swims about. Temporal sensing depends on an adaptation mechanism that cancels stimulus responses by resetting the detection threshold of the signaling system, enabling the organism to "forget" past environments. Cells adapt to MCP-detected stimuli by changing the methylation states of the signaling receptors (cf. ref. 1). Adaptation to PTS stimuli occurs through a methylation-independent process whose mechanism is unknown (9–11). In terms of our model, adaptation could take place by restoring the prestimulus level of unphosphorylated EI or by stimulating CheA to offset EI inhibition. Both mechanisms would come

into play as soon as the PEP-generating machinery compensates for transport-imposed drains on phosphodonor levels (4, 38). Alternatively, the build-up of pyruvate from PEP consumption could be a feedback signal for adaptation. It might accelerate PEP production or activate CheA or even enhance the switching behavior of the flagellar motors, as fumarate reportedly does (39). Whatever the mechanism(s) involved, PEP metabolism may well play an important role in sensory adaptation to PTS stimuli (32).

How Might EI Inhibit CheA? Although there is no detailed structural information available for either protein, their overall domain organizations could accommodate several simple control strategies. The EI molecule is composed of two domains, possibly joined by a flexible linker (40). The N-terminal domain contains the site of autophosphorylation, His-189, and determinants for promoting phosphotransfer interactions with HPr. The C-terminal domain is probably involved in PEP-binding and dimerization. The CheA molecule has at least four functional domains with intervening linkers (41). The N-terminal P1 domain contains His-48, the autophosphorylation site. The adjacent P2 domain binds CheY to assist the phosphotransfer reaction. The catalytic domain is located in the middle of the CheA sequence, followed by a C-terminal segment that couples CheA to chemoreceptor control.

EI inhibition of CheA presumably involves a binding interaction between one or more of these domains in each protein. The receptor coupling segment at the C terminus of CheA seems an unlikely target for EI control because it is normally bound to receptor and CheW molecules in a stable ternary complex (42). Most of the CheA molecules in wild-type cells are located in these MCP–CheW–CheA complexes (43). Even though MCPs are not needed for PTS signaling, EI must be able to interact with and control such CheA molecules. Initial *in vitro* studies indicate that EI inhibits receptor-coupled CheA as readily as free CheA (unpublished results), so EI may be targeted to parts of the CheA molecule, such as the N-terminal P1 or P2 domains, that are not directly involved in receptor coupling control. EI might block interaction between the autophosphorylation site and catalytic center of CheA by binding either to P1, perhaps directly occluding His-48, or to P2, which could prevent access through steric hindrance. P2 is the more intriguing candidate because its tertiary structure, recently determined by NMR studies, resembles that of HPr (44). Thus, the phosphotransfer domain of EI, which interacts with HPr, may also interact with the similarly shaped P2 domain of CheA.

Inhibition of CheA by unphosphorylated EI would seem to provide a simple mechanism for cross-circuiting the PTS phospho-relay to the chemotaxis signaling pathway. Whether the cell actually uses this signaling strategy is not yet clear, but the model makes some unique and easily tested predictions. It predicts, for example, that a large intracellular pool of unphosphorylated EI molecules should disrupt PTS- and MCP-dependent chemotaxis by constantly inhibiting the CheA kinase. Such experiments should determine whether or not EI is the long-sought key component in the signaling pathway for PTS chemotaxis.

We fondly dedicate this paper to Julius Adler on the occasion of his 65th year. This work was supported by the Feodor-Lynen Program of the Alexander von Humboldt Foundation (K.J.), by Research Grant GM19559 from the National Institutes of Health (J.S.P.), and by Deutsche Forschungsgemeinschaft through Sonderforschungsbereich 171, TPC3 (J.W.L.).

1. Hazelbauer, G. L. (1992) *Curr. Opin. Struct. Biol.* **2,** 505–510.
2. Parkinson, J. S. (1993) *Cell* **73,** 857–871.
3. Bourret, R. B., Borkovich, K. A. & Simon, M. I. (1991) *Annu. Rev. Biochem.* **60,** 401–441.
4. Roseman, S. & Meadow, N. D. (1990) *J. Biol. Chem.* **265,** 2993–2996.
5. Adler, J. & Epstein, W. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2895–2899.
6. Lengeler, J. W., Auburger, A.-M., Mayer, R. & Pecher, A. (1981) *Mol. Gen. Genet.* **183,** 163–170.
7. Postma, P. W., Lengeler, J. W. & Jacobson, G. R. (1993) *Microbiol. Rev.* **57,** 543–594.
8. Lengeler, J. W. & Vogler, A. P. (1989) *FEMS Microbiol. Rev.* **63,** 81–89.
9. Niwano, M. & Taylor, B. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 11–15.
10. Pecher, A., Renner, I. & Lengeler, J. (1983) *Mobility and Recognition in Cell Biology*, eds. Sund, H. Veegher, C. (de Gruyter, Berlin).
11. Taylor, B. L., Johnson, M. S. & Smith, J. M. (1988) *Bot. Acta* **101,** 101–104.
12. Lengeler, J. W., Bettenbrock, K. & Lux, R. (1994) *Phosphate in Microorganisms: Cellular and Molecular Biology*, eds. Torriani-Gorini, A., Yagil, E. & Silver, S. (Am. Soc. Microbiol., Washington, DC).
13. Grisafi, P. L., Scholle, A., Sugiyama, J., Briggs, C., Jacobson, G. R. & Lengeler, J. W. (1989) *J. Bacteriol.* **171,** 2719–2727.
14. Weng, Q.-P., Elder, J. & Jacobson, G. R. (1992) *J. Biol. Chem.* **267,** 19529–19535.
15. Grübl, G., Vogler, A. P. & Lengeler, J. W. (1990) *J. Bacteriol.* **172,** 5871–5876.
16. Studier, F. W. & Moffatt, B. A. (1986) *J. Mol. Biol.* **189,** 113–130.
17. Smith, R. A. & Parkinson, J. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5370–5374.
18. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1074–1078.
19. Morrison, T. B. & Parkinson, J. S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5485–5489.
20. Adler, J. (1973) *J. Gen. Microbiol.* **74,** 77–91.
21. Ames, P. & Parkinson, J. S. (1994) *J. Bacteriol.* **176,** 6340–6348.
22. Hess, J. F., Oosawa, K., Kaplan, N. & Simon, M. I. (1988) *Cell* **53,** 79–87.
23. Waygood, E. B. & Steeves, T. (1980) *Can. J. Biochem.* **58,** 40–48.
24. Beyreuther, K., Raufuss, H., Schrecker, O. & Hengstenberg, W. (1977) *Eur. J. Biochem.* **75,** 275–286.
25. Sutrina, S. L., Chin, A. M., Esch, F. & Saier, M. H., Jr. (1988) *J. Biol. Chem.* **263,** 5061–5069.
26. Lengeler, J. W. (1986) *Methods Enzymol.* **125,** 473–485.
27. Lengeler, J. W. (1975) *J. Bacteriol.* **124,** 26–38.
28. Eiserman, R. (1989) Ph.D. thesis (Ruhruniversität Bochum, Bochum, Germany).
29. Anderson, J. W., Bhanot, P., Georges, F., Klevi, R. E. & Waygood, E. B. (1991) *Biochemistry* **30,** 9601–9607.
30. Johnson, M. S. & Taylor, B. L. (1991) *FASEB J.* **5,** A427.
31. Lowry, O. H., Carter, J., Ward, J. B. & Glaser, L. (1971) *J. Biol. Chem.* **246,** 6511–6521.
32. Weigel, N., Kukuruzinsak, M. A., Nakazawa, A., Waygood, E. B. & Roseman, S. (1982) *J. Biol. Chem.* **257,** 14477–14491.
33. Chauvin, F., Brand, L. & Roseman, S. (1994) *J. Biol. Chem.* **269,** 20270–20274.
34. Segall, J. E., Block, S. M. & Berg, H. C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8987–8991.
35. Gegner, J. A. & Dahlquist, F. W. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 750–754.
36. Mattoo, R. L. & Waygood, E. B. (1983) *Can. J. Biochem.* **61,** 29–37.
37. Bray, D., Bourret, R. B. & Simon, M. I. (1993) *Mol. Biol. Cell* **4,** 469–482.
38. Pertierra, A. G. & Cooper, R. A. (1977) *J. Bacteriol.* **129,** 1208–1214.
39. Barak, R. & Eisenbach, M. (1992) *J. Bacteriol.* **174,** 643–645.
40. LiCalsi, C., Crocenzi, T. S., Freire, E. & Roseman, S. (1991) *J. Biol. Chem.* **266,** 19519–19527.
41. Parkinson, J. S. & Kofoid, E. C. (1992) *Annu. Rev. Genet.* **26,** 71–112.
42. Gegner, J. A., Graham, D. R., Roth, A. F. & Dahlquist, F. W. (1992) *Cell* **70,** 975–982.
43. Maddock, J. R. & Shapiro, L. (1993) *Science* **259,** 1717–1723.
44. McEvoy, M. M., Zhou, H., Roth, A. F., Lowry, D. F., Morrison, T. B., Kay, L. E. & Dahlquist, F. W. (1995) *Biochemistry*, in press.
45. Van der Vlag, J., Van't Hof, R., Van Dam, K. & Postma, P. W. (1995) *Eur. J. Biochem.* **230,** 170–182.