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Biochemistry

Liberation of an interaction domain from the phosphotransfer region of CheA, a signaling kinase of *Escherichia coli*

(chemotaxis/flagellar rotation/protein linkers/inhibitory polypeptides/epistasis)

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ABSTRACT **The CheA protein of *Escherichia coli* is a histidine autokinase that donates its phosphate groups to two target proteins, CheY and CheB, to regulate flagellar rotation and sensory adaptation during chemotactic responses. The amino-terminal third of CheA contains the autophosphorylation site, determinants needed to interact with the catalytic center of the molecule, and determinants needed for specific recognition of its phosphorylation targets. To understand the structural basis for these activities, we examined the domain organization of the CheA phosphotransfer region by using DNA sequence analysis, limited proteolytic digestion, and a genetic technique called domain liberation. Comparison of the functionally interchangeable CheA proteins of *E. coli* and *Salmonella typhimurium* revealed two extensively mismatched segments within the phosphotransfer region, 22 and 25 aa long, with sequences characteristic of domain linkers. Both segments were readily susceptible to proteases, implying that they have an extended, flexible structure. In contrast, the intervening segments of the phosphotransfer region, designated P1 and P2 (roughly 140 and 65 aa, respectively), were relatively insensitive, suggesting they correspond to more compactly folded structural domains. Their functional properties were explored by identifying portions of the *cheA* coding region capable of interfering with chemotactic behavior when "liberated" and expressed as polypeptides. P1 fragments were not inhibitory, but P2 fragments blocked the interaction of CheY with the rotational switch at the flagellar motor, leading to incessant forward swimming. These results suggest that P2 contains CheY-binding determinants which are normally responsible for phosphotransfer specificity. Domain-liberation approaches should prove generally useful for analyzing multidomain proteins and their interaction targets.**

The chemotaxis machinery of *Escherichia coli* and *Salmonella typhimurium* has afforded substantial insight into the information-processing strategies and molecular workings of protein-based signaling circuits (1). These bacteria continuously monitor their chemical environment as they swim about, moving toward beneficial compounds and away from harmful ones. Chemoeffector gradients elicit appropriate swimming responses by changing the cell's pattern of flagellar rotation. Counterclockwise (CCW) rotation produces forward swimming; clockwise (CW) rotation initiates tumbling motions and random directional changes. Most attractants and repellents are detected by a family of transmembrane receptors known as methyl-accepting chemotaxis proteins (MCPs), which communicate with rotational switches at the flagellar motors through a network of cytoplasmic signaling proteins. As in higher organisms, intracellular signaling by bacterial MCPs involves protein phosphorylation and dephosphorylation reactions.

CheA, a 71-kDa cytoplasmic protein, is the central component in the *E. coli* chemotaxis signal relay. CheA autophosphorylates at a specific histidine residue, using ATP as the phosphodonor (2). It then donates its phosphate groups to two aspartate autokinases, CheB and CheY, thereby activating their signaling functions. Phospho-CheY interacts with flagellar switch proteins to augment CW rotation (3, 4); phospho-CheB removes methyl groups from MCP molecules to bring about sensory adaptation (5). These phosphorylated effectors are intrinsically short-lived, enabling MCPs to modulate the phosphorylation states of CheY and CheB by regulating the autophosphorylation activity of CheA. Attractant-occupied receptors inhibit CheA activity, whereas unliganded MCP molecules stimulate CheA (6). A small soluble protein, CheW, is needed to couple CheA activity with chemoreceptor control (7–9).

The various signaling functions of CheA appear to reside in different parts of the molecule (Fig. 1). The central third corresponds to the "transmitter" sequence motif characteristic of a large family of bacterial histidine kinases and most likely contains the ATP-binding and catalytic centers needed for autophosphorylation (10, 11). The carboxyl-terminal third of the CheA molecule is involved specifically in chemoreceptor control and may contain the contact sites that promote coupling interactions with CheW and chemoreceptor molecules (12, 13). The amino-terminal third of CheA contains the autophosphorylation site (His^{48}) and other determinants that promote interaction with the catalytic center and presumably contains sites that participate in the CheB and CheY phosphotransfer reactions (2, 14).

Because of its segmental functional organization, CheA provides a good model for exploring the structural basis of signaling specificity, a fundamental but still poorly understood property of bacterial sensory systems. *E. coli* probably contains 50 or more pairs of signaling proteins that employ the same phosphotransfer chemistry as the CheA–CheB and CheA–CheY reactions (11). However, inappropriate crosstalk between signaling pathways is minimal, indicating that phosphodonors and phosphoacceptors have highly specific recognition mechanisms for promoting interactions with their proper partners. In CheA the determinants of signaling specificity most likely reside in the phosphotransfer portion of the molecule. To test this idea, we analyzed the structural and functional organization of the CheA phosphotransfer region with several physical and genetic methods. Using an approach dubbed domain liberation, we were able to identify a segment of the phosphotransfer region, distinct from the site of autophosphorylation, that may contribute to signaling specificity. It should be possible to characterize interaction domains in other proteins with the same experimental strategy.

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Abbreviations: CCW, counterclockwise; CW, clockwise; MCP, methyl-accepting chemotaxis protein; IPTG, isopropyl β-D-thiogalactopyranoside.

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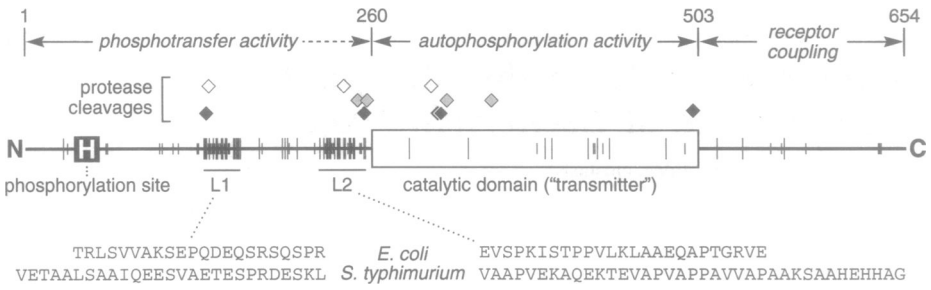

FIG. 1. Functional organization and important structural features in CheA. Vertical lines indicate the positions of sequence mismatches between the *E. coli* and *S. typhimurium* proteins. Long lines represent completely dissimilar residues; short lines represent residues with similar chemical properties. Prominent cleavage sites are indicated for trypsin (◆), proteinase K (◇), and V8 protease (◈). N, amino terminus; C, carboxyl terminus; H, phosphorylatable histidine residue.

MATERIALS AND METHODS

Bacterial Strains. All strains were derivatives of RP437, an *E. coli* K-12 strain that is wild type for chemotaxis (15). Strains for epistatic studies (see Fig. 6) were constructed by introducing different combinations of mutations into recipients by generalized transduction with phage P1, using linked, selectable markers (16). The following nonpolar deletions were used to eliminate chemotaxis functions: Δ(*cheW–tap*)*2217* (7), Δ(*cheZ*)*6725* (7), Δ(*tsr*)*7028* (17), Δ(*tar–tap*)*5201* (18), Δ(*trg*)*100* (19), Δ(*cheA*)*1643* (13), and Δ(*cheA–cheZ*)*2209* (15, 20, 21). Other markers used were *pcnB1*, which reduces plasmid copy numbers by a factor of about 15 (22), and *fliG1010* and *fliM1003* (formerly *scyB10* and *scyA3*, respectively), which impart a CW rotational bias to the flagellar switch (23, 24).

Plasmids. Fragments of the *cheA* coding region were cloned and expressed in pTM30, a high-copy-number plasmid derived from a fusion of pBR322 (25) and pUC119 (26). Details of its construction will be presented elsewhere. In essence, pTM30 contains a multiple cloning site flanked on one side by the strong ribosome-binding site and translational start of the *cheY* gene (27) and on the other side by TAA stop codons in all reading frames. In-frame inserts produce polypeptide products with different amino and carboxyl residues specified by codons in the vector, depending on the point of insertion and the exiting reading frame. Transcription of insert fragments is driven by a p_{tac} promoter (28), which is in turn controlled by lactose repressor expressed constitutively from a $lacI^q$ gene also carried on the plasmid. Cells carrying pTM30 or one of its derivatives were identified and maintained by selecting for resistance to ampicillin at 50–100 μg/ml. In addition to clones randomly generated by domain liberation (see below), the following *cheA* subclones were constructed in pTM30 through specific manipulations: pTM22 [CheA-(124–257)] and pTM52 [CheA-(1–169)] by partial digests of *cheA* DNA, and pTM36 [CheA-(1–121)] by deleting a restriction fragment from pTM33-13 (see Fig. 2). Other plasmids used in this work were pTM45 [CheA-(1–97)], a derivative of pJL163 (7) carrying a TAG stop codon at the internal translation start site of *cheA* (29); pTM46, a derivative of pACYC184 (30) carrying a constitutively expressed *cheY* gene from pRL22 (27); pTM47, a pTM46 control lacking the *cheY* insert; and pTM48, a pTM46 derivative carrying the cheY $Asp^{13} \rightarrow Lys$ mutation from pRBB40-DK13 (31).

CheA Proteolysis. Samples containing 18 μg of purified CheA in 5 mM $MgCl_2$/50 mM KCl/50 mM Tris, pH 7.5, were treated with either 40 ng of proteinase K (Sigma), 2 ng of trypsin (Sigma), or 4 μg of *Staphylococcus aureus* V8 protease (Boehringer Mannheim) in 40 μl. Reaction mixtures were incubated at room temperature for 5 and 30 min, and reactions were halted by boiling for 5 min in sample loading buffer (32). Proteolysis products separated by electrophoresis in 10% polyacrylamide gels containing 1% SDS were transferred to poly(vinylidine difluoride) membranes and visualized by Coomassie staining. Polypeptide bands were excised and their amino-terminal sequences were determined by Robert Shackmann (Protein–DNA Core Facility, University of Utah Cancer Center).

Behavioral Assays. Chemotactic ability was assessed by rate of colony swarming on semisolid tryptone "swarm" agar (consisting of tryptone, 10 mg/ml; NaCl, 5 mg/ml; and agar, 2.6 mg/ml) (16). Each plate was inoculated with four samples of one experimental strain and a wild-type control and incubated at 35°C. After 3–4 hr, colony diameters were measured at several 45-min intervals and swarm rate was calculated from a plot of swarm size against time, with normalization to the control swarm on the same plate. Flagellar rotation patterns were assessed by observing cells tethered by a single flagellum to microscope coverslips with flagellar antiserum (16). Torque determinations of plasmid-containing cells were done in strain RP4160, which has a chemoreceptor alteration (*tsr-192*) with locked CCW signal output (33) to eliminate reversals that interfere with measurement of rotational speed. Torque calculations were as described (34, 35).

Domain Liberation. A *cheA*-containing DNA segment was subjected to partial digestion with restriction endonuclease *Msp* I (pTM33 series) or *Fnu*4HI (pTM34 series) to generate pseudo-random coding fragments. Digestion products were purified by phenol/chloroform extraction and ethanol precipitation, treated with S1 nuclease to create blunt ends, and repurified. Fragments were then mixed and ligated in a roughly 2:1 ratio with pTM30 DNA that had been digested at the single *Eco*RV site in the cloning region. Ligation products were treated with *Eco*RV to linearize vector molecules that had not acquired an insert, and recombinant plasmids were introduced into strain RP437 by transformation and selection for ampicillin resistance. Individual transformant colonies were transferred by toothpick to a series of swarm plates containing ampicillin at 50 μg/ml and isopropyl β-D-thiogalactopyranoside (IPTG) at various concentrations to screen for clones carrying inducibly expressed inhibitory domains. After incubation at 35°C for about 8 hr, about 1% of the colonies exhibited substantially reduced chemotactic swarming on plates containing IPTG and were saved for further analysis.

RESULTS

Domain Linkers in the CheA Phosphotransfer Region. The *E. coli* (36) and *S. typhimurium* (10) CheA proteins are functionally interchangeable (37). Their primary structures are 86% identical, with most of the sequence mismatches clustered in two regions: L1, in the middle of the phosphotransfer region, and L2, at the boundary between the phosphotransfer and catalytic regions (Fig. 1). Length differences within these segments account for the overall size difference of the two proteins (654 vs. 671 aa). The L1 segments are 22 and 27 aa, respectively; the L2 segments are 25 and 37 aa, respectively. All four segments have similar amino acid contents, including one or more prolines and a preponderance of charged and polar residues (Fig. 1). Interdomain segments in other bacterial proteins often exhibit

similar properties, suggesting that L1 and L2 might serve as domain linkers in CheA (38).

Protein domains are typically more compact than the segments connecting them, which often have more extended, flexible structures. If L1 and L2 are domain linkers, they might be more susceptible to proteolytic attack than other parts of the CheA molecule. To test this possibility, we treated native CheA with different proteases, purified the first large proteolytic fragments to appear, and determined the amino acid sequences of their amino termini. The predominant cleavage sites for V8 protease, trypsin, and proteinase K are shown in Fig. 1. Two of the enzymes cleaved within the L1 segment, and all three cleaved within the L2 segment. Only a few other sites in the CheA molecule, notably in the amino-terminal half of the transmitter domain, were as susceptible to cleavage, consistent with the premise that L1 and L2 are flexible linkers between subdomains of the phosphotransfer region.

Detection of CheA Domains Through Domain Liberation. The L1 and L2 linkers define two putative domains in the CheA phosphotransfer region (Fig. 2): P1, an amino-terminal segment containing the autophosphorylation site, and P2, the segment between L1 and L2. If P1 and P2 correspond to discrete structural domains, they might be stable when expressed as isolated polypeptides within cells, and the polypeptides might retain their functional activities, including the ability to interact with other chemotaxis proteins. Accordingly, we scanned the *E. coli* CheA phosphotransfer region for functional domains by looking for portions of the *cheA* coding region capable of interfering with chemotactic behavior when expressed as independent polypeptides.

Fragments of *cheA* DNA were generated by partial digestion with restriction endonucleases and inserted into a plasmid (pTM30) that provided an IPTG-regulatable promoter (p_{tac}) and efficient translation initiation signals. Recombinant plasmids were transferred to a chemotactically wild-type strain and examined at different induction levels for inhibitory effects on chemotaxis. The extents of inhibitory inserts obtained from the *cheA* phosphotransfer region are summarized in Fig. 2. DNA sequence determinations at their promoter-proximal ends showed that most were in frame with respect to the translation start provided by the vector. However, some were not (indicated by open rectangles in Fig. 2). The *cheA* locus contains an efficient, in-frame translation start at codon 98, within the P1 portion of the coding region (36). The apparently out-of-frame inserts probably make CheA polypeptides from this internal start site.

All inhibitory clones contained a common subset of the *cheA* coding region corresponding to the P2 segment and much of the flanking L2 linker (Fig. 2). Although we cannot exclude the possibility that L2 contributes to the inhibitory effects, it seems likely that the P2 segment is primarily responsible. Four inhibitory clones, three with minimal P2 inserts (pTM34-21, pTM34-39, pTM22) and one with a P1–P2 insert (pTM34-55), were sequenced from both ends and examined for polypeptide products inducible by IPTG. All made stable proteins of the predicted size (Fig. 3). The overall stability of P2-containing fragments and their ability to inhibit chemotaxis indicate that P2 is most likely a discrete structural and functional domain within the CheA phosphotransfer region.

Our failure to obtain inhibitory P1 clones is surprising in view of the fact that P1 fragments can be phosphorylated *in vitro* and can donate their phosphate groups to CheB and CheY (2, 14). To ascertain the stability of P1 polypeptides in our experimental system, we constructed several specific P1 subclones and characterized their products (Fig. 3). Plasmids containing most [pTM36; CheA-(1–121)] or all [pTM52; CheA-(1–169)] of the P1 coding region made polypeptides of appropriate size, whereas a clone corresponding to the first

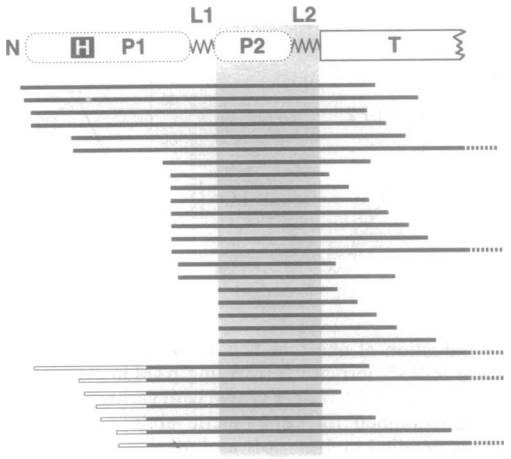

FIG. 2. Inhibitory clones from the phosphotransfer region of *cheA*. Filled bars represent inserts that are in frame with the translational start site of the expression vector. Open bars represent the out-of-frame portions of inserts that most likely initiate CheA translation at a secondary start site within the P1 coding region. T, transmitter domain.

two-thirds of P1 [pTM45; CheA-(1–97)] made no product detectable by immunoblotting or by Coomassie staining (Fig. 3). Thus, large P1 fragments are stable but evidently were not detected with the domain-liberation technique because, unlike P2 fragments, they do not interfere with chemotactic signaling.

Inhibition of Chemotaxis by P2 Fragments of CheA. The effects of representative P1 and P2 fragments on chemotaxis are contrasted in Fig. 4. At moderate induction levels the P1 clone [pTM52; CheA-(1–169)] had no discernible effects either on chemotactic swarming (Fig. 4*a*) or on flagellar rotation pattern (Fig. 4*b*). At high inducer levels both chemotactic swarming and cell growth were impaired. At 1 mM IPTG, generation times were about doubled, but the flagellar rotation pattern of the cells remained normal (data not shown), suggesting that the reduction in swarming speed at high induction levels is largely or entirely due to a slower growth rate.

Plasmids expressing the entire P1–P2 phosphotransfer region [pTM34-55; CheA-(3–283)] or just the P2 segment [pTM22; CheA-(124–257)] inhibited swarming at moderate induction levels (Fig. 4*a*). Although high level expression of these fragments proved toxic, they specifically inhibited chemotaxis at inducer concentrations that had no effect on growth rate (Fig. 4*a* and data not shown). This inhibition was

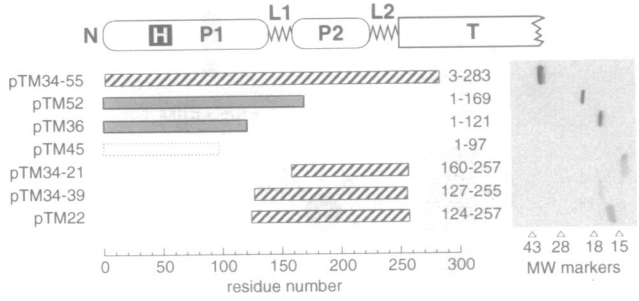

FIG. 3. Properties of P1 and P2 polypeptides. pTM30 derivatives carrying the indicated portions of the *cheA* coding region were tested in strain RP437 for inhibition of chemotactic swarming, and in strain RP3098 (39) for production of CheA peptides, visualized by immunoblotting with polyclonal antiserum. Insert products that were both stable and inhibitory are indicated by diagonal hatching; those that were stable but not inhibitory are indicated by shaded bars; one that was unstable and not inhibitory is indicated by a dotted outline.

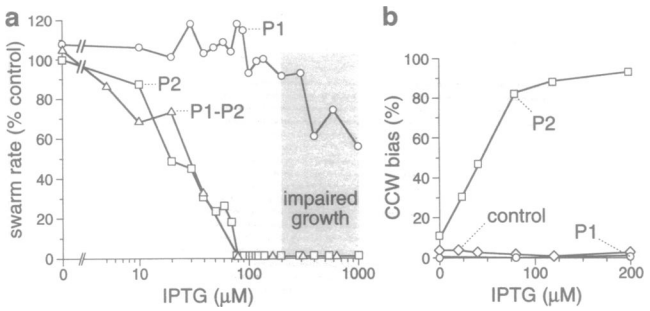

FIG. 4. Induction of aberrant behavioral effects by CheA fragments. Strain RP437 derivatives carrying pTM30 (◇), pTM22 (□), pTM34-55 (△), or pTM52 (○) were grown with various concentrations of IPTG and examined for chemotactic ability (*a*) and flagellar rotation (*b*) as detailed in *Materials and Methods*.

accompanied by a reduction in CW flagellar rotation. Rotation data for cells containing the P2 plasmid (pTM22) are shown in Fig. 4*b*; the P1–P2 plasmid (pTM34-55) produced similar effects (data not shown). To test the possibility that P2-mediated inhibition of CW rotation might be due to a reduction in cellular levels of protonmotive force, the energy source for flagellar rotation, we compared the rotation speeds of antibody-tethered cells containing pTM22 (expressing P2) with those of tethered cells containing pTM30 (control vector). At 80 μM IPTG, pTM22 caused nearly complete CCW rotational bias and loss of chemotaxis, but angular velocity, and, by inference, the torque output of the flagellar motors was not significantly different from the control (data not shown). This implies that P2 polypeptides inhibit chemotaxis by interfering directly with the production or control of CW flagellar rotation.

***In Vivo* Target of P2 Inhibition.** To identify the site of P2 action, we examined the effects of P2 induction on the flagellar rotation patterns of mutant strains lacking various phosphorelay proteins. Ordinarily, all of the components shown in Fig. 5, except for CheZ, are essential for CW rotation. Our strategy in these experiments was to devise strains that retained detectable CW rotation even though missing components of the CW pathway. Then we could determine whether or not the CW rotation generated by the remaining components was sensitive to P2 inhibition. The most extensive studies were done with pTM22 and are summarized in Fig. 6. Other P2 plasmids produced similar results (data not shown).

Overproduction of the P2 domain inhibited the highly CW-biased rotation of a *cheZ* deletion mutant (Fig. 6, line 2), indicating that its effect does not depend on CheZ function.

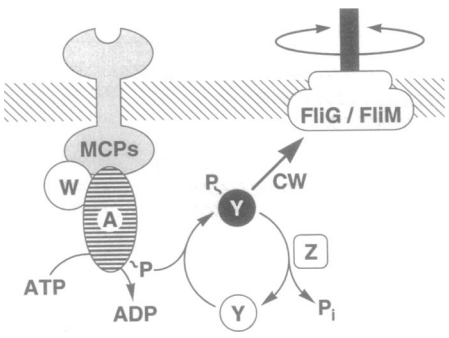

FIG. 5. Signaling pathway for the production of CW flagellar rotation. Hatched area represents the cytoplasmic membrane. Cytoplasmic Che components are indicated by one-letter designations (e.g., A = CheA). The default direction of flagellar rotation is CCW. Interaction of phospho-CheY with the FliG/FliM proteins in the switch/basal body complex produces CW rotation.

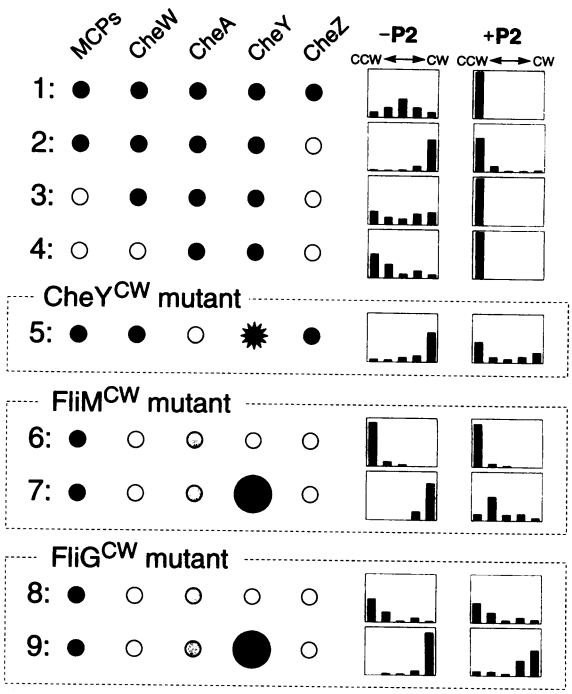

FIG. 6. Epistatic analysis of P2-mediated inhibition of CW flagellar rotation. Strains containing pTM22 (CheA P2) were tethered to microscope slides with anti-flagellar serum and examined for rotation pattern in the absence of chemotactic stimuli. For each strain, at least 100 rotating cells were individually observed for 15 sec and classified as exclusively CCW or CW, predominantly CCW or CW but with occasional reversals, or frequently reversing with no obvious rotational bias. The resulting rotational profiles are presented as histograms giving the percentage of cells in each rotational category when grown in the absence (−P2) or presence (+P2) of 80 μM IPTG. Each line represents the behavior of a strain with a different combination of the signaling components shown in Fig. 5. Open circles denote missing components, large filled symbols denote plasmid-encoded components present in stoichiometric excess, and shaded circles indicate subnormal levels of a component or its activity. The recipient strains were as follows: line 1, RP437 (wild type for chemotaxis); line 2, RP9349 [*Δ(cheZ)6725*]; line 3, RP9352 [*Δ(tsr)7028 Δ(tar–tap)5201 Δ(trg)100 Δ(cheZ)6725*]; line 4, RP9411 [*Δ(tsr)7028 Δ(trg)100 Δ(cheW–tap)2217 Δ(cheZ)6725*]; line 5, RP9843 [*pcnB1 Δ(cheA)1643*]/pTM48 (*cheY$_{D13K}$*); line 6, RP732 [*Δ(cheA–cheZ)2209 fliM1003*]/pTM47 [*Δ(cheY)*]; line 7, RP732 [*Δ(cheA–cheZ)2209 fliM1003*]/pTM46 (*cheY$^+$*); line 8, RP733 [*Δ(cheA–cheZ)2209 fliG1010*/pTM47 [*Δ(cheY)*]; line 9, RP733 [*Δ(cheA–cheZ)2209 fliG1010*]/pTM46 (*cheY$^+$*). The *Δ(cheA–cheZ)2209* deletion in strains 6–9 produces a CheA–CheZ fusion protein with about 5% of normal CheA activity (20, 21).

In strains lacking CheZ, CW rotation is no longer strictly dependent on stimulation of CheA activity by chemoreceptors and CheW (7). Under CheZ-less conditions, CW rotation in strains lacking all MCPs (Fig. 6, line 3) or all MCPs and CheW (Fig. 6, line 4) was fully inhibited by P2, indicating that the sensitive step is subsequent to chemoreceptor coupling control of CheA. The next steps in the pathway, CheA autophosphorylation and phosphotransfer to CheY, were bypassed by using a mutant form of CheY ($Asp^{13} \rightarrow Lys$, D13K) that produces CW rotation in the unphosphorylated state (31). In a *cheA* deletion background, the CheY D13K mutant caused highly CW-biased rotation, which was extensively inhibited upon P2 induction (Fig. 6, line 5). This result indicates that the P2-sensitive step involves either the interaction of CheY with the flagellar switch or the subsequent production of CW rotation by the flagellar motor. To determine whether the motor itself could be the P2 target, we looked for P2 effects in flagellar switch mutants able to carry out CheY-independent CW rotation. In the absence of CheY, P2 had no effect on the extent of CW rotation in a FliM^{CW}

mutant (Fig. 6, line 6) or a FliG^{CW} mutant (Fig. 6, line 8). The same switch mutants exhibited considerably more CW rotation in the presence of CheY (and CheA to phosphorylate it), but the CheY-dependent increment in CW rotation was largely abolished by P2 induction (Fig. 6, lines 7 and 9). Taken together, these results show that P2 fragments inhibit CW rotation by interfering with the interaction between phospho-CheY and the flagellar switch.

DISCUSSION

Structural Organization of the CheA Phosphotransfer Region. These studies show that the phosphotransfer region of the *E. coli* CheA protein contains two structural domains (P1 and P2) connected by an ≈25-aa linker (L1). A similar linker (L2) joins the P1–L1–P2 segment to the transmitter domain that catalyzes the CheA autophosphorylation reaction. The CheA protein of *S. typhimurium* has the same overall domain organization, including linker sequences in corresponding positions, implying that these flexible domain connectors are important to CheA function. They might, for example, permit movements of the P1 and P2 domains relative to each other and the rest of the molecule that enable CheA to cycle between autophosphorylation and phosphotransfer modes. Such conformational changes may underlie the coupling mechanisms that regulate CheA signaling activity in response to chemoreceptor input.

Roles of the P1 and P2 Domains in CheA Signaling. Swanson *et al.* (14) recently showed that CheA binds with high affinity ($K_d < 10^{-6}$ M) to its phosphorylation targets, CheB and CheY. These binding interactions presumably confer signaling specificity to the phosphotransfer reactions. Because a CheA fragment containing only the P1 and P2 domains [CheA-(1–233)] also binds tightly to CheY, the specificity determinants most likely reside within the phosphotransfer region (14). However, a P1 fragment failed to bind CheY (14), suggesting that the specificity determinants reside in P2. The properties of liberated P1 and P2 domains *in vivo* support this idea. Upon high-level expression in wild-type cells, P2 polypeptides are potent inhibitors of chemotactic behavior, whereas P1 fragments are not. Conceivably, P2 inhibits chemotaxis by binding to CheY, blocking its interaction with the flagellar switch.

Use of Domain Liberation to Detect Functional Domains in Proteins. As demonstrated here for the P2 domain of CheA, domain liberation can be a useful genetic tool for analyzing the functional architecture of a multidomain protein. This approach is based on the premise that protein domains invariably function through specific interactions with some partner, either a small molecule, another macromolecule, or another part of the same protein. When subcloned and overexpressed, a liberated domain should compete with its counterpart in the intact protein, disrupting its activity. This could happen in a number of ways—for example, through formation of nonfunctional heterooligomers with the parent protein, through stoichiometric titration of a common interaction target, or through creation of an aberrant or unregulated catalytic activity. In any event, the resultant mutant phenotype can provide a genetic handle for identifying the target of inhibition and for investigating the normal function of the domain. For example, mutations that alter the inhibitory properties of the liberated domain could be isolated to identify structural determinants involved in its interaction with target proteins.

Domain liberation does not always lead to pathological effects. Although both P1 and P2 proved stable as polypeptides, only P2 jammed chemotaxis. The reason for this difference in behavior may be that P2 interacts specifically with other proteins, whereas P1 interacts mainly with the catalytic domain of CheA. In reactions *in vitro*, P1 polypeptides can be phosphorylated in trans by an isolated catalytic domain (14), so P1 evidently contains the determinants needed for recognition and interaction with the catalytic center. Perhaps P1 fragments cannot displace their normal counterparts from the catalytic centers of intact CheA molecules. Alternatively, trans phosphorylation may occur *in vivo* but may fail to disrupt sensory signaling because phosphorylated P1 fragments can still function as phosphotransfer substrates.

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