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CheA, CheW, and CheY Are Required for Chemotaxis to Oxygen and Sugars of the Phosphotransferase System in *Escherichia coli*

EDWARD H. ROWSELL,[1]† JIMMIE M. SMITH,[1]‡ ALAN WOLFE,[2] AND BARRY L. TAYLOR[1]*

Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, California 92350,[1] and Department of Microbiology and Immunology, Loyola University Chicago School of Medicine, Maywood, Illinois 60153[2]

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We carried out studies with *Escherichia coli* to determine the site at which the methylation-independent pathways for taxis to oxygen and to sugars of the phosphoenolpyruvate:sugar phosphotransferase transport system converge with the methylation-dependent chemotaxis pathways. Using genetic reconstitution of the pathways in a null strain, we determined that all pathways examined required the products of the genes *cheA*, *cheW*, and *cheY*. Thus, we conclude that both the methylation-independent and methylation-dependent pathways converge at CheA, the histidine kinase product of *cheA*.

In *Escherichia coli* and *Salmonella typhimurium*, taxis to either oxygen (aerotaxis) or substrates of the phosphoenolpyruvate phosphotransferase transport system (PTS) does not require chemoreceptor methylation and demethylation for adaptation (4, 8, 13). In contrast, chemotactic adaptation to certain amino acids, dipeptides, or non-PTS sugars requires methylation and demethylation. (For a review of chemotaxis see references 2, 7, and 14.) In *E. coli*, the *tsr*, *tar*, *trg*, and *tap* genes encode the methylation-dependent chemoreceptors (in *S. typhimurium*, *tip* substitutes for *tap*) while the genes *cheR*, *cheB*, *cheA*, *cheW*, *cheY*, and *cheZ* encode the cytoplasmic proteins that make up the methylation-dependent pathway. Of these cytoplasmic proteins, CheR and CheB catalyze chemoreceptor methylation and demethylation, respectively (12, 17). CheW, in association with the signaling domain located on the cytoplasmic surface of each chemoreceptor, modulates the rate of CheA autophosphorylation of the CheA residue His-48 (2). Phospho-CheA then serves as the phosphodonor for CheY autophosphorylation of the CheY residue Asp-57. Phospho-CheY binds to the flagellar motor switch, increasing the probability of clockwise (CW) rotation (5). Finally, the CheZ protein accelerates dephosphorylation of phospho-CheY, a process that restores counterclockwise (CCW) rotation (5). Whereas bacteria swim in gently curved paths when their motors rotate CCW, they abruptly change direction by tumbling chaotically when some of the motors rotate briefly in a CW direction (7).

Aerotaxis in both *E. coli* and *S. typhimurium* requires an electron transport system (11). Chemotaxis to a PTS substrate requires both a functional PTS and the transport of that substrate (9, 16). The signal transduction pathways by which the electron transport system and the PTS communicate with the flagellar motors remain unknown, although evidence that the former involves the proton motive force exists (10). Since all chemotaxis pathways utilize the same motor and flagellar structures, the aerotaxis and phosphotransferase pathways must converge with the methylation-dependent pathway at, or before, the flagellar motor switch. We have investigated this point of convergence, determining which signal transduction components of the methylation-dependent pathways are also required for aerotaxis and PTS chemotaxis.

Table 1 lists the strains, plasmids, and phages used in the present study. Cells were grown at 30°C in tryptone broth supplemented with mannose or fructose, to induce expression of the appropriate PTS components, and other requisite inducers (i.e., isopropyl-β-D-thiogalactopyranoside [IPTG] or arabinose) and antibiotics. At an optical density at 600 nm of 0.2 to 0.4 the cells were harvested, washed, and resuspended in chemotaxis buffer for analysis of free-swimming cells (11) or resuspended in tethering buffer for tethered-cell assays (1). For tethering, the bacterial flagella were sheared and the bacteria were attached to the glass surface of a laminar flow chamber with antiflagellum antibody (1). The change in rotational behavior of cells in response to the addition or removal of PTS sugars was captured on a time-tagged video recording. Frame-by-frame analysis was used to score the bacteria as rotating either CW or CCW. Cells that rotated too rapidly to analyze, that stopped momentarily, or that detached from the glass during the analysis were scored as indeterminate.

Figure 1A shows the response by cells which are wild type for chemotaxis (strain RP437) to the addition of 56 μM mannose and the response to its removal. After the addition of mannose these cells increased transiently their CCW bias before adapting back to the original baseline behavior. After removal of mannose these cells decreased transiently their CCW bias before returning to baseline behavior. These observations document for the first time a negative response involving a PTS sugar. Similar results were obtained in response to the addition and removal of fructose (data not shown).

To identify the minimal excitatory pathway for oxygen and PTS substrates, we began by constructing a strain (HCB349) with many, but not all, of the chemotaxis genes deleted. Cells of this strain expressed CheA, CheW, Tar, and Trg from their respective genes, each located at its normal position on the chromosome under control of its native promoter. We transformed these cells with plasmids that expressed, under control of the arabinose promoter, either CheY and CheZ (pJH123)

* Corresponding author. Phone: (909) 824-4480. Fax: (909) 824-4035. Electronic mail address (Internet): blTaylor@ccmail.llu.edu.

† Present address: University of Minnesota Medical School, Department of Laboratory Medicine and Pathology, UMHC, Minneapolis, MN 55455.

‡ Present address: Coleman Clinic, Ltd., Canton, IL 61520-2690.

TABLE 1. Bacterial strains, plasmids, and phages used in this study

Strain, plasmid, or phage	Relevant genotype	Chemotaxis genes present[a]	Reference or source
Strains			
HCB326	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209 trg*::Tn*10*	None	18
HCB437	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209* Δ(*trg*)*100 zbd*::Tn*5*	None	18
HCB484	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209* Δ(*trg*)*100 zbd*::Tn*5 fliG*(*scyB10*)	None; carries *fliG*(*scyB10*)	18
HCB627	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209* Δ(*trg*)*100 zbd*::Tn*5* λDFB19	*cheY*	This study
HCB628	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209* Δ(*trg*)*100 zbd*::Tn*5* λDFB19/pDFB8	*cheW cheY*	This study
HCB673	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209* Δ(*trg*)*100 zbd*::Tn*5* λDFB19/pDFB41	*cheA cheY*	This study
HCB661	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209* Δ(*trg*)*100 zbd*::Tn*5* λDFB19/pDFB38	*cheA cheW cheY*	This study
HCB660	Δ(*tsr*)*7021* Δ(*cheA-cheZ*)*2209* Δ(*trg*)*100 zbd*::Tn*5* λAJW3/pDFB38	*tar cheA cheW cheY*	3
HCB349	Δ(*tsr*)*7021* Δ(*tap-cheZ*)*2206*	*trg tar cheA cheW*	This study
HCB513	Δ(*tsr*)*7021* Δ(*tap-cheZ*)*2206* Δ(*trg*)*100 zbd*::Tn*5* pJH123	*tar cheA cheW cheY cheZ*	This study
HCB525	Δ(*tsr*)*7021* Δ(*tap-cheZ*)*2206* Δ(*trg*)*100 zbd*::Tn*5* pJH120	*tar cheA cheW cheY*	18
HCB526	Δ(*tsr*)*7021* Δ(*tar-tap*)*5201* Δ(*trg*)*100 zbd*::Tn*5* λgt4.*tar*101	*tar cheA cheW cheR cheB cheY cheZ*	This study
RB437	Wild type for chemotaxis	All	15
Plasmids			
pDFB8	p*ara-cheW*		D. F. Blair
pDFB38	p*ara-motA motB cheA cheW*		3
pDFB41	p*ara-motA motB cheA*		3
pJH120	p*ara-cheY*		F. W. Dahlquist
pJH123	p*ara-cheY cheZ*		F. W. Dahlquist
Phages			
λAJW3	λgt4-p*meche-tar* p*lac-cheY*		3
λDFB19	λgt4-p*lac-cheY*		3

[a] Out of the following set: *tsr tar tap trg cheA cheW cheR cheB cheY cheZ.*

or CheY alone (pJH120) to yield the strains HCB513 and HCB525, respectively. Cells of these strains exhibited an extreme CW bias which made observing CCW responses difficult. We alleviated this extreme bias by pretreating cells of these strains with 0.5 μM aspartate (4). Under such conditions, cells of strain HCB513 responded to oxygen and mannose on a time scale similar to that exhibited by wild-type cells (strain RP437) and by cells lacking all the chemoreceptors except Tar (strain HCB526) (Table 2). Cells of strain HCB525 responded similarly to both mannose and fructose. They did not respond, however, to oxygen.

To further dissect the minimal excitatory pathway, we adopted a strategy similar to that used to define the minimal set of genes required for signaling in the methylation-dependent pathway (3, 18). Subsets of the *che* genes were restored via multicopy plasmids or lambda hybrids to cells of the null (or gutted) strain HCB437. Cells of this strain lack all the known chemoreceptors (i.e., Tsr, Tar, Trg, and Tap) and the cytoplasmic chemotaxis proteins (i.e., CheR, CheB, CheA, CheW, CheY, and CheZ) yet retain a functional flagellar motor and switch. We demonstrated previously that cells of a chemotaxis null strain (HCB326) did not respond either to oxygen or to fructose during a temporal assay of free-swimming cells (15). We obtained similar results in this study with the null strain HCB437 (data not shown). Unfortunately, cells of these null strains exhibit such a strong CCW bias that responses to oxygen or PTS substrates are likely masked. Therefore, we tested cells of strain HCB484, a null strain isogenic to HCB437 that possesses the *scyB10* suppressor mutation in the switch gene *fliG*. Although these cells reversed their flagellar rotation spontaneously (18), they did not respond to mannose or 56 μM fructose (data not shown).

By transformation with plasmid pDFB8, pDFB38, or pDFB41 and transduction with λDFB19 or λAJW3 we added back subsets of *cheA*, *cheW*, and *cheY* to cells of the null strain HCB437. Cells that expressed CheY (HCB627), CheW and CheY (HCB628), or CheA and CheY (HCB673) did not respond to the addition or removal of 56 μM mannose or 56 μM fructose. Figure 1B shows a set of representative experiments in which cells that expressed CheA and CheY did not respond to their exposure to mannose. In contrast, cells that expressed CheA, CheW, and CheY (HCB661) did respond to mannose (Fig. 1C). Intriguingly, however, this response was inverted relative to that of wild-type cells and the cells did not adapt (cf. Fig. 1A). After the addition of mannose, cells of strain HCB661 decreased their CCW bias and, unlike wild-type cells, did not return to baseline behavior until after the mannose was removed. Interestingly, we observed no peak of CCW bias after removal of mannose, as would be expected if this response were a mirror image of the response exhibited by wild-type cells. We observed a similar response to the addition or removal of 5.6 mM mannose (data not shown).

To investigate the nature of the inverted response, we manipulated the ratio of CheA and CheW expression relative to that of CheY in cells of strain HCB661 by varying independently the concentrations of the inducers arabinose and IPTG between 0 and 50 μM. By doing so, we observed that cells responded to the addition of mannose only when CheY expression was induced by 50 μM IPTG and CheA and CheW expression was induced by 10 or 25 μM arabinose. These responses also were inverted. Similarly, cells of strain HCB660 (which, in addition to CheA, CheW, and CheY, also expressed the aspartate receptor, Tar) exhibited an inverted response to mannose. In contrast, HCB660 cells responded normally to the addition of 2.5 μM aspartate (data not shown).

In summary, the minimal excitatory pathway for methylation-independent chemotaxis parallels that required for methylation-dependent chemotaxis, i.e., it requires CheA, CheW,

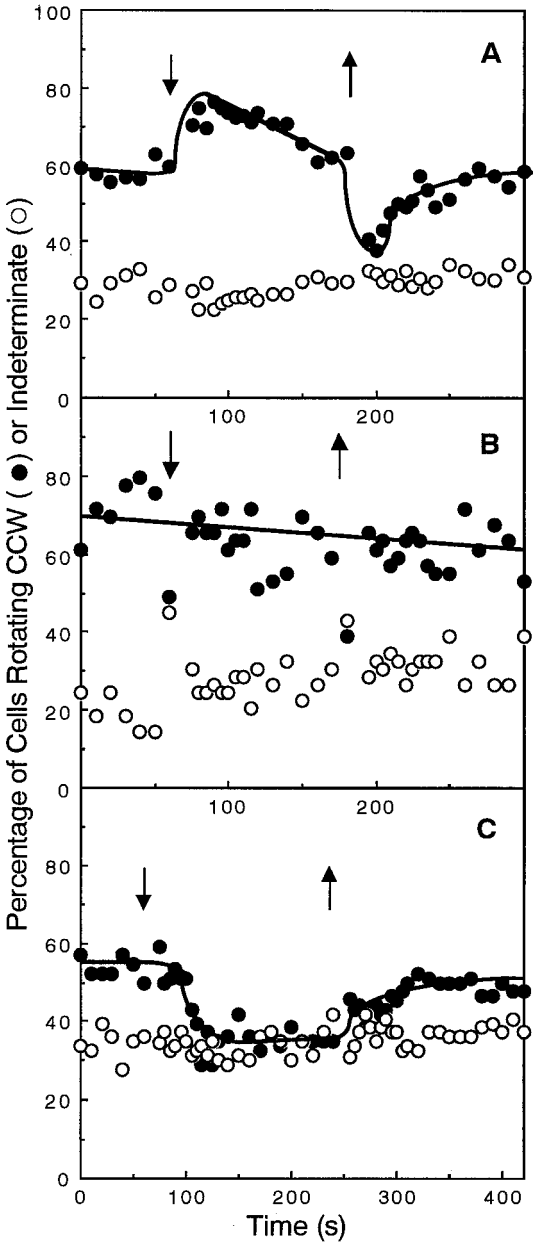

FIG. 1. Response of tethered *E. coli* cells to the addition (↓) or removal (↑) of 56 μM mannose (see text). (A) Cells wild-type for chemotaxis (strain RP437) (n = 148); (B) null cells which express CheA and CheY (strain HCB673) (n = 37); (C) null cells which express CheA, CheW, and CheY (strain HCB661) (n = 86). In cells of strains HCB673 and HCB66, expression of CheA and CheW was induced with 25 μM arabinose and expression of CheY was induced with 50 μM IPTG.

and CheY. It appears, then, that the methylation-dependent and methylation-independent pathways converge at CheA and also share both CheW and CheY.

The pathways by which the receptors for aerotaxis and chemotaxis to PTS substrates link with the CheA-CheW complex remain elusive. HPr and enzyme I represent the most attractive candidates for this linker role in the PTS (16). Initial genetic studies suggested that HPr was the linker; however, later genetic studies implicated enzyme I, and Lengeler and coworkers demonstrated recently that nonphosphorylated enzyme I inhibits CheA autophosphorylation in vitro (6). Extensive studies performed in one of our laboratories indicate that phosphoenolpyruvate, phospho-enzyme I, and phospho-HPr do not phosphorylate the CheA-CheW complex and CheA does not phosphorylate enzyme I. These results support a model in which methylation-independent signaling interacts with methylation-dependent signaling without phosphotransfer between the pathways.

TABLE 2. Response of *E. coli* strains to oxygen, mannose, and fructose

Strain	Chemotaxis genes present[a]	Response time (s)[b]		
		Oxygen	Mannose	Fructose
RP437	All	12.7	24.9	17.0
HCB526	*tar cheA cheW cheR cheB cheY cheZ*	15.7	26.3	
HCB513[c]	*tar cheA cheW* para-*cheY cheZ*[d]	10.5	14.5	
HCB525[c]	*tar cheA cheW* para-*cheY*[d]	NR	16.0	12.0

[a] Out of the following set: *tsr tar tap trg cheA cheW cheR cheB cheY cheZ*. Wild-type flagellar motor and switch genes are present in all strains listed.
[b] Time required for 50% of free-swimming *E. coli* cells to return to the prestimulus motility following the abrupt addition of 21% oxygen, 56 μM mannose, or 56 μM fructose. NR, no response observed.
[c] Cells were pretreated in 0.5 μM aspartate.
[d] Induced by 50 μM arabinose.

The nature of the inverted response remains a mystery. This is not the first reported instance of such an inverted response (see reference 4 for a summary of various conditions that result in inverted chemotactic responses). For example, cells lacking the methylesterase, CheB, respond to oxygen by increasing their CW bias instead of the usual increase in CCW bias exhibited by wild-type cells (4). The absence of CheB in the presence of the methylation-dependent chemoreceptors, however, cannot explain our results. Cells that expressed CheA, CheW, Tar, and Trg from the chromosome and CheY from a plasmid (but not CheB, CheR, or CheZ) (strain HCB525) responded normally to the addition of mannose or fructose. Only null cells transformed with a plasmid that expressed both CheA and CheW and transduced with a bacteriophage that expressed either CheY alone (strain HCB661) or CheY and Tar (strain HCB660) exhibited this anomalous response. Thus, we suspect that such a response occurred as a result of an imbalance between the expression levels of CheA and CheW relative to those of the PTS components. While this newly reported inverted response may be related to the interaction between certain components of the PTS system and chemotactic signaling, we cannot rule out the possibility that it results from some other process not directly related to signaling. Clearly, understanding the mechanism(s) responsible for the inverted response requires further study. We believe, however, that future models for bacterial chemotaxis should address the issue of inverted responses.

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