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The short form of the CheA protein restores kinase activity and chemotactic ability to kinase-deficient mutants

ALAN J. WOLFE*† AND RICHARD C. STEWART‡

*Department of Microbiology and Immunology, Loyola University Chicago, Maywood, IL 60153; and ‡Department of Microbiology and Immunology, McGill University, Montreal, Quebec, H3A 2B4, Canada

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ABSTRACT ***Escherichia coli*** **expresses two forms of the chemotaxis-associated CheA protein, $CheA_L$ and $CheA_S$, as the result of translational initiation at two distinct, in-frame initiation sites in the gene *cheA*. The long form, $CheA_L$, plays a crucial role in the chemotactic signal transduction mechanism by phosphorylating two other chemotaxis proteins: CheY and CheB. $CheA_L$ must first autophosphorylate at amino acid His-48 before transferring its phosphono group to these other signal transduction proteins. The short form, $CheA_S$, lacks the N-terminal 97 amino acids of $CheA_L$ and, therefore, does not possess the site of autophosphorylation. Here we demonstrate that although it lacks the ability to autophosphorylate, $CheA_S$ can mediate phosphorylation of kinase-deficient variants of $CheA_L$ each of which retains a functional autophosphorylation site. This transphosphorylation enables these kinase-deficient $CheA_L$ variants to phosphorylate CheY. Because it mediates this activity, $CheA_S$ can restore to kinase-deficient *E. coli* cells the ability to tumble and, thus, to perform chemotaxis in swarm plate assays.**

The chemotaxis-associated gene *cheA* of *Escherichia coli* encodes two proteins, $CheA_L$ (78 kDa) and $CheA_S$ (69 kDa), translated in-phase from two different initiation sites (1, 2). Found *in vitro* as a dimer (3), $CheA_L$ autophosphorylates a histidine residue (His-48) located near its N terminus (4). Phosphorylated $CheA_L$, in turn, phosphorylates two other chemotaxis proteins: CheY, required for clockwise signal generation, and CheB, required for adaptation (reviewed in refs. 5 and 6). $CheA_S$ lacks the site of autophosphorylation; however, it retains domains that in $CheA_L$ are required for both autophosphorylation and the transfer of phosphate to CheY and CheB (2, 4, 5).

In contrast to that of $CheA_L$, the role of $CheA_S$ in chemotactic signal transduction remains unknown. However, some clues concerning its function exist. Analysis of the interallelic complementation behavior of a large family of *cheA* mutants indicated that, *in vivo*, $CheA_S$ can perform some function associated with the C terminus of $CheA_L$ (1). *In vitro*, $CheA_S$ interacts physically with several essential components of the signal transduction pathway. First, complexes of CheW with both $CheA_L$ (3, 7, 8) and $CheA_S$ (7, 8) have been detected. Second, Matsumura and coworkers (7, 8) have suggested that a $CheA_L$/$CheA_S$/CheW complex binds to CheY to effect its phosphorylation. Third, they also have found evidence for formation of a complex between $CheA_S$ and CheZ, a protein that accelerates CheY dephosphorylation. Thus, $CheA_S$ might serve some role in regulating the activities of these components.

We report that $CheA_S$ can mediate *in vitro* phosphorylation of kinase-deficient $CheA_L$ mutant proteins which retain the site of autophosphorylation. Furthermore, *in vivo*, $CheA_S$ restores to cells that express these same $CheA_L$ mutants the ability to perform chemotaxis in swarm agar.

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MATERIALS AND METHODS

Bacterial Strains and Plasmids. All strains used as recipients in plasmid transformations or in the construction of those recipients were derivatives of *E. coli* K-12. J. S. Parkinson (University of Utah) kindly provided the $Pol^+Rec^+Che^+$ strain RP437 (9) and the Δ(*flhA–flhD*) strain RP3098 (1); C. Park (Korea Advanced Institute for Science and Technology, South Korea) the $polA^{ts}$ strain CP366 (10); and A. Binney (Purdue University) the *recA::cml* strain $MH6^-$. Transformants were constructed as described (11).

All alleles used are shown in Fig. 1. R. R. Bourret and M. I. Simon (California Institute of Technology) generously provided plasmids carrying the alleles *cheA*, *cheA501*, and *cheA504*. Wild-type allele *cheA* expresses both $CheA_L$ and $CheA_S$. Allele *cheA501* possesses a mutation that resides between codons 430 and 547. Cells that carry this allele express proteins $CheA_L501$ and $CheA_S501$, which lack the ability to autophosphorylate. Cells that carry this mutant allele exhibit nonchemotactic behavior as determined by swarm assays (12).

Allele $cheA_S$ was constructed by deleting the 5′ portion of the wild-type allele *cheA*. The polymerase chain reaction (13) was used to generate a *Hin*dIII site just upstream of the $CheA_S$ translational start site. In conjunction with the *Sal* I site located about 300 bp downstream of that start site, this engineered *Hin*dIII site was used to replace the 1358-bp *Hin*dIII–*Sal* I fragment of plasmid pMPC3 (kindly provided by M. P. Conley and H. C. Berg, Harvard University; Fig. 1) with a 5′ truncated 330-bp *Hin*dIII–*Sal* I fragment. Therefore, allele $cheA_S$ expresses only $CheA_S$. The amber mutation in allele *cheA503*(am) and the unique *Nhe* I used to track this mutation during various *in vitro* manipulations were generated by standard oligonucleotide-directed mutagenesis (14).

Strains AJW372 and AJW377 were constructed as follows. Alleles *cheA501* and *cheA503*(am), respectively, were introduced into the chromosome by means of homologous recombination in the $polA^{ts}$ host, strain CP366 (10). The resultant recombinants were tested for nonchemotactic behavior. Nonchemotactic recombinants were then tested for their ability to generate wild-type recombinants when transformed with plasmids (Fig. 1) carrying alleles *cheA* (pMPC3) or $cheA_S$ (pAF1), but not with a plasmid carrying $cheA_S501$ (pAF1*cheA501*). P1 transduction was used to transfer each mutant allele out of its Pol^{ts} host into the $Pol^+Rec^+Che^+$ recipient, strain RP437 (9), and to make the resultant transductants *recA* by using strain $MH6^-$ as the donor.

Plasmids (Fig. 1) used for swarm assays were derivatives of pDV4 (15) in which the *cheA* alleles were under the control of the tryptophan promoter, P_{trp}, of *Serratia marcescens*. P_{trp} was not induced by the addition of 3-β-indoleacrylic acid.

†To whom reprint requests should be addressed.

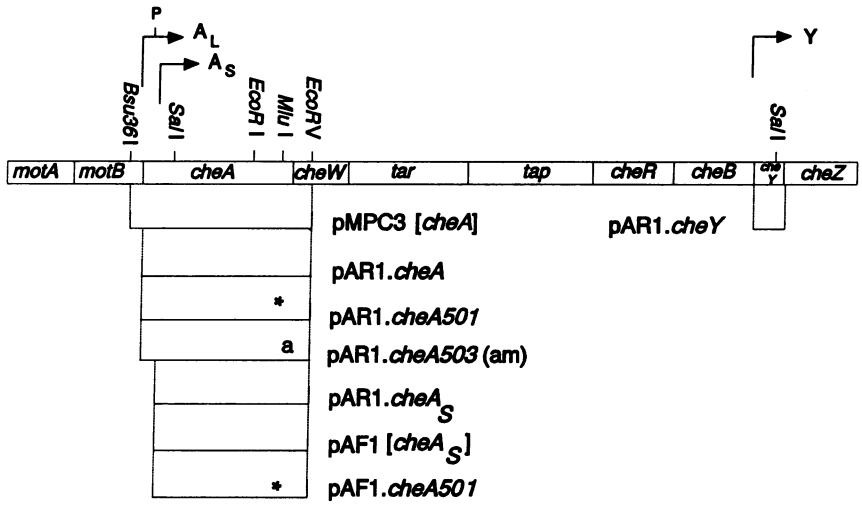

FIG. 1. Portions of the *mocha* and *meche* operons encoded by plasmids used for this work. The allele carried by each plasmid either is part of that plasmid's designation or is identified within brackets following the designation. Arrows indicate the locations of translational initiation sites for $CheA_L$, $CheA_S$, and CheY, respectively. P indicates the approximate location of the codon for the phosphorylation site in $CheA_L$. Also shown are the approximate locations of various *cheA* mutations: a, amber mutation; *, missense mutation.

Plasmids (Fig. 1) designed to overexpress either CheY or various forms of CheA by means of the *tac* promoter were generated by inserting *cheY*, *cheA*, *cheA$_S$*, *cheA501*, or *cheA503*(am) into the plasmid pAR1 (a derivative of pCW provided by A. Roth and F. W. Dahlquist, University of Oregon; ref. 3).

Swarm Assays. Cells were grown at 35°C to mid-exponential phase in tryptone broth (1% tryptone/0.5% NaCl) supplemented with the requisite antibiotics. Tryptone swarm plates were 0.20% agar in the same broth. Antibiotics were not added. A 5-μl aliquot of the culture (10^6–10^7 cells) was placed on the surface of a swarm plate near its center and the plate was incubated at 35°C in a humid environment. Swarm plates were handled and measured as described (16).

Protein Purification. The CheA and CheY overexpression plasmids were transformed into the Δ(*flhA–flhD*) strain RP3098 (1), which expresses no chemotaxis proteins. The resultant transformants were grown to ≈10^8 cells per ml at 30°C in L broth (1% tryptone/0.5% yeast extract/0.5% NaCl) containing ampicillin (0.1 mg/ml). Expression was induced by the addition of 1 mM isopropyl β-D-thiogalactopyranoside and the cells were harvested by centrifugation 6 hr later.

The various forms of CheA were purified as described (17), except for the addition of a Whatman DE-52 column after elution from the Affi-Gel Blue column. Over 95% of the purified wild-type CheA was $CheA_L$ as determined by Coomassie blue staining of polyacrylamide gels. CheY was purified by a combination of the procedures reported by Matsumura *et al.* (15) and Stock *et al.* (18). Protein concentrations were determined by the bicinchoninic acid (BCA) assay (Pierce) and by measuring absorbance at 280 nm. The molar extinction coefficients of CheY (8.25 $mM^{-1}\cdot cm^{-1}$) and the various forms of CheA (16.3 $mM^{-1}\cdot cm^{-1}$ for the wild-type protein) were calculated as described (19).

Protein Labeling, SDS/PAGE, Protein Visualization, and Immunoblotting. Phosphorylation reaction mixtures contained the indicated proteins in TKMD buffer (50 mM Tris·HCl/50 mM KCl/5 mM $MgCl_2$/0.5 mM dithiothreitol, pH 7.5) and 50 μM [γ-^{32}P]ATP (≈2500 cpm/pmol). Reactions were terminated by the addition of 2× SDS/PAGE sample buffer (20). Samples were subjected to SDS/PAGE (10% or 15% polyacrylamide gels) at 4–10°C in a Bio-Rad minigel apparatus under the conditions of Laemmli (21) except that Chromaphor protein stain (Promega) was added to the buffer in the upper electrode compartment. This enabled visualization of the protein bands during electrophoresis. Liquid scintillation counting of excised protein bands was performed using BioLite cocktail (ICN) and a Beckman LS6800 counter.

Immunoblot analyses (20) used affinity-purified rabbit anti-CheA antibodies.

RESULTS

***In Vivo* Complementation by $CheA_S$ of a Kinase-Deficient CheA Mutant.** Oosawa *et al.* (12) demonstrated the inability of mutant $CheA_L$501 protein to autophosphorylate. They generated *cheA501*, the mutant allele that expresses $CheA_L$501, by random hydroxylamine mutagenesis; the resultant mutation resides somewhere between codons 430 and 547. They also demonstrated that cells which carry allele *cheA501*, instead of the wild-type *cheA*, are nonchemotactic as measured by their inability to migrate in swarm assays. To test whether plasmid-encoded $CheA_S$ could restore chemotactic ability to mutant cells deficient in $CheA_L$ kinase activity, we constructed the kinase-deficient strain AJW372. By means of homologous recombination in the $PolA^{ts}$ strain CP366 (10) followed by P1 transduction (11), we replaced the chromosomally encoded wild-type *cheA* allele of strain RP437 (9) with the mutant allele *cheA501*. Plasmids (Fig. 1) that overexpressed $CheA_S$ (pAF1), $CheA_S$501 (pAF1cheA501), and $CheA_L$ and $CheA_S$ (pMPC3) were transformed into strain AJW372 to produce strains AJW374, AJW375, and AJW376, respectively. Cells of these strains were inoculated at the center of tryptone swarm plates and their swarms were compared (Fig. 2). Cells which overexpressed $CheA_S$ (strain AJW374) formed two concentric bands indicating that such cells were chemotactic to both L-serine and L-aspartate. The outermost band formed by these cells migrated faster (0.13 cm/hr) than those formed by cells which overexpressed the $CheA_S$ mutant $CheA_S$501 (AJW375). These latter cells formed dense irregular swarms that migrated at a rate of 0.02 cm/hr, characteristic of nonchemotactic cells that cannot tumble. Microscopic inspection revealed that cells which overexpressed $CheA_S$ tumbled occasionally; cells which overexpressed $CheA_S$501 did not. Cells that overexpressed both $CheA_L$ and $CheA_S$ (strain AJW376) formed two concentric bands indicating that such cells also were chemotactic to both L-serine and L-aspartate. The outermost band formed by these cells migrated faster (0.39 cm/hr) than those formed by cells that overexpressed only $CheA_S$. The outer band produced by cells that overexpressed $CheA_S$ (strain AJW374) migrated at about 33% the rate of the outer band produced by cells that overexpressed both $CheA_L$ and $CheA_S$ (strain AJW376). Qualitatively similar results were obtained with transformants of a strain (AJW377) that expressed the chromosomally encoded kinase-deficient CheA variant CheA503(am) (data not shown).

To determine whether the bands produced by cells which overexpressed $CheA_S$ (strain AJW374) resulted from chemotactic behavior and not simply a restoration of tumbling ability, we challenged these cells with a range of concentrations of the chemoattractant L-serine. Although the presence in the me-

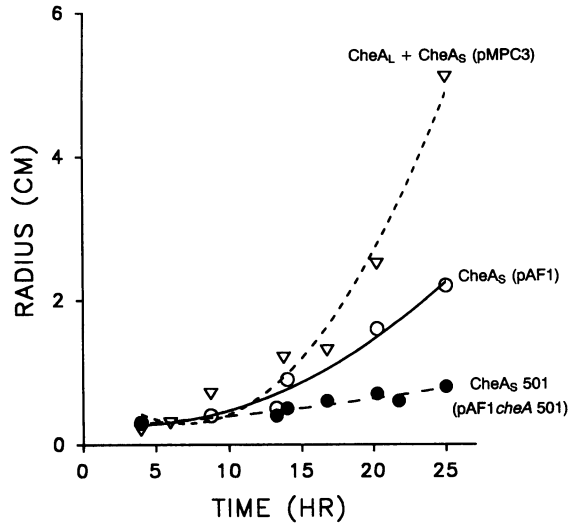

FIG. 2. Restoration of swarming ability in *cheA501* strains expressing CheA$_S$. Plots indicate the time course of displacement (radius) of the outermost edges of swarms produced by cells deficient for CheA kinase activity (strain AJW372) transformed with plasmids that direct expression of CheA$_S$ (strain AJW374, ○), CheA$_S$501 (strain AJW375, ●), or CheA$_L$ and CheA$_S$ (strain AJW376, ▽). Cells were inoculated near the center of tryptone swarm plates containing 0.20% agar and incubated at 35°C. The standard errors of the mean displacements were <0.05 cm.

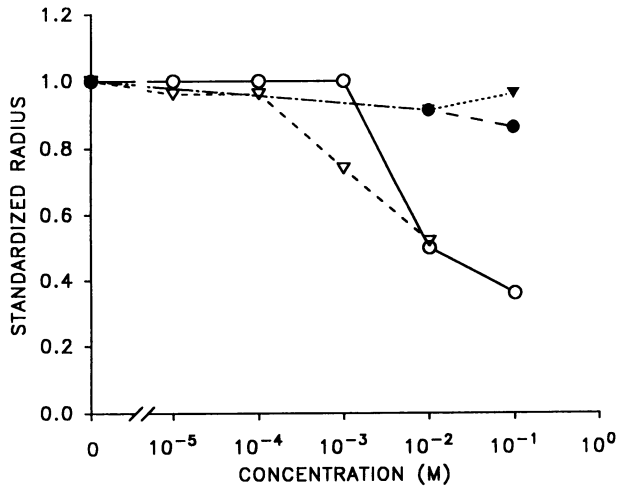

FIG. 3. Influence of L-serine upon the chemotactic ability of cells wild-type for chemotaxis and cells deficient for CheA$_L$ kinase activity that overexpress CheA$_S$. Plots indicate the displacement of the outermost edges of swarms produced by cells wild-type for chemotaxis (strain RP437) or deficient for CheA kinase activity and transformed with pAF1, a plasmid that expressed CheA$_S$ (strain AJW374). Cells were inoculated near the center of tryptone swarm plates containing 0.20% agar and various concentrations of either L-serine (open symbols) or sodium lactate (filled symbols). Radial displacements were measured after 7 hr (for RP437, triangles) and 16 2/3 h (for AJW374, circles) at 35°C. For each strain, these measurements were standardized to the mean displacement observed when neither L-serine nor sodium lactate was present. The standard errors of the mean displacements for duplicate swarms were <0.05 cm.

dium of up to 1 mM L-serine had no effect upon the ability of cells of strain AJW374 to produce an L-serine band, higher concentrations reduced that band until, at 10 mM L-serine, only the inner, L-aspartate band remained (Fig. 3). Similar behavior was observed when we challenged these cells with L-aspartate concentrations, except that the band affected was the inner one (data not shown). Equivalent concentrations of a compound that does not act as a chemoattractant, sodium lactate, had little effect upon the chemotactic ability of these cells (Fig. 3). To verify that saturating concentrations of a chemoattractant actually reduce chemotactic ability, we challenged wild-type cells (strain RP437; ref. 9) with the same conditions. Similar results were observed with these cells. The swarms produced by wild-type cells grown under these saturating conditions resembled those produced by cells deleted for the serine-specific chemoreceptor, Tsr, and the aspartate-specific chemoreceptor, Tar, respectively.

***In Vitro* Phosphorylation of Kinase-Deficient CheA Mutant Proteins in the Presence of CheA$_S$.** Wild-type CheA$_L$, wild-type CheA$_S$, and two kinase-deficient CheA$_L$ variants, CheA$_L$501 and CheA$_L$503(am), were purified to near homogeneity, as judged by SDS/PAGE (Fig. 4*A*), and confirmed as CheA variants by immunoblotting with anti-CheA antibody (Fig. 4*B*). These immunoblots also confirmed that our CheA$_S$ preparation was free of CheA$_L$ and that the CheA$_L$503(am) preparation contained only the expected truncated forms of CheA.

To assess the ability of CheA$_S$ to promote phosphorylation of the kinase-deficient variants CheA$_L$501 and CheA$_L$503(am), [γ-^{32}P]ATP was added to purified preparations of both mutant proteins in the absence and presence of CheA$_S$. In addition, [γ-^{32}P]ATP was incubated with the mutant protein CheA$_L$503(am) in the presence of wild-type CheA$_L$. At the conclusion of a 60-min labeling period at room temperature, each reaction was terminated by the addition of 2× SDS/PAGE sample-loading buffer and analyzed by SDS/PAGE followed by autoradiography (Fig. 5). As expected, in the reaction mixture containing wild-type CheA$_L$ (lane 1) about 45% of the wild-type protein became phosphorylated. No phosphorylation was exhibited by reactions with CheA$_S$ alone (lane 2), CheA$_L$501 alone (lane 3), or CheA$_L$503(am) alone (lane 4). In contrast, about 40% of CheA$_L$501 (lane 5) and 15% of CheA$_L$503(am) (lane 6) were phosphorylated when CheA$_S$ was present. When incubated with CheA$_L$503(am) (lane 7), wild-type CheA$_L$ phosphorylated both itself (about 30%) and the truncated protein (about 7.5%).

To monitor the kinetics of these reactions, we removed aliquots from reaction mixtures at specified times, terminated

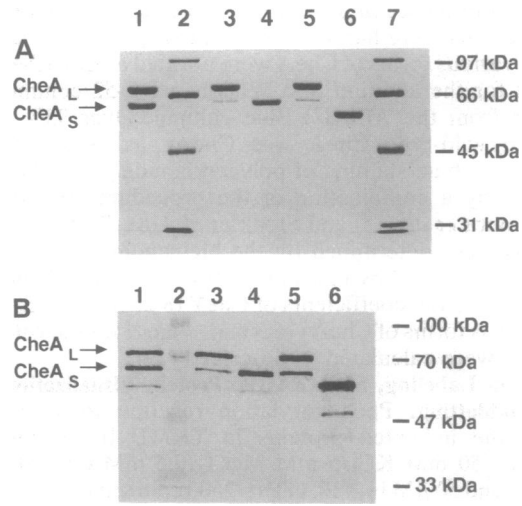

FIG. 4. Purified CheA variant proteins isolated from strain RP3098 carrying various CheA expression vectors. Lanes 1, mixture of CheA$_L$ and CheA$_S$ proteins encoded by plasmid pAR1.*cheA*; lanes 2, molecular mass standards (labeled in kilodaltons at right); lanes 3, purified wild-type CheA$_L$ encoded by plasmid pAR1.*cheA*; lanes 4, purified wild-type CheA$_S$ encoded by plasmid pAR1.*cheA$_S$*; lanes 5, purified CheA$_L$501 encoded by plasmid pAR1.*cheA501*; lanes 6, purified CheA$_L$503(am) encoded by pAR1.*cheA503*(am); lane 7, molecular mass standards (*A* only). (*A*) SDS/10% polyacrylamide gel stained with Coomassie blue. (*B*) Immunoblot analysis using rabbit anti-CheA (IgG) following SDS/10% PAGE.

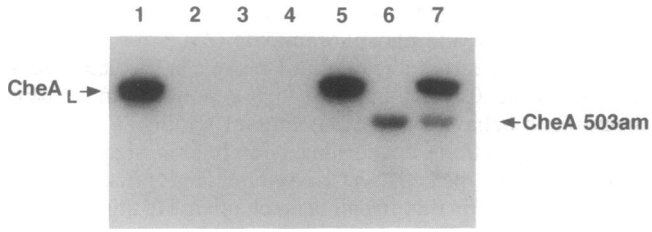

FIG. 5. Phosphorylation of $CheA_L$ variants in the absence or presence of $CheA_S$. CheA samples (5 μM final concentration of each protein) were incubated with 50 μM [γ-^{32}P]ATP (2500 cpm/pmol) in TKMD buffer (see *Materials and Methods*), the reactions were terminated after 60 min at room temperature by the addition of 2× SDS/PAGE sample loading buffer, and the samples were analyzed by SDS/10% PAGE followed by autoradiography for 60 min. Lane 1, wild-type $CheA_L$; lane 2, wild-type $CheA_S$; lane 3, $CheA_L501$; lane 4, $CheA_L503$(am); lane 5, wild-type $CheA_S$ plus $CheA_L501$; lane 6, wild-type $CheA_S$ plus $CheA_L503$(am); lane 7, wild-type $CheA_L$ plus $CheA_L503$(am). Correspondence was established between autoradiography bands and Chromaphor-visualized protein bands.

the reactions, separated the proteins by SDS/PAGE, and analyzed the extent of CheA phosphorylation by liquid scintillation (Fig. 6). Phosphorylation of wild-type $CheA_L$ exhibited an exponential time course with a $t_{1/2}$ of about 2 min and a final phosphorylation level of 45%. Although $CheA_S$-mediated phosphorylation of $CheA_L501$ was somewhat slower ($t_{1/2}$ = 7 min), the final level of phosphorylation (38%) was similar to that observed with wild-type $CheA_L$. $CheA_S$-mediated phosphorylation of $CheA_L503$(am) was considerably slower ($t_{1/2}$ = 38 min) and reached a final level of only about 15%.

***In Vitro* Phosphorylation of CheY by Phosphorylated Kinase-Deficient $CheA_L$ Mutant Proteins.** To enable clockwise flagellar rotation, the $CheA_S$-mediated phosphorylated forms of $CheA_L501$ and $CheA_L503$(am) each must be able to efficiently donate its phosphono group to CheY. To test this prediction, we generated phosphorylated $CheA_L501$ and phosphorylated $CheA_L503$(am) by incubating the unphosphorylated forms of each variant protein with [γ-^{32}P]ATP in the presence of $CheA_S$. At the conclusion of a 60-min labeling period, we added a substoichiometric amount of purified CheY, terminated the CheA → CheY phosphotransfer after

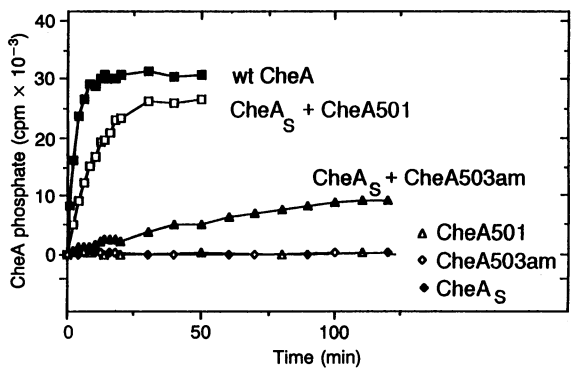

FIG. 6. Kinetics of mutant $CheA_L$ phosphorylation mediated by $CheA_S$ compared with kinetics of wild-type (wt) $CheA_L$ autophosphorylation. CheA samples (5 μM final concentration of each protein indicated) were mixed with 50 μM [γ-^{32}P]ATP (2740 cpm/pmol) in TKMD buffer (final volume, 200 μl) at room temperature. At the indicated times, 10-μl aliquots were removed and mixed with 10 μl of 2× SDS/PAGE sample buffer to terminate the reaction. Ten microliters of each sample was then analyzed by SDS/10% PAGE with simultaneous protein staining using the Chromaphor system. Bands containing CheA were excised and ^{32}P covalently attached to $CheA_L$ was determined by liquid scintillation. Values have been corrected for a small background (500 cpm) observed with $CheA_L$ samples that had been denatured before addition of ATP.

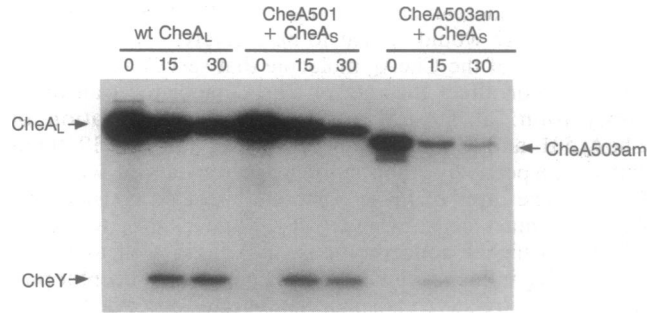

FIG. 7. Phosphorylation of CheY by wild-type (wt) and mutant CheA proteins. Various combinations of CheA variants (6 μM final concentration of each CheA variant indicated) were incubated with 50 μM [γ-^{32}P]ATP (2740 cpm/pmol) in TKMD buffer (final volume, 30 μl). Each labeling mixture was divided into three 10-μl aliquots: to the first aliquot, 8 μl of TKMD buffer was added and after 30 sec, 18 μl of 2× SDS/PAGE sample buffer was added to terminate the reaction (such samples correspond to the 0 time point); to the second aliquot, 8 μl of 2.25 μM CheY (in TKMD buffer) was added and after 15 sec, 18 μl of 2× SDS/PAGE sample buffer was added to terminate the reaction (such samples correspond to the 15-sec time point); and to the third aliquot, 8 μl of 2.25 μM CheY (in TKMD buffer) was added and after 30 sec, 18 μl of 2× SDS/PAGE sample buffer was added to terminate the reaction (such samples correspond to the 30-sec time point). Ten microliters of each sample was then analyzed by SDS/15% PAGE followed by autoradiography. Correspondence was established between autoradiography bands and Chromaphor-visualized protein bands.

either 15 or 30 sec, and analyzed the samples by SDS/PAGE followed by autoradiography (Fig. 7). Because phosphorylated CheY rapidly catalyzes its own dephosphorylation (22, 23), the levels of CheY phosphorylation observed reflect the relative rates of CheA-mediated CheY phosphorylation and CheY autodephosphorylation. The phosphorylated forms of wild-type $CheA_L$ (lanes 1–3), $CheA_L501$ (lanes 4–6), and $CheA_L503$(am) (lanes 7–9) rapidly donated phosphate to CheY. It is not possible from these results to make rigorous comparisons of phosphotransfer kinetics from the various phosphorylated CheA variants to CheY. For both wild-type $CheA_L$ and the mutant $CheA_L501$, however, the reaction was sufficiently rapid to enable phosphorylation of CheY to reach similar maximal levels within 15 sec after CheY addition. In contrast, the level of CheY phosphorylation obtained with $CheA_L503$(am) was considerably lower, suggesting that the phosphotransfer may be slower with this variant.

DISCUSSION

We examined the ability of $CheA_S$ to interact productively with other selected components of the *E. coli* chemotactic signal transduction pathway in the absence of $CheA_L$ kinase activity. *In vivo*, the presence of $CheA_S$ restored chemotactic ability to $CheA_L$ kinase-deficient cells, presumably by enabling the cells to tumble. These cells, like those wild-type for chemotaxis, produced chemotactic bands and sensed the presence of saturating concentrations of chemoattractants in the medium. Thus, $CheA_S$ restored to these cells the ability to both tumble and respond to chemotactic stimuli. Our *in vitro* investigations of the underlying biochemistry indicated that $CheA_S$ mediates ATP-dependent phosphorylation of $CheA_L$ variants that are incapable of autophosphorylation. This phosphorylation appears to result from transphosphorylation of $CheA_L$ by $CheA_S$ and can occur on a time scale similar to that observed for autophosphorylation of $CheA_L$. Conceivably, $CheA_S$-mediated phosphorylation of $CheA_L$ could occur by resurrecting a cryptic or latent kinase activity associated with the various $CheA_L$ mutants, although the

existence of such an activity in both of the $CheA_L$ mutants examined here would appear to be unlikely.

Properties of the $CheA_L$ molecule that serves as the phospho acceptor affect the rate of $CheA_S$-mediated transphosphorylation: at 50 μM ATP, transphosphorylation of $CheA_L$501 and $CheA_L$503(am) is about 3.5 and 19 times slower, respectively, than autophosphorylation of wild-type $CheA_L$. The rates of these reactions might be considerably faster in intact cells, where $CheA_L$ and $CheA_S$ would be subject to higher concentrations of ATP as well as to the accelerating influence of CheW and chemotaxis receptor proteins (5). The relative phosphorylation rates observed *in vitro* paralleled the relative ability of each $CheA_L/CheA_S$ combination to support chemotaxis in swarm assays: chemotaxis was maximal when wild-type $CheA_L$ was present, somewhat reduced when $CheA_S$ was paired with $CheA_L$501, and markedly reduced when $CheA_S$ was paired with $CheA_L$503(am).

We have not determined the location of the $CheA_L$ phosphono group resulting from $CheA_S$-mediated transphosphorylation; however, this phosphono group is readily available for transfer to CheY. This suggests that $CheA_S$-mediated transphosphorylation occurs at the same position as does $CheA_L$-mediated autophosphorylation (His-48; ref. 4). Our results with various mutant CheA proteins suggest that, in wild-type cells, both transphosphorylation of $CheA_L$ by $CheA_S$ and transphosphorylation of one $CheA_L$ molecule by another can occur. Such reactions could play significant roles in controlling cell swimming behavior.

Evidence exists that the osmoregulation-associated CheA homolog, EnvZ, uses a similar transphosphorylation mechanism (24). That transphosphorylation occurs in both the chemotaxis and osmoregulatory systems does not preclude the possibility that intramolecular phosphorylation plays an equally important or even dominant role in these signal transduction pathways. Hess *et al.* (22) demonstrated that the rate of $CheA_L$ autophosphorylation is insensitive to the effects of dilution and concluded that this reaction proceeds via an intramolecular mechanism. However, in view of the demonstration that $CheA_L$ exists as a stable dimer (3), transphosphorylation of one member of a dimer by the other member could also occur in a dilution-independent manner.

The mechanism of $CheA_S$ transphosphorylation of $CheA_L$ remains unclear. It could involve formation of $CheA_L/CheA_S$ heterodimers. For example, it is possible that a functional kinase active site requires amino acid residues from both protomers of a CheA dimer. Alternatively, it could result from transient interaction of a $CheA_L$ dimer with a distinct $CheA_S$ molecule, present as either a monomer or a dimer. There is no clear-cut evidence of the existence of $CheA_L/CheA_S$ heterodimers, although some form of interaction may be indicated by the ability of excess $CheA_L$ to suppress the deleterious effects of $CheA_S$ overproduction *in vivo* (A.J.W., M. P. Conley, and H. C. Berg, unpublished results).

The ability of CheY to acquire an activating phosphono group from various kinase-deficient $CheA_L$ mutants phosphorylated by $CheA_S$ raises several interesting possibilities. First, these mutant CheA proteins may be functional as CheY kinases despite being incompetent in autophosphorylation. Second, $CheA_S$ may transfer to CheY the phosphono group of the mutant phosphorylated $CheA_L$. Third, CheY phosphorylation in these experiments may reflect the ability of CheY to catalyze its own phosphorylation by using phosphorylated $CheA_L$ as a high-energy phospho donor. In this scenario, CheY functions as an autokinase and the primary role of $CheA_L$ is to provide phosphohistidine for CheY autophosphorylation. Thus, CheA would not be thought of as a CheY kinase, but rather as an autokinase that, when phosphorylated, serves as a substrate for CheY. Indeed, evidence exists that suggests that CheY has the ability to function as an autokinase: CheY can acquire a high-energy phosphono group from small organic molecules such as acetyl phosphate and phosphoramidate (25).

Our results clearly indicate that $CheA_S$ can mediate phosphorylation of $CheA_L$ and that this reaction can restore chemotactic behavior in various mutant strains. This raises interesting questions concerning chemotactic signal transduction in wild-type cells. Is $CheA_S$-mediated transphosphorylation involved in this signaling mechanism? Is this reaction regulated in response to chemotactic stimuli? Experiments to address these questions will better define the possible role(s) of $CheA_S$ in chemotactic signal transduction.

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