doi:10.1006/jmbi.2000.3595 available online at http://www.idealibrary.com on IDEAL® J. Mol. Biol. (2000) 297, 543–551

JMB

COMMUNICATION

NMR Structure of Activated CheY

Ho S. Cho[1], Seok-Yong Lee[1,2], Dalai Yan[3], Xiaoyu Pan[3] John S. Parkinson[4], Sydney Kustu[3], David E. Wemmer[1,5] and Jeffrey G. Pelton[1]*

[1]*Physical Biosciences Division Lawrence Berkeley National Laboratory, 1 Cyclotron Rd Berkeley, CA 94720, USA*

[2]*Graduate Group in Biophysics University of California Berkeley, CA 94720, USA*

[3]*Department of Plant and Microbial Biology, University of California, 111 Koshland Hall, Berkeley, CA 94720, USA*

[4]*Department of Biology University of Utah, Salt Lake City, UT 84112, USA*

[5]*Department of Chemistry University of California Berkeley, CA 94720, USA*

**Corresponding author*

The CheY protein is the response regulator in bacterial chemotaxis. Phosphorylation of a conserved aspartyl residue induces structural changes that convert the protein from an inactive to an active state. The short half-life of the aspartyl-phosphate has precluded detailed structural analysis of the active protein. Persistent activation of *Escherichia coli* CheY was achieved by complexation with beryllofluoride (BeF_3^-) and the structure determined by NMR spectroscopy to a backbone r.m.s.d. of 0.58(±0.08) Å. Formation of a hydrogen bond between the Thr87 OH group and an active site acceptor, presumably Asp57·BeF_3^-, stabilizes a coupled rearrangement of highly conserved residues, Thr87 and Tyr106, along with displacement of β4 and H4, to yield the active state. The coupled rearrangement may be a more general mechanism for activation of receiver domains.

Keywords: response-regulator; signal transduction; beryllofluoride; two-component; NMR spectroscopy

Bacteria move toward favorable chemical environments by modulating smooth swimming and tumbling motions in a process known as chemotaxis (Falke *et al*., 1997). Ligand binding to a transmembrane chemoreceptor elicits a signal that is ultimately carried to the flagellar motor by the phosphorylated form of the response regulator, CheY. The phosphorylation state of CheY is controled by the opposing activities of the sensor kinase CheA and the phosphatase CheZ (Djordjevic & Stock, 1998). CheA and CheY comprise a two-component signaling system, a pervasive signal transduction system in bacteria, which is also utilized in some eukarya (Nixon *et al*., 1986; Ota & Varshavsky, 1993; Parkinson & Kofoid, 1992; Stock *et al*., 1995).

CheY has been extensively studied as a model for understanding the link between phosphorylation and the activation of response regulators. Phosphorylation of Asp57 (Sanders *et al*., 1989) induces a structural change (Drake *et al*., 1993; Lowry *et al*., 1994) that enhances the interaction of CheY with the flagellar basal body protein FliM (Welch *et al*., 1993) and with CheZ (Blat & Eisenbach, 1994), but reduces its affinity for CheA (Li *et al*., 1995). Studies of mutant forms suggest that the structural changes caused by phosphorylation, but not the phosphoryl group itself, are important for activation (Djordjevic & Stock, 1998). Functional roles have been assigned for several highly conserved residues, including Asp12 and Asp13 (Stock *et al*., 1993), Asp57 (Sanders *et al*., 1989), and Lys109 (Lukat *et al*., 1991). The importance of Thr87 and Tyr106 has also been noted (Appleby & Bourret, 1998; Zhu *et al*., 1997), but due to the transient nature of phosphorylated CheY, a detailed understanding of their roles in the activation process has been difficult to attain.

Recently, Yan *et al*. (1999) discovered that BeFx forms a persistent complex with response regulators to yield an acyl-phosphate analog. Functionally, NtrC, OmpR, and NarL complexed to BeFx are very similar to their phosphorylation-activated

E-mail address of the corresponding author: jgpelton@lbl.gov

0022-2836/00/030543–9 $35.00/0

counterparts. For CheY, several lines of evidence show that BeFx·CheY mimics P-CheY. BeFx·CheY has the same affinity as P-CheY for a 16-residue N-terminal peptide derived from FliM (Yan *et al.*, 1999); BeFx·CheY has increased binding affinity for CheZ and magnesium (Figure 1, and data not shown); BeFx·CheY shows very similar changes in amide chemical shifts upon beryllofluoride binding as upon phosphorylation (Lowry *et al.*, 1994; Yan *et al.*, 1999). Although technical difficulties prevented determination of whether both BeF_4^{-2} and BeF_3^- generate active complexes with NtrC, monitoring of BeFx-dependent quenching of CheY fluorescence at different fluoride concentrations provided evidence that only BeF_3^- can generate an active species (Figure 1(b)). Here, we report the solution structure of BeF_3-activated CheY and interpret it in the light of previous structural, biochemical and genetic data. Our results suggest a mechanism by which phosphorylation induces the active conformation *via* a coupled structural rearrangement involving Thr87 and Tyr106, along with displacement of β4 and H4.

Triple-resonance NMR methods with uniformly ^{15}N-labeled or $^{15}N/^{13}C$-labeled samples were used to assign all of the backbone amide ^1H and ^{15}N resonances except for Ala2, Ser15, and Thr16 (Figure 2(a)), and 89% of the non-exchangeable side-chain resonances. Distance and torsion angle restraints derived from the NMR data were used to calculate 60 structures using DYANA (Güntert *et al.*, 1997). The 27 structures with a residual target function of less than 1.0 Å^2 were subjected to energy minimization using the program OPAL (Luginbühl *et al.*, 1996) (Table 1). The ensemble of structures (Figure 2(b)) reveals the $(\beta/\alpha)_5$ fold of receiver domains. The five helices (H1 (15-26); H2 (39-46); H3 (65-73); H4 (92-101); and H5 (113 -126)) and the five-stranded parallel β-sheet (β1 (7-11); β2 (33-35); β3 (53-57); β4 (83-87); and β5 (105 -108)) are well defined by the data, yielding a backbone r.m.s.d. of 0.40(±0.06) Å (Table 1).

Comparison of backbone coordinates for BeF_3·-CheY with previously determined X-ray structures of apo protein (Volz & Matsumura, 1991), magnesium-bound (Bellsolell *et al.*, 1994), CheA-complexed (Welch *et al.*, 1997), hyperactive mutant form Tyr106Trp (Zhu *et al.*, 1997), as well as with a magnesium-bound NMR structure (Moy *et al.*, 1994) revealed low r.m.s.d. values (range 0.9 to 1.4 Å). In addition, $^{13}C^\alpha$ shifts, which are sensitive to changes in electronic environment and structure, are similar (difference less than 1 ppm) for most, but not all residues of the BeF_3-activated and magnesium-bound forms of the protein. The largest backbone structural change is movement of the C-terminal portion of β4, which includes Thr87, toward Asp57 on β3. This is likely due to interaction of Thr87 with the Asp57·BeF_3^- group (see below).

Small differences observed in the backbone conformation of inactive CheY, especially in H4 and the loop between β4 and H4, led to the notion that

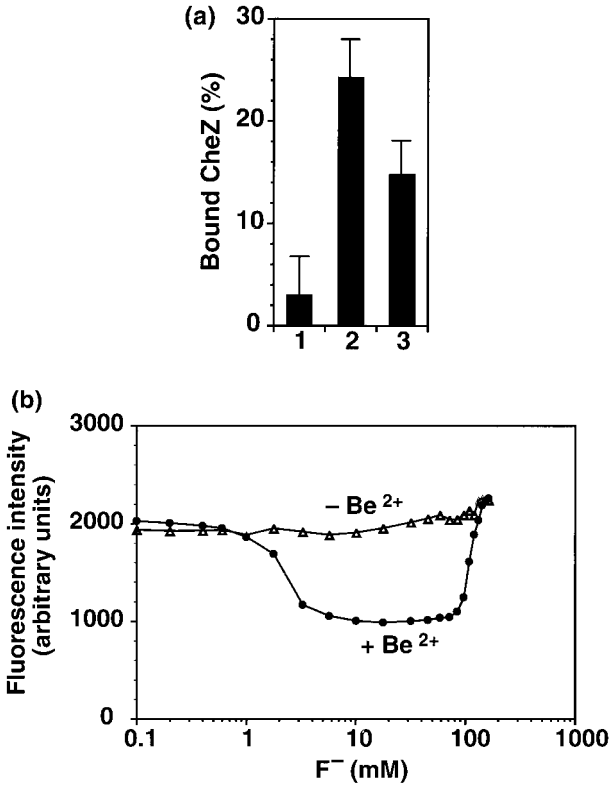

Figure 1. Biochemical assays of BeF_3·CheY. (a) Binding of CheZ to CheY. The assay was carried out exactly as described by Blat & Eisenbach (1994) and Halkides *et al.* (1998). CheZ (3 nmol) was incubated with immobilized CheY (3 nmol) in the presence of (1) buffer alone (as control); (2) 0.2 mM $BeCl_2$ and 10 mM NaF; and (3) 20 mM acetyl phosphate. (b) Response of fluorescence intensity of CheY to the concentration of F^- in the presence or absence of Be^{2+}. Fluorescence intensity was determined at 345 nm with excitation at 280 nm on a Hitachi F-4500 fluorescence spectrophotometer. The CheY samples (10 μM CheY in 50 mM sodium phosphate (pH 7.2) and 5 mM $MgCl_2$) were titrated with 1 M NaF in the presence (filled circles) or absence (open triangles) of 0.1 mM $BeCl_2$. Note that quenching occurs above 1 mM F^-, where BeF_3^- is the predominant species and is lost above 100 mM F^-, where BeF_4^{2-} is the predominant species (Goldstein, 1964; Martin, 1988). The gene for *E. coli* CheY was cloned into a PET21a vector (Novagen, Madison, WI) using PCR and standard molecular biology techniques. The sequence was confirmed by DNA sequencing. The protein was over-expressed in *E. coli* strain BL21(DE3)/pACYC and was purified as described by Bruix *et al.* (1993).

the protein is structurally malleable, and that this particular region can adopt multiple conformations (Djordjevic & Stock, 1998). For example, in the NMR structure of magnesium-bound CheY (Moy *et al.*, 1994) and the X-ray structures of apo CheY (Volz & Matsumura, 1991) and magnesium-bound (10 mM Mg^{2+}) CheY (Stock *et al.*, 1993), H4 begins at Lys92, but in another X-ray structure of magnesium-bound (100 mM Mg^{2+}) CheY (Bellsolell

Table 1. Structural statistics of the energy-minimized NMR structures of BeF_3 -activated *E. coli* CheY

Parameter	27 Conformers[a]
Residual distance restraint violations	
Number $\geqslant$0.1 Å	0.2(±0.4)
Maximum (Å)	0.1(±0.01)
Residual dihedral angle violations	
Number $\geqslant$2.5°	0.1(±0.3)
Maximum (deg.)	2.1(±0.3)
AMBER energies (kcal mol^{-1})	
Total	−5184 ± 95)
van der Waals	−467(±24)
Electrostatic	−5609(±87)
r.m.s. deviation from ideal geometry	
Bond lengths (Å)	0.0070(±0.0001)
Bond angles (deg.)	1.87(±0.04)
Peptide bonds (deg.)	0.16(±0.04)
Atomic r.m.s. differences[b] (Å)	
Backbone residues 6-129	0.58(±0.08)
Backbone for helices and β-sheet	0.40(±0.06)
Backbone-side-chain <40 % solvent-exposed	1.0(±0.1)

The average residual target function value for the 27 best DYANA conformers before energy minimization was 0.3(± 0.2) $Å^2$.

[a] For each entry the average for the 27 conformers and the standard deviation among the 27 conformers is given.

[b] Backbone atoms include N, C^α, and C′.

et al., 1994), H4 begins at residue Asn94 (Figure 2(c)). The conformational flexibility of the β4-H4 loop and marginal stability of H4 are also supported by ^{15}N NMR relaxation and hydrogen-deuterium exchange studies of the inactive magnesium-bound protein (Moy *et al*., 1994).

Similar to the apo crystal and magnesium-bound X-ray and NMR structures, in BeF_3-activated CheY, H4 begins at residue Lys92. However, significant differences in $^{13}C^\alpha$ shifts were observed between the BeF_3-activated and inactive magnesium-bound (Moy *et al*., 1994) forms for Val86 in β4 as well as Asn94 and Lys91, which are located in H4 and the loop between β4 and H4, respectively. These data point to possible structural changes resulting from activation. Comparison of BeF_3-activated CheY with the inactive structures shown in Figure 2(c) reveals movement of H4 up and toward the active site. In addition, when compared to NMR studies of the inactive magnesium-bound protein (Moy *et al*., 1994), activation increases the stability of this helix, as evidenced by substantial protection for the amides of residues Ile95 through to Ala99. Interestingly, phosphorylation of the homologous domain of NtrC appears to induce large conformational changes in the H4 region (Kern *et al*., 1999). In contrast, our data show that activation of CheY induces small but significant structural rearrangements, but no large conformational changes. This is consistent with previous biochemical data which show only a 20-fold increase in FliM binding upon phosphorylation (Welch *et al*., 1993).

In virtually all known receiver domains the residue equivalent to position 87 in CheY is conserved as either threonine or serine, and position 106 is conserved as either tyrosine or phenylalanine (Volz, 1993). Previous biochemical and genetic data suggested that the side-chain of Tyr106 (Zhu *et al*., 1996) and the hydroxyl group of Thr87 (Appleby & Bourret, 1998) participate in conformational changes leading to the active structure of CheY. In addition, structural studies of inactive wild-type and mutant forms of CheY indicated that there is steric competition between these two side-chains for a hydrophobic pocket created by β4, H4, and the intervening loop (β4-H4-pocket) (Ganguli *et al*., 1995; Zhu *et al*., 1996, 1997). Except for Tyr106Trp, which is hyperactive *in vivo*, other mutational substitutions at this position caused either a major decrease (Phe) or complete loss of activity (Zhu *et al*., 1996). In the crystal structure of unphosphorylated (inactive) mutant form Tyr106Trp, the Trp residue side-chain was found in the β4-H4-pocket, whereas in the mutant forms Thr87Ile and Thr87Ile/Tyr106Trp, which are inactive *in vivo*, the aromatic ring of 106 was solvent exposed, and the pocket occupied by the side-chain of Ile87 (Zhu *et al*., 1997). Analogous to the case for Ile87 in Thr87Ile and Thr87Ile/Tyr106Trp, in inactive magnesium-bound CheY, Tyr106 was exposed, and a significant portion of the β4-H4 pocket was occupied by the methyl group of Thr87 (Bellsolell *et al*., 1994; Moy *et al*., 1994). Our structure of BeF_3-activated CheY is more similar to Tyr106Trp, in that the side-chain of Tyr106 is buried in the β4-H4-pocket, making contacts with Tyr58, Thr87, Asn94, Ile95 and Ala98 of H4, whereas the methyl group of Thr87 is displaced from the pocket (see below).

We have identified the resonance for the hydroxyl proton of Thr87 in BeF_3-activated CheY as an exchangeable, non-nitrogen-bound proton with NOEs to the amide protons of Trp58, Ala88, and Glu89 (data not shown). These data strongly suggest that a hydrogen bond is formed between Thr87 and an active site acceptor, presumably $Asp57 \cdot BeF_3^-$. In the inactive state, the methyl group of Thr87 is in the β4-H4-pocket, with Tyr106 in the solvent-exposed position (Figure 3(a)). Activation moves the hydroxyl group of Thr87 (and β4) toward the active site, promoting formation of a hydrogen bond. This results in the removal of the Thr87 methyl group from the β4-H4-pocket, which is then filled by Tyr106 (Y-T coupling) (Figure 3(b)). Thus, the buried-exposed conformation of Tyr106 is coupled *via* Thr87 to phosphorylation of Asp57 (Sanders *et al*., 1989). A similar arrangement of the equivalent threonine and tyrosine in the phosphorylated response regulators Spo0A (Lewis *et al*., 1999) and FixJ (Birk *et al*., 1999) was recently reported. The high degree of conservation of these residues, combined with the structural data suggest that this may be a general mechanism for inducing structural changes leading to the activation of receiver domains. Although

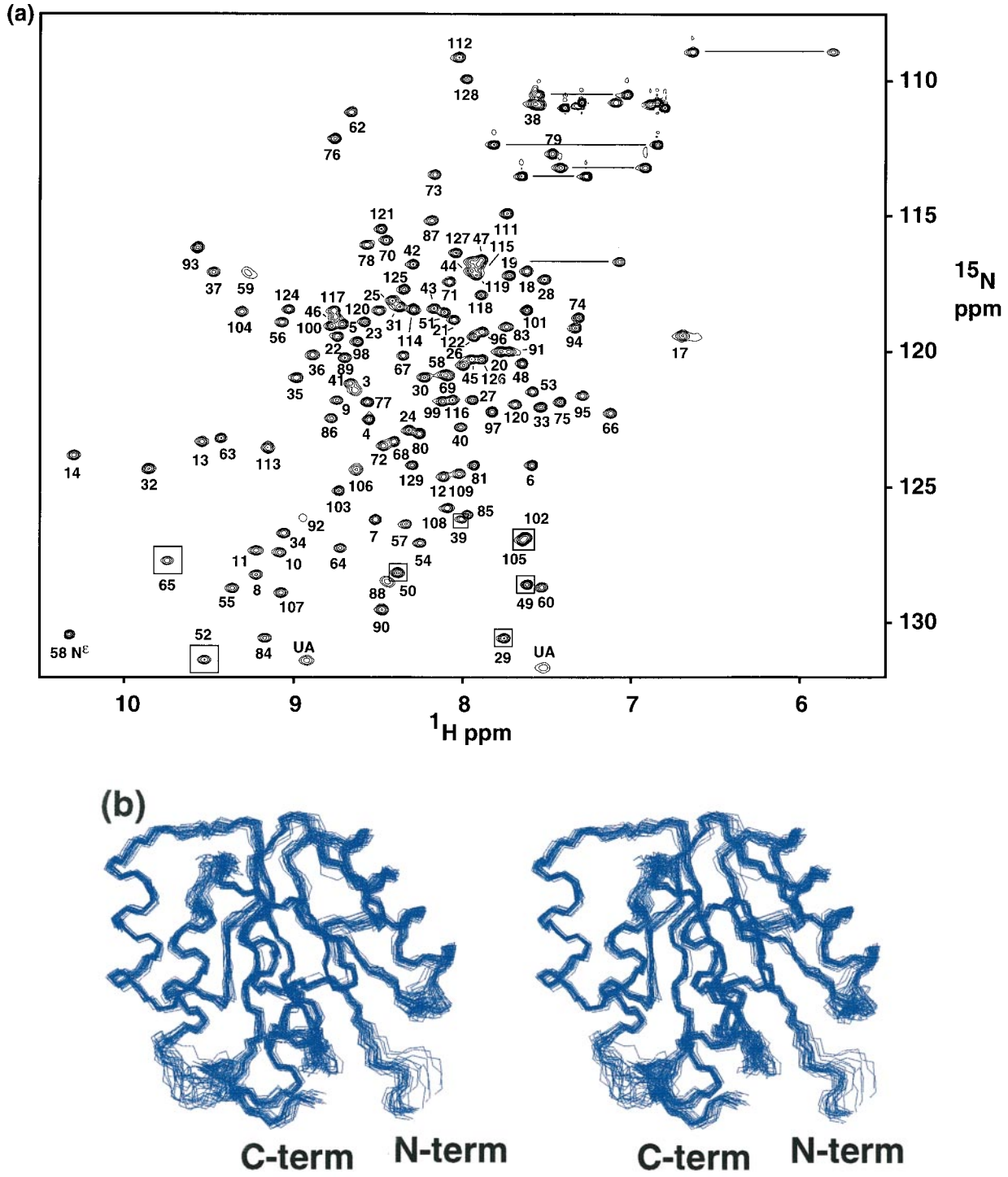

Figure 2 (*legend opposite*)

resolution in the active site of the phosphorylated receiver domain of NtrC was not sufficient to define the mechanism of activation, both structural (Kern *et al.*, 1999) and genetic/biochemical studies (Klose *et al.*, 1993; Moore *et al.*, 1993; D.Y. & S.K., unpublished data) indicate that there are significant differences between the activation of NtrC and the activation of both CheY and the receiver domain of Spo0A (Lewis *et al.*, 1999). Whether or not FixJ is more similar to CheY or NtrC remains to be determined.

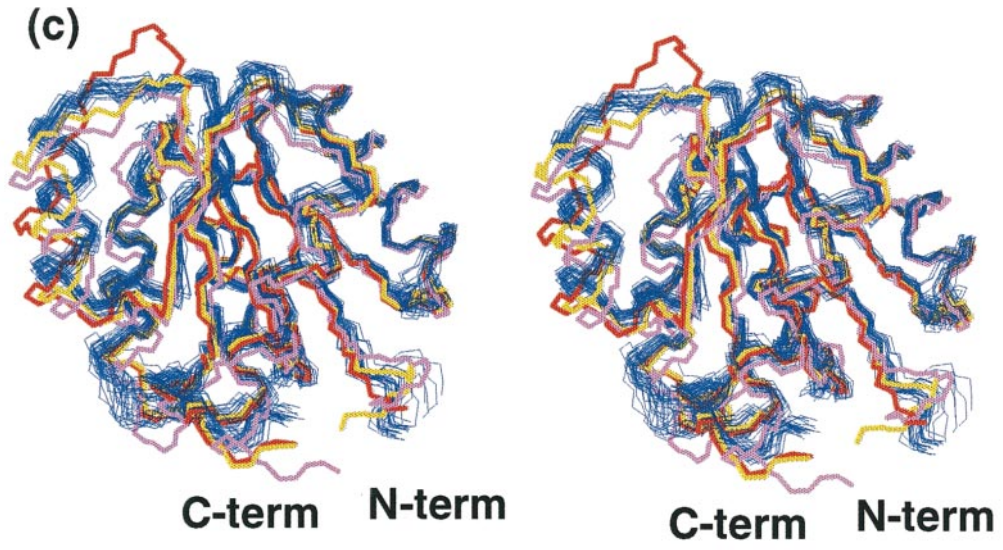

Figure 2. (a) ^{15}N-^{1}H FHSQC spectrum of BeF_3-CheY along with (b) superpositions of backbone N, C^{α}, and C′ coordinates for BeF_3-activated CheY and (c) comparison of BeF_3-activated and inactive CheY structures shown in stereoview. (a) In the FHSQC, spectrum peaks are labeled with residue numbers. Unassigned backbone resonances are labeled UA. Pairs of side-chain NH_2 resonances are connected by horizontal lines. Signals enclosed in boxes are folded in the ^{15}N dimension. (b) The 27 structures of BeF_3-activated CheY. Backbone coordinates for residues in the five helices and five-stranded β-sheet were superimposed. (c) Superposition of the 27 structures of BeF_3-activated CheY (blue), with apo X-ray (Volz & Matsumura, 1991) (gold), magnesium-bound X-ray (Bellsolell *et al.*, 1994) (red), and mean magnesium-bound NMR (Moy *et al.*, 1994) (magenta) structures. Superposition included backbone coordinates for residues in H1, H2, β1, β2, and β3. Considering the mean coordinates obtained from the family of magnesium-bound (Moy *et al.*, 1994) and BeF_3-activated NMR structures, backbone superposition of H1, H2, β1, β2, and β3 yields an r.m.s.d. value of 2.4 Å for the backbone coodinates of residues in H3, β4, H4, β5, and H5. The Figure was produced with the program MOLMOL (Koradi *et al.*, 1996). Uniformly ^{15}N and $^{15}N/^{13}C$-labeled samples were prepared by growth in M9 minimal medium supplemented with biotin and either [^{15}N]ammonium chloride or [^{15}N]ammonium chloride and [^{13}C]glucose. The BeF_3-activated sample conditions were 4 mM CheY, 16 mM $BeCl_2$, 100 mM NaF, 20 mM $MgCl_2$, at pH 6.7, and 10 % $^{2}H_2O$. NMR spectra were recorded on AMX 600 and DRX 500 NMR spectrometers at 25 °C. Backbone resonances were assigned with 3D ^{15}N NOESY-FHSQC (Talluri & Wagner, 1996), HNCACB (Wittekind & Mueller, 1993), CBCA(CO)NH (Grzesiek & Bax, 1992a), and HNCA (Grzesiek & Bax, 1992b) spectra. Side-chain aliphatic $^{13}C/^{1}H$ pairs were assigned with 3D ^{15}N TOCSY-HSQC (Driscoll *et al.*, 1990), HCCH-TOCSY (Kay *et al.*, 1993) and CBCA(CO)NH spectra. In each of the experiments above, purge-type pulsed-field gradients were used to suppress artifacts and the solvent signal (Bax & Pochapsky, 1992). Aromatic assignments were obtained from DQF-COSY (Rance *et al.*, 1983) and $^{13}C/^{1}H$ HMQC spectra (Bax *et al.*, 1990). The assignment process was also aided by making reference to published chemical shifts for CheY (Bruix *et al.*, 1993; Moy *et al.*, 1994). Phi torsion angle restraints were obtained from a ^{15}N HMQC-J spectrum (Kay & Bax, 1990). Stereospecific assignments for Val and Leu methyl groups were obtained by comparison of ct-HSQC spectra of uniformly ^{13}C-labeled and 10 % uniformly ^{13}C-labeled samples (Neri *et al.*, 1989; Szyperski *et al.*, 1992). χ^1 restraints for the Val, Ile, and Thr residues were obtained from ct-HMQC-J spectra (Grzesiek *et al.*, 1993; Vuister *et al.*, 1993a). NOEs identified in 3D NOESY-FHSQC, 4D $^{13}C/^{15}N$ HMQC-NOESY-FHSQC and 4D $^{13}C/^{13}C$ HMQC-NOESY-HMQC (all recorded with a 100 ms mixing time) (Vuister *et al.*, 1993b) spectra were classified as strong (2.9 Å upper distance limit), medium (3.3 Å), or weak (5.0 Å). A total of 972 non-trivial NOE restraints (213 intraresidue, 271 sequential, 238 medium-range, and 250 long-range) were used as input to DYANA (Güntert *et al.*, 1997), along with 78 phi torsion angle restraints and 17 χ^1 restraints for the Val, Ile, and Thr residues. Once sets of 20 (of 60) structures reached a backbone r.m.s.d. of 1 Å, 47 hydrogen bonds (94 upper and 94 lower distance restraints (H-O distance restraint 1.8-2.0 Å; N-O 2.7-3.0 Å)), identified on the basis of slow amide proton exchange rates (protection factors greater than 75) and short donor/acceptor distances were included in the calculations. Structures resulting from DYANA calculations with a pseudoatom (van der Waals radius 2.5 Å) corresponding to BeF_3^- attached to the side-chain of Asp57 resulted in a backbone r.m.s.d. value of only 0.4 Å when compared to structures without the additional pseudoatom. The 27 of 60 structures (BeF_3^- pseudoatom not included) with residual target function values less than 1.0 $Å^2$ (Table 1; target function before energy minimization was 0.3(±0.2) $Å^2$) were subjected to restrained energy minimization using the AMBER94 forcefield (Cornell *et al.*, 1995) implemented in the program OPAL (Luginbühl *et al.*, 1996). Conjugate gradient minimization (1500 steps) included bond, angle, dihedral, improper dihedral, van der Waals, electrostatic, NMR distance, and NMR torsion angle terms. The minimization was performed in a shell of water at least 6 Å thick, with the dielectric constant set to 1, and with no cut-off for non-bonded interactions. PROCHECK analysis (Laskowski *et al.*, 1993) of the structures revealed that 99 % of the residues fall within the allowed or generously allowed regions of the Ramachandran map. The 27 energy-minimized structures are used to represent the solution structure of CheY complexed with beryllofluoride and magnesium.

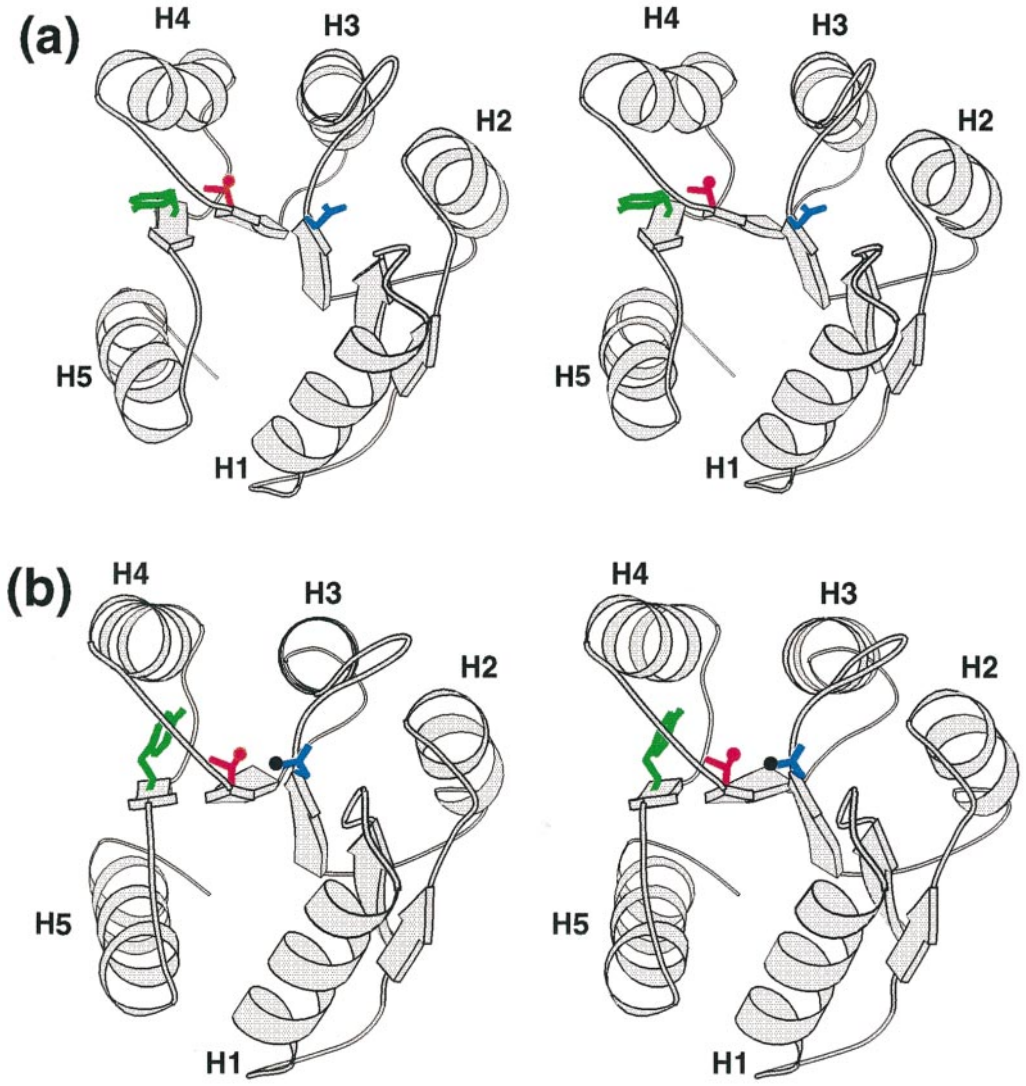

Figure 3. Ribbon diagrams of CheY in stereo showing movement of side-chains Thr87 and Tyr106 upon activation. Superposition included backbone coordinates for residues in H1, H2, β1, β2, and β3. Relative that depicted in Figure 2, the structures are rotated 90° about a horizontal axis in the page, affording a view (top) of the active site. The loops between β3 and H3 and between H3 and β4 are ill-defined by the NMR data, and should not be used for comparison. (a) CheY taken from the inactive magnesium-bound NMR structure (Moy *et al*., 1994) and (b) representative NMR structure of BeF_3-activated CheY. Asp57 (blue) is the site of phosphorylation. Highly conserved Tyr106 (green) and Thr87 (red) are also shown. The Thr87 hydroxyl group is represented by a small ball. BeF_3^- is modeled as a black ball attached to Asp57. The Figure was created with the program MOLSCRIPT (Kraulis, 1991).

The H4-β5-H5 face of CheY is an important interaction surface for FliM binding (Djordjevic & Stock, 1998; Shukla *et al*., 1998). Similar to results for apoCheY (McEvoy *et al*., 1999), titrations of BeF_3-activated CheY with the 16 N-terminal residues of the FliM protein resulted in large shifts for amide resonances of many residues in this face, including Lys92, Tyr106, and Val107 (data not shown). In our activated structures, Tyr106 (in β5) is exclusively in the buried conformation. It is therefore plausible that Tyr106 in the exposed position (magnesium-bound inactive state) contributes to a decrease in the affinity of FliM for CheY by sterically blocking interaction of FliM with the H4-β5-H5 face. However, failure of other residues to substitute for Tyr106 indicates that other subtle structural changes which occur upon re-orientation of the tyrosine side-chain are likely to be important for normal function (Zhu *et al*., 1996). For example, upon activation there is stabilization of H4, presumably due to hydrophobic interactions between Tyr106 and the β4-H4-pocket, and there is displacement of H4 in conjunction with β4. There is also a significant $^{13}C^{\alpha}$ chemical shift change for highly conserved Lys109 and a slight distortion of the backbone in the region near it in activated CheY compared to inactive CheY (Bellsolell *et al*., 1994; Moy *et al*., 1994). Unfortunately, the side-chain res-

onances for Lys109 are weak, fall in a very crowded region, and could not be assigned. Consequently, we were unable to define its position in the active state.

Our structural studies of BeF_3-activated CheY are consistent with the notion that the rotameric position of the Tyr106 side-chain is important in determining the signaling state of CheY and, directly or indirectly, controls its affinity for FliM. The recent discovery that BeF_3^- can be used to activate CheY (Yan *et al*., 1999) will facilitate study of complexes such as CheY with FliM, and will provide a more detailed understanding of the mechanism by which CheY transmits a signal to the flagellar motor assembly. It should also facilitate assessing whether CheZ acts allosterically to stimulate the autophosphatase activity of CheY, or whether it directly catalyzes dephosphorylation.

Accession codes

Coordinates have been deposited in the Protein Data Bank under accession number 1DJM. The chemical shift assignments have been deposited in the BioMagResBank (BMRB) under accession number 4472.

Acknowledgments

We thank D. King, C. Hastings, C. Liu, B. Volkman, and H. Yokota for helpful discussions. This work was supported by grants from the Office of Energy Research, Office of Health and Environmental Research, Health Effects Research Division of the US Department of Energy to D.E.W., and through instrumentation grants from the US Department of Energy and the National Science Foundation to D.E.W. This work was also supported by an NIH grant to S.K.

References

Appleby, J. L. & Bourret, R. B. (1998). Proposed signal transduction role for conserved CheY residue Thr87 a member of the response regulator active-site quintet. *J. Bacteriol.* **180**, 3563-3569.

Bax, A. & Pochapsky, S. (1992). Optimized recording of heteronuclear multidimensional NMR spectra using pulsed field gradients. *J. Magn. Reson. ser. B,* **99**, 638-643.

Bax, A., Ikura, M., Kay, L. E., Torchia, D. A. & Tschudin, R. (1990). Comparison of different modes of two-dimensional reverse-correlation NMR for the study of proteins. *J. Magn. Reson. ser. B,* **86**, 304-318.

Bellsolell, L., Prieto, J., Serrano, L. & Coll, M. (1994). Mg^{2+} binding to the bacterial chemotaxis protein CheY results in large conformational changes involving its functional surface. *J. Mol. Biol.* **238**, 489-495.

Birk, C., Mourey, L., Gouet, P., Fabry, B., Schumacher, J., Rousseau, P., Kahn, D. & Samama, J.-P. (1999). Conformational changes induced by phosphorylation of the FixJ receiver domain. *Structure,* **7**, 1505-1515.

Blat, Y. & Eisenbach, M. (1994). Phosphorylation-dependent binding of the chemotaxis signal molecule CheY to its phosphatase CheZ. *Biochemistry,* **33**, 902-906.

Bruix, M., Pascual, J., Santoro, J., Prieto, J., Serrano, L. & Rico, M. (1993). ^1H and ^{15}N NMR assignment and solution structure of the chemotactic *E. coli* CheY protein. *Eur. J. Biochem.* **215**, 573-585.

Cornell, W., Cioplak, P., Bayly, C., Gould, I., Merz, K. J., Fergusan, D., Spellmeyer, D., Fox, T., Caldwell, J. & Kollman, P. (1995). A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.* **117**, 5179-5197.

Djordjevic, S. & Stock, A. M. (1998). Structural analysis of bacterial chemotaxis proteins: components of a dynamic signalling system. *J. Struct. Biol.* **124**, 189-200.

Drake, S. K., Bourret, R. B., Luck, L. A., Simon, M. I. & Flake, J. J. (1993). Activation of the phosphosignaling protein CheY. *J. Biol. Chem.* **268**, 13081-13088.

Driscoll, P. C., Clore, G. M., Marion, D., Wingfield, P. T. & Gronenborn, A. M. (1990). Complete resonance assignments for the polypeptide backbone of interleukin-1β using 3D heteronuclear NMR spectroscopy. *Biochemistry*, **29**, 3542-3556.

Falke, J. J., Bass, R. B., Butler, S. L., Chervitz, S. A. & Danielson, M. A. (1997). The two-component signaling pathway of bacterial chemotaxis. *Annu. Rev. Cell. Dev. Biol.* **13**, 457-512.

Ganguli, S., Wang, H., Matsumura, P. & Volz, K. (1995). Uncoupled phosphorylation and activation in bacterial chemotaxis. The 2.1 Å structure of a threonine to isoleucine mutant at position 87 of CheY. *J. Biol. Chem.* **270**, 17386-17393.

Goldstein, G. (1964). Equilibrium distribution of metal-fluoride complexes. *Anal. Chem.* **36**, 243-244.

Grzesiek, S. & Bax, A. (1992a). Correlating backbone amide and sidechain resonances in larger proteins by multiple relayed triple resonance NMR. *J. Am. Chem. Soc.* **114**, 6291-6293.

Grzesiek, S. & Bax, A. (1992b). An efficient experiment for sequential backbone assignment of medium-sized isotopically enriched proteins. *J. Magn. Reson.* **99**, 201-207.

Grzesiek, S., Vuister, G. & Bax, A. (1993). A simple and sensitive experiment for measurement of JCC couplings between backbone carbonyl and methyl carbons in isotopically enriched proteins. *J. Biomol. NMR*, **3**, 487-493.

Güntert, P., Mumenthaler, C. & Wüthrich, K. (1997). Torsion angle dynamics for NMR structure calculation with the new program DYANA. *J. Mol. Biol.* **273**, 283-298.

Halkides, C. J., Zhu, X., Phillion, D. P., Matsumura, P. & Dahlquist, F. W. (1998). Synthesis and biochemical characterization of an analogue of CheY-phosphate, a signal transduction protein in bacterial chemotaxis. *Biochemistry,* **37**, 13674-13680.

Kay, L. E. & Bax, A. (1990). New methods for the measurement of NH-CaH coupling constants in ^{15}N-labeled proteins. *J. Magn. Reson.* **86**, 110-126.

Kay, L. E., Xu, G.-Y., Singer, A., Muhandram, D. & Forman-Kay, J. (1993). A gradient-enhanced HCCH-TOCSY experiment for recording side-chain ^1H and ^{13}C correlations in H_2O samples of proteins. *J. Magn. Reson. ser. B,* **101**, 333-337.

Kern, D., Volkman, B. F., Luginbühl, P., Nohaile, M. J., Kustu, S. & Wemmer, D. E. (1999). Structure of the

transiently phosphorylated bacterial receiver domain of NtrC. *Nature,* **402**, 894-898.

Klose, K. E., Weiss, D. S. & Kustu, S. (1993). Glutamate at the site of phosphorylation of nitrogen-regulatory protein NtrC mimics aspartyl-phosphate and activates the protein. *J. Mol. Biol.* **232**, 67-78.

Koradi, R., Billeter, M. & Wüthrich, K. (1996). MOLMOL: a program for display and analysis of macromolecular structures. *J. Mol. Graph.* **14**, 51-55.

Kraulis, P. (1991). MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallog.* **24**, 946-950.

Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallog.* **26**, 283-291.

Lewis, R. J., Brannigan, J. A., Muchova, K., Barak, I. & Wilkinson, A. J. (1999). Phosphorylated aspartate in the structure of a response regulator protein. *J. Mol. Biol.* **294**, 9-15.

Li, J., Swanson, R. V., Simon, M. I. & Weis, R. M. (1995). The response regulators CheB and CheY exhibit competitive binding to the kinase CheA. *Biochemistry,* **34**, 14626-14636.

Lowry, D. F., Roth, A. F., Rupert, P. B., Dahlquist, F. W., Moy, F. J., Domaille, P. J. & Matsumura, P. (1994). Signal transduction in chemotaxis. *J. Biol. Chem.* **269**, 26358-26362.

Luginbühl, P., Güntert, P., Billeter, M. & Wüthrich, K. (1996). The new program OPAL for molecular dynamics simulations and energy refinements of biological macromolecules. *J. Biomol. NMR,* **8**, 136-146.

Lukat, G. S., Lee, B. H., Mottonen, J. M., Stock, A. M. & Stock, J. B. (1991). Roles of the highly conserved aspartate and lysine residues in the response regulator of bacterial chemotaxis. *J. Biol. Chem.* **266**, 8348-8354.

Martin, R. B. (1988). Ternary hydroxide complexes in neutral solutions. *Biochem. Biophys. Res. Commun.* **155**, 1194-1200.

McEvoy, M. M., Bren, A., Eisenbach, M. & Dahlquist, F. W. (1999). Identification of the binding interfaces on CheY for two of its targets, CheZ and FliM. *J. Mol. Biol.* **289**, 1423-1433.

Moore, J. B., Shiau, S. P. & Reitzer, L. J. (1993). Alterations of highly conserved residues in the regulatory domain of nitrogen regulator I (NtrC) of *E. coli*. *J. Bacteriol.* **175**, 2692-2701.

Moy, F. J., Lowry, D. F., Matsumura, P., Dahlquist, F. W., Krywko, J. E. & Domaille, P. J. (1994). Assignments and secondary structure, global fold, and dynamics of chemotaxis Y protein using 3D and 4D heteronuclear NMR spectroscopy. *Biochemistry,* **33**, 10731-10742.

Neri, D., Szyperski, T., Otting, G., Senn, H. & Wüthrich, K. (1989). Stereospecific NMR assignments of the methyl groups of valine and leucine in the DNA-binding domain of the 434 repressor by biosynthetic directed fractional ^{13}C labeling. *Biochemistry,* **28**, 7510-7516.

Nixon, B. T., Ronson, C. W. & Ausubel, F. M. (1986). Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes ntrB and ntrC. *Proc. Natl Acad. Sci. USA,* **83**, 7850-7854.

Ota, I. M. & Varshavsky, A. (1993). A yeast protein similar to bacterial two-component regulators. *Science,* **262**, 566-569.

Parkinson, J. S. & Kofoid, E. C. (1992). Communication modules in bacterial signaling proteins. *Annu. Rev. Genet.* **26**, 71-112.

Rance, M., Sorensen, O. W., Bodenhausen, G., Wagner, G. & Ernst, R. R. (1983). Improved spectral resolution in COSY ^{1}H NMR spectra of proteins *via* double quantum filtering. *Biochem. Biophys. Res. Commun.* **117**, 479-485.

Sanders, D. A., Gillece-Castro, B. L., Stock, A. M., Burlingame, A. L. & Koshland, D. E., Jr (1989). Identification of the site of phosphorylation of the chemotaxis response regulator protein, CheY. *J. Biol. Chem.* **264**, 21770-21778.

Shukla, D., Zhu, X. Y. & Matsumura, P. (1998). Flagellar motor switch binding face of CheY and the biochemical basis of suppression by CheY mutants that compensate for motor switch defects in *E. coli*. *J. Biol. Chem.* **273**, 23993-23999.

Stock, A. M., Martinez-Hackert, E., Rasmussen, B. F., West, A. H., Stock, J. B., Ringe, D. & Petsko, G. A. (1993). Structure of the Mg^{2+} bound form of CheY and mechanism of phosphoryl transfer in bacterial chemotaxis. *Biochemistry,* **32**, 13375-13380.

Stock, J. B., Surette, M. G., Levit, M. & Park, P. (1995). In *Two-component Signal Transduction* (Hoch, J. A. & Silhavy, T. J., eds), pp. 25-30, ASM, Washington DC, USA.

Szyperski, T., Neri, D., Leiting, B., Otting, G. & Wüthrich, K. (1992). Support of ^{1}H NMR assignments in proteins by biosynthetic directed fractional ^{13}C-labeling. *J. Biomol. NMR,* **2**, 323-334.

Talluri, S. & Wagner, G. (1996). An optimized 3D NOESY-HSQC. *J. Magn. Reson. ser. B,* **112**, 200-205.

Volz, K. (1993). Structural conservation in the CheY superfamily. *Biochemistry,* **32**, 11741-11753.

Volz, K. & Matsumura, P. (1991). Crystal structure of *Escherichia coli* CheY refined at 1.7 Å resolution. *J. Biol. Chem.* **266**, 15511-15519.

Vuister, G., Wang, A. & Bax, A. (1993a). Measurement of 3-bond nitrogen carbon-J couplings in proteins uniformly enriched in ^{15}N and ^{13}C. *J. Am. Chem. Soc.* **115**, 5334-5335.

Vuister, G. W., Clore, G. M., Gronenborn, A. M., Powers, R., Garrett, D. S., Tschudin, R. & Bax, A. (1993b). Increased resolution and improved spectral quality in 4D $^{13}C/^{13}C$-separated HMQC-NOESY-HMQC spectra using pulsed field gradients. *J. Magn. Reson. ser. B,* **101**, 210-213.

Welch, M., Oosawa, K., Aizawa, S.-I. & Eisenbach, M. (1993). Phosphorylation dependent binding of a signal molecule to the flagellar switch of bacteria. *Proc. Natl Acad. Sci. USA,* **90**, 8787-8791.

Welch, M., Chinardet, N., Mourey, L., Birck, C. & Samama, J.-P. (1997). Structure of the CheY binding domain of histidine kinase CheA in complex with CheY. *Nature Struct. Biol.* **5**, 25-29.

Wittekind, M. & Mueller, L. (1993). HNCACB, a high sensitivity 3D NMR experiment to correlate amide proton and nitrogen resonances with the alpha-carbon and beta-carbon resonances in proteins. *J. Magn. Reson. ser. B,* **101**, 201-205.

Yan, D., Cho, H. S., Hastings, C. A., Igo, M. M., Lee, S.-Y., Pelton, J. G., Stewart, V., Wemmer, D. E. & Kustu, S. (1999). Beryllofluoride mimics phosphorylation of NtrC and other bacterial response regulators. *Proc. Natl Acad. Sci. USA,* **96**, 14789-14794.

Zhu, X., Amsler, C. D., Volz, K. & Matsumura, P. (1996). Tyrosine 106 of CheY plays an important role in chemotaxis signal transduction in *Escherichia coli*. *J. Bacteriol.* **178**, 4208-4215.

Zhu, X., Rebello, J., Matsumura, P. & Volz, K. (1997). Crystal structures of CheY mutants Y106W and T87I/Y106W. *J. Biol. Chem.* **272**, 5000-5006.

Edited by P. E. Wright

(Received 22 December 1999; received in revised form 31 January 2000; accepted 4 February 2000)

http://www.academicpress.com/jmb

Supplementary material for this paper is available from JMB Online.