Position 106 in CheY is highly conserved as an aromatic residue in the response regulator superfamily. In the structure of the wild-type, apo-CheY, Tyr\textsuperscript{106} is a rotamer whose electron density is observed in both the inside and the outside positions. In the structure of the T87I mutant of CheY, the threonine to isoleucine change at position 87 causes the side chain of Tyr\textsuperscript{106} to be exclusively restricted to the outside position. In this report we demonstrate that the T87I mutation causes cells to be smooth swimming and non-chemotactic. We also show that another CheY mutant, Y106W, causes cells to be more tumbling than wild-type CheY, and impairs chemotaxis. In the structure of Y106W, the side chain of Trp\textsuperscript{106} stays exclusively in the inside position. Furthermore, a T87I/Y106W double mutant, which confers the same phenotype as T87I, restricts the side chain of Trp\textsuperscript{106} to the outside position. The results from these behavioral and structural studies indicate that the rotameric nature of the Tyr\textsuperscript{106} residue is involved in activation of the CheY molecule. Specifically, CheY's signaling ability correlates with the conformational heterogeneity of the Tyr\textsuperscript{106} side chain. Our data also suggest that these mutations affect the signal at an event subsequent to phosphorylation.

How regulatory proteins are involved in signal transduction events is a fundamental question for both prokaryotic and eukaryotic intracellular signaling pathways. In most microorganisms, cells adaptively respond to a wide variety of environmental stimuli through “two-component” regulatory systems. During chemotaxis in \textit{Escherichia coli}, the sensor, CheA, receives signals from transmembrane chemoreceptors with the help of CheW (1–4), and then transfers the signal to the response regulator, CheY, by transient phosphorylation (5, 6). Phosphorylated CheY (CheY-P) interacts with the switch motor to reverse the direction of flagella rotation from counterclockwise (CCW)\textsuperscript{1} to clockwise (CW). This causes the bacteria to tumble rather than swim smoothly (7, 8). CheY-P is dephosphorylated by its autophosphatase activity, a reaction enhanced by CheZ (5, 6). CheY’s return to its resting form results in normal CCW flagella rotation and smooth swimming of the bacteria.

More than 80 different two-component systems have been reported in bacterial and yeast signal transduction pathways, regulating many different activities such as chemotaxis, osmo-regulation, sporulation, and virulence. All response regulators in this superfamily are homologous to CheY. CheY is the only member of the response regulator superfamily for which a detailed three-dimensional structure is known (9–11). Both genetic and structural studies demonstrate that the highly conserved residues in response regulators, such as Asp\textsuperscript{12}, Asp\textsuperscript{15}, Asp\textsuperscript{57}, Thr\textsuperscript{27}, and Lys\textsuperscript{149}, play important roles in CheY activation (12–17). Mutagenesis studies indicate that substitutions of any one of those residues cause functional defects in CheY (13, 16, 17).

Position 106 is another conserved site in the CheY superfamily; this position is occupied by an aromatic residue (tyrosine or phenylalanine) greater than 80% of the time (9). When residue 106 is substituted with a non-aromatic residue, the mutant CheY loses its signaling ability (18). The three-dimensional structure of wild-type CheY (Mg\textsuperscript{2+}-free form) shows that Tyr\textsuperscript{106} is a rotamer whose electron density is found both in an inside (solvent inaccessible) and an outside (solvent exposed) position (10). When the tyrosine side chain is in the inside, the hydroxyl group forms a hydrogen bond with the O\textsubscript{6} of the conserved Thr\textsuperscript{97} residue through one intervening solvent molecule. In the structure of the T87I mutant of CheY, the Tyr\textsuperscript{106} side chain is well ordered, exclusively occupying the external position, forced out by the increased bulk and hydrophobicity of the isoleucine at position 87 (17). The functional defect caused by the T87I mutation (17) correlates with this structural change.

We report here the behavioral effects, biochemical properties, and crystal structures of two very different CheY mutants. The first mutant has a tyrosine to tryptophan substitution at position 106 (Y106W). The second mutant bears a double mutation: T87I and Y106W (called T87I/Y106W). The Y106W mutant exhibits a hyperactive phenotype, while the T87I/Y106W double mutant causes a loss of activity. Both mutants are phosphorylatable \textit{in vitro}. Structurally, the only significant differences between these molecules and wild-type CheY are the conformations of the Trp\textsuperscript{106} side chain. In Y106W, Trp\textsuperscript{106} is located in the inside position only, while in T87I/Y106W, Trp\textsuperscript{106} is forced to the outside position by the bulk of isoleucine at position 87, similar to that previously seen in the T87I mutant. Thus, results from our combined behavioral and structural studies support our proposal that movement of the side chain of residue 106 modulates the activation state of CheY.

**MATERIALS AND METHODS**

**Strains, Plasmids, and Mutant Constructions**—Bacterial strains and plasmids are listed in Table I. pXYZ301, containing the CheY double mutant T87I/Y106W, was constructed by recombining appropriate restriction fragments containing the CheY mutations using standard techniques (19), then confirmed by DNA sequencing.

The plasmid-born mutations were transferred into the host chromosome of \textit{E. coli} by gene conversion to obtain single copy mutations (20, 21). The EcoRI-SalI fragments of either pYM31(T87I) or pXYZ301

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1 The abbreviations used are: CCW, counterclockwise; CW, clockwise; r.m.s., root mean square.
X-ray Structures of CheY Mutants Y106W and T87I/Y106W

Table I

Bacterial strains and plasmids

| Strain/plasmid | Relevant genotype and description | Source/reference |
|---------------|----------------------------------|------------------|
| 594           | cheB+ cheZ+ recD1903 cheA         | Laboratory collection |
| D345          | cheY+ cheZ+ recD1903 cheA         | Laboratory collection |
| XYZ6          | cheY+ cheZ+ recD1903 cheA         | Laboratory collection |
| XYZ23         | cheY+ cheZ+ recD1903 cheA         | Laboratory collection |
| XYZ27         | cheY+ cheZ+ recD1903 cheA         | Laboratory collection |
| XYZ9          | cheY+ cheZ+ recD1903 cheA         | Laboratory collection |
| pXY22z4       | cheZ deletion, wild-type CheY    | Laboratory collection |
| pYM113        | cheY+ recD1903 cheA               | Laboratory collection |
| pXY22z201     | T87I mutation                      | Laboratory collection |
| pMC100        | cheA+ tap cheB+ tar+ cheR+        | Laboratory collection |
| pXY22z112     | cheA+ tap cheB+ tar+ cheR+        | Laboratory collection |
| pXY22z21      | cheA+ tap cheB+ tar+ cheR+        | Laboratory collection |

Table II

Statistics for data and final refinement of E. coli CheY mutant Y106W

| Resolution limits | No. of reflections predicted | Complete | $\geq 2\sigma$ | Refinement R-value |
|-------------------|------------------------------|----------|---------------|-------------------|
| $\AA$              |                             | %        | %             |                   |
| $\infty \rightarrow 10.00$ | 174                         | 90.2     | 90.2          | 20.8              |
| 10.00 $\rightarrow 4.43$ | 1528                        | 81.6     | 81.6          | 14.7              |
| 3.54 $\rightarrow 3.10$ | 1511                        | 78.7     | 78.6          | 17.0              |
| 3.10 $\rightarrow 2.81$ | 1557                        | 75.5     | 75.0          | 18.1              |
| 2.81 $\rightarrow 2.61$ | 1513                        | 74.0     | 73.8          | 20.8              |
| 2.61 $\rightarrow 2.45$ | 1556                        | 62.8     | 62.6          | 23.2              |
| 2.45 $\rightarrow 2.33$ | 1474                        | 43.2     | 42.7          | 22.2              |
| 10.00 $\rightarrow 2.33$ | 10838                       | 72.5     | 71.0          | 18.6              |

Table III

Statistics for data and final refinement of E. coli CheY double mutant T87I/Y106W

| Resolution limits | No. of reflections predicted | Complete | $\geq 2\sigma$ | Refinement R-value |
|-------------------|------------------------------|----------|---------------|-------------------|
| $\AA$              |                             | %        | %             |                   |
| $\infty \rightarrow 10.00$ | 174                         | 90.2     | 90.2          | 20.8              |
| 10.00 $\rightarrow 4.43$ | 1528                        | 81.6     | 81.6          | 14.7              |
| 3.54 $\rightarrow 3.10$ | 1511                        | 78.7     | 78.6          | 17.0              |
| 3.10 $\rightarrow 2.81$ | 1557                        | 75.5     | 75.0          | 18.1              |
| 2.81 $\rightarrow 2.61$ | 1513                        | 74.0     | 73.8          | 20.8              |
| 2.61 $\rightarrow 2.45$ | 1556                        | 62.8     | 62.6          | 23.2              |
| 2.45 $\rightarrow 2.33$ | 1474                        | 43.2     | 42.7          | 22.2              |
| 10.00 $\rightarrow 2.33$ | 10838                       | 72.5     | 71.0          | 18.6              |

(Continued)
TABLE IV

| Parameter                              | Target σ | Final value |
|----------------------------------------|----------|-------------|
| Distance restraints (Å)                |          |             |
| Bond distance                          | 0.020    | 0.012       |
| Angle distance                         | 0.040    | 0.044       |
| Planar distance                        | 0.050    | 0.045       |
| Plane restraint (Å)                    | 0.020    | 0.009       |
| Chiral-center restraint (Å³)           | 0.150    | 0.138       |
| Non-bonded contact restraints (Å)      |          |             |
| Single torsion contact                 | 0.500    | 0.209       |
| Multiple torsion contact               | 0.500    | 0.251       |
| Possible hydrogen bond                  | 0.500    | 0.214       |
| Conformational torsion angle restraint (*) |          |             |
| Planar (α₀°, 180°)                     | 3.0      | 1.8         |
| Staggered (±60°, 180°)                 | 15.0     | 18.9        |
| Orthornormal (±90°)                    | 20.0     | 24.0        |
| Isotropic thermal factor restraints (Å²) |          |             |
| Main-chain bond                        | 1.000    | 0.788       |
| Main-chain angle                       | 1.500    | 1.414       |
| Side-chain bond                        | 1.000    | 0.925       |
| Side-chain angle                       | 1.500    | 1.490       |
| X-ray                                  | 0.8 × (Fo² - Fc²) | 18.92%         |

TABLE V

| Parameter                              | Target σ | Final value |
|----------------------------------------|----------|-------------|
| Distance restraints (Å)                |          |             |
| Bond distance                          | 0.020    | 0.014       |
| Angle distance                         | 0.040    | 0.051       |
| Planar distance                        | 0.050    | 0.053       |
| Plane restraint (Å)                    | 0.020    | 0.012       |
| Chiral-center restraint (Å³)           | 0.150    | 0.175       |
| Non-bonded contact restraints (Å)      |          |             |
| Single torsion contact                 | 0.500    | 0.202       |
| Multiple torsion contact               | 0.500    | 0.250       |
| Possible hydrogen bond                  | 0.500    | 0.276       |
| Conformational torsion angle restraint (*) |          |             |
| Planar (α₀°, 180°)                     | 3.0      | 2.0         |
| Staggered (±60°, 180°)                 | 15.0     | 21.0        |
| Orthornormal (±90°)                    | 20.0     | 27.9        |
| Isotropic thermal factor restraints (Å²) |          |             |
| Main-chain bond                        | 1.000    | 0.667       |
| Main-chain angle                       | 1.500    | 1.122       |
| Side-chain bond                        | 1.000    | 0.659       |
| Side-chain angle                       | 1.500    | 1.086       |
| X-ray                                  | 0.7 × (Fo² - Fc²) | 18.90%         |

RESULTS

Genetic and Behavioral Characterization of Mutations—All strains were constructed in single copy to ensure wild-type levels of expression. The resulting strains were XYZ7 (for T87I, T87I, and T87I/Y106W) as the above mutant strains except for its wild-type cheY gene to serve as a control for wild-type chemotaxis. A spectrophotometric assay was used to detect chemotactic ability for the wild-type and all three mutant CheY strains. As shown in Fig. 1, the Y106W mutant showed about 80% chemotaxis of wild-type strain XYZ6, while neither the T87I nor the T87I/Y106W mutant had any chemotactic ability.

In wild-type cells, phosphorylated CheY interacts with the flagellar motor to change the direction of rotation from CCW to CW and cause the cells to make a tumble motion. After dephosphorylated CheY returns to its resting state, the cells change back to a CCW flagellar rotation, resulting in smooth swimming. Thus, normal chemotaxis behavior depends on the balance of cell tumbling and smooth swimming. Since chemotaxis is impaired by either excessive tumbling or excessive smooth swimming, mutations in cheY can cause cells to be dominant tumble (13, 18) or dominant smooth swimming (17, 18). To determine if the reductions of chemotaxis in the cheY mutants were because of extra tumbles or extra smooth swimming, the flagellar rotations of the mutant strains were counted under the bacterial tethering method. In comparison with the wild-type strain XYZ6, the Y106W mutant showed increased CW-biased flagellar rotation, while both the T87I and the T87I/Y106W mutants had extreme CCW rotation (Fig. 2). These results show that Y106W is an activated mutant, which increases cell tumble frequency and diminishes cell chemotaxis. On the other hand, T87I and T87I/Y106W are non-activated mutants, which

FIG. 1. Chemotactic ability of cheY mutants versus wild-type based on spectrophotometric assay. ● denotes wild-type cheY (see Ref. 18). The cheY mutants are Y106W in strain XYZ3 (Ref. 18, ○), T87I in strain XYZ7 (this work; ●), and T87I/Y106W in strain XYZ9 (this work; □). The results are the averages of three individual experiments.

were excluded, and residues 87 and 106 were changed to alanines. A number of resolution ranges and I/σI cutoffs were explored. The rotation function searches consistently gave one strong peak, accompanied by a variety of minor peaks. A systematic cross-translation function test of the strong rotation function peak combined with each of the minor peaks finally yielded two convincing solutions. These solutions were confirmed with packing analyses and a polar rotation function calculation. R-value minimization refinement of the rotation and translation parameters gave a 2-molecule model with an R-factor at 39%.

Structural Refinements—The Y106W CheY mutant structure refined without difficulty using the restrained least-squares method (29), combined with 23 instances of manual intervention and partial rebuilding with omit maps. The final R-factor was 18.5% for the 7,156 reflections in the 10–2.0 Å range with intensities greater than 2σI (Table IV). Refinement of the T87I/Y106W double mutant CheY was more problematic due to the poor molecular replacement model and the underdetermined ratio of observations/variables of 0.90. Refinement was done using both the conventional restrained least-squares method and the simulated annealing method (30), interspersed with 37 rounds of partial rebuilds based on omit maps. The final R-factor was 18.6% for the 7,698 reflections in the 10–2.3 Å range with intensities greater than 2σI (Table V).

Calculations of electron density maps and other data processing were done with the XTA1 (31), CCP4 (32), and X-PLOR (33) software packages, and several locally developed programs. Refinement was done with the software packages PROTIN and PROFFIT (29, 34) and X-PLOR. Display of electron density maps and model rebuilding were done using the graphics package QUANTA (35) on a Silicon Graphics Indigo² workstation. All water molecules were built manually, using the following rules: 1) electron density must appear in both the Fo and Fc maps, 2) there must be proper hydrogen bonding distances and geometries, and 3) molecules that refine to unreasonable temperature factors must be removed. The presence of a SO₄²⁻ ion in the T87I/Y106W structure was indicated in the final rounds of refinement by electron density greater than any other solvent density, located in a positively charged environment, with hydrogen bond distances too great for a water molecule.
result in smooth swimming cells with a non-chemotactic phenotype.

In Vitro Phosphorylation Biochemistry of the Mutants—CheY is phosphorylated by CheA plus ATP, and dephosphorylated by its autophosphatase activity and by CheZ (5, 6). A mutation in CheY that abolishes its ability to be phosphorylated (e.g. D57C) results in smooth swimming cells that are non-chemotactic (14, 15). In order to determine whether the smooth-swimming phenotypes of the T87I and T87I/Y106W mutants are due to defects in their phosphoryl-group transfer abilities, the mutant molecules were combined with CheA and ATP in vitro. Both T87I and T87I/Y106W were found to be phosphorylatable to a level of wild-type CheY in vitro, as well as the Y106W mutant (data not shown). Next, the stabilities of the phosphorylated forms of the wild-type, T87I, Y106W, and T87I/Y106W mutant CheYs were compared. Phosphorylated Y106W was 2 times more stable than wild-type CheY-P, while phosphorylated T87I and T87I/Y106W were 5 times more stable (Fig. 3). These results indicate different levels of autophosphatase activities of these mutants. Note, however, that the variation in the mutants’ phosphorylation half-lives correlates negatively with their signaling abilities, so it is not a factor in this structure/function analysis.

Description of Structural Results—The final electron density maps for the refined structures of Y106W and T87I/Y106W were clear and well defined for the entire backbones of all molecules. All side chain atoms in the Y106W structure were located within interpretable electron density, and only the side chain Lys53 of molecule A and Arg35 of molecule B in the T87I/Y106W structure were not identifiable. The final coordinate set for the Y106W mutant consists of 1,068 atoms, 83 of which are solvent, with one Ca$^{2+}$ ion. The T87I/Y106W mutant has 1,951 atoms for the two protein molecules in the asymmetric unit, 177 solvent molecules, and one SO$_4^{2-}$ ion.

The crystal packing of the Y106W structure is the same as wild-type, apo-CheY, the two being essentially isomorphous. This is surprising given that the crystallization conditions were completely different (calcium acetate/polyethylene glycol 8000 versus ammonium sulfate). For the T87I/Y106W structure, the molecules pack as dimers in the asymmetric unit, with an almost perfect non-crystallographic two-fold axis parallel to the ab plane. The two molecules superimpose with an r.m.s. deviation of 0.42 Å. The local dyad involves close associations between hydrophobic residues of the α4 helices, plus extensive solvent-mediated interactions of side chains from the α4β2α5 surfaces of the molecules.

A least-squares superposition of α-carbons of the Y106W mutant and wild-type CheY structures yields an r.m.s. agreement of 0.33 Å. Thus, their backbone conformations are the same, within the limits of error. Comparisons of the T87I/Y106W molecules with wild-type require an analysis similar to that for the previously reported T87I mutant structure (17). The T87I molecules showed distinct backbone conformational changes in the β4-α4 loop that were directly attributed to that substitution (17). Those same localized changes also appear in the T87I/Y106W double mutant structure. Note that these changes do not occur in the Y106W mutant. This confirms the original assertion concerning the effects of the threonine to isoleucine substitution at position 87, and provides further evidence that the Y106W mutation by itself does not introduce measurable backbone conformational changes. Therefore, as with the T87I to wild-type comparison, the T87I/Y106W molecules can be divided into NH2-terminal segments (residues 2–86) and CO2-terminal segments (residues 87–129). The r.m.s. deviations of α-carbon atoms from wild-type for the first segment are 0.42 Å for molecule A and 0.47 Å for molecule B. These differences are not significantly greater than the 0.42 Å r.m.s. deviations from the full-length comparison of T87I/Y106W molecule A versus molecule B. Because of the relatively large (up to 2 Å) localized changes in the β4-α4 loops, the carboxyl-terminal segments cannot be least-square superimposed with any validity.

Active Site Structures—One prominent feature of the structure of the Y106W mutant was the presence of a Ca$^{2+}$ ion in the Mg$^{2+}$ ion binding site (the crystallization conditions included 0.12 M calcium acetate). The Ca$^{2+}$ ion has an octahedral coordination sphere similar to the Mg$^{2+}$ ions in the two previously reported CheY-Mg$^{2+}$ structures (36, 37). Its presence does not influence the conformation in the region of residue 106, which is more than 11 Å away. The active site structures of the two T87I/Y106W double mutant molecules are similar to wild-type, apo-CheY. There are minor differences in side chain conforma-

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**Fig. 2.** Flagellar rotation bias of cells with either wild-type or mutant CheY. Tethered cells that were actively spinning were videotaped through a light microscope within a 30-min period. The flagellar rotations of each cell was quantitated for 10 continuous s. More than 100 tethered cells were analyzed for each sample. The flagellar rotation biases were classified into five different categories: exclusive counterclockwise rotation (CCW), counterclockwise bias with rotation reversals (CCW-R), rotation reversals with no bias direction (R), clockwise bias with rotation reversals (CW-R), or exclusive clockwise rotation (CW). The strains were the same as described in Fig. 1.

**Fig. 3.** The stability of phosphorylated wild-type and mutant CheY proteins. Reactions were carried out with 0.5 mM Mg$^{2+}$ at room temperature. The intensity of each band was determined by an Ambis β-scanner, and the percentage of CheY-P concentration was plotted versus time. The half-lives of phosphorylated forms were calculated as $t_{1/2} = \ln(2)/k$. For wild-type CheY, $t_{1/2} = 15.5$ s (18); Y106W, $t_{1/2} = 33$ s (18); T87I, $t_{1/2} = 102$ s (18); and T87I/Y106W, $t_{1/2} = 110$ s (this work).
tions of Asp$^{13}$, Phe$^{14}$, and Asn$^{59}$, due to intermolecular contacts. A detailed discussion of solvent structure is not warranted at these resolution limits.

**Structural Differences at the Mutation Sites**—The most obvious and unambiguous results of these two structure determinations are the repositionings of the side chain of residue 106 relative to wild-type CheY (Fig. 4). In the Y106W mutant CheY, the tryptophan side chain at position 106 is clearly in the $g_1$ position ($\chi_1 = -69^\circ$), neatly packed in a hydrophobic cavity lined by residues Trp$^{58}$, Met$^{85}$, Thr$^{87}$, Ile$^{95}$, and Ala$^{98}$. Restriction of the tryptophan ring to the internal cavity appears to be driven by these hydrophobic interactions. There are van der Waals contacts between the C$_g$ and C$_a$ atoms of Thr$^{87}$ and the six-membered ring of Trp$^{106}$, which appear to stabilize the inside position, but there are no obvious steric barriers that would prevent the tryptophan ring from assuming the external position in this crystal packing. In contrast, the T87I/Y106W mutant shows the tryptophan side chain in the highly solvent exposed $g_2$ position ($\chi_1 = 76^\circ$ and $79^\circ$) for both molecules of the asymmetric unit. Clearly the presence of the isoleucine side chain at position 87 sterically prevents the tryptophan ring from entering the cavity. The electron density for the Ile$^{87}$ side chain shows them to have the same conformations as seen in the previously reported T87I mutant structures, and the C$_a$ atom of Ile$^{87}$ has slight van der Waals contacts with the ring of Trp$^{106}$ as it occupies the outside position.

**DISCUSSION**

**CheY's Signaling Ability Correlates with the Position of Residue 106**—Our behavioral studies demonstrate that both the Y106W and T87I CheY mutations impair cell chemotaxis, but they cause the functional defect in opposite ways. Y106W is an activating mutant, which causes the cells to be more tumbling, while the T87I mutant loses signaling ability, resulting in completely smooth swimming.

Our previous structural reports showed that in wild-type CheY the side chain of Tyr$^{106}$ was observed in both the inside and the outside positions (Ref. 10; Fig. 4a), and also that the side chain of Tyr$^{106}$ in the T87I mutant was forced to the outside position by the bulk of the isoleucine side chain at position 87 (Ref. 17; Fig. 4b). Here we show that the crystal structure of Y106W has the same overall structure as wild-type CheY, except for the side chain of residue 106. The tryptophan side chain of the Y106W mutant was found buried in the
internal cavity, apparently driven by hydrophobic forces, and assisted by local rearrangements (Fig. 4c). The functional relationship between residues Thr87 and Tyr106 suggested by these results led us to design the T87I/Y106W double mutant. As expected, the T87I/Y106W mutant is non-chemoactive with a smooth swimming phenotype like the T87I mutant. The side chain of residue 106 in T87I/Y106W is restricted to the outside position (Fig. 4d), as in T87I (Fig. 4b).

These combined results from both behavioral and structural studies suggest that the signaling state of the CheY molecule correlates with the nature of the side chain of Tyr106. The simplest model would be that the “in” position is the activated form, whereas the “out” position is the inactive form. But it may be the dynamic act of switching between the two conformations that is important in CheY activation. We propose that wild-type CheY can alter its signaling state by freely rotating the side chain of Tyr106 between inside and outside position, resulting in the optimum chemotaxis activity for the cells. Presumably, the position of the Tyr106 side chain would be modulated by the phosphorylation state of the molecule. In the Y106W mutant, the side chain of Trp106 could rotate to the external environment, but instead remains in the internal cavity, resulting in hyperactive signaling and impaired chemotaxis. In contrast, for both the T87I and T87I/Y106W mutants, this cavity is partially filled by the bulk of the Ile87 side chain, which forces residue 106 exclusively to the outside position, resulting in total loss of chemotaxis.

Very few activation mechanisms based on rotameric rearrangements have been proposed for other proteins. Some examples involving catalytic mechanisms include ribonuclease A, which has a mobile histidine residue in the active site whose conformation is modulated by pH (38, 39). A similar case of pH-dependent conformational mobility of a histidine in the active site of carbonic anhydrase II has been reported (40). An induced-fit type of conformational change occurs in ribonuclease T1 upon binding of guanose to the recognition site (41). The importance of mobile aromatic residues in antibody-antigen recognition has also been discussed (42). Aromatic residues (tyrosine and tryptophan) have high occurrence in the antigen binding sites of antibodies, especially in the heavy chain. Movements of their side chains may play key roles in the variation of antibody-antigen recognition. One explanation for the specific use of aromatic residues is that their large rotatable volumes affect variation in the surface contours of the antigen binding site without alterations in backbone conformation. In addition, their amphiphilicity allows them to form hydrogen bonds, hydrophobic interactions, and electrostatic interactions with other residues, and contact easily with other molecules (42). Thus, the special role of aromatic residues may be a central component in protein-protein recognition, and CheY provides the best documented example.

Position 106 is occupied by an aromatic residue (tyrosine or phenylalanine) in more than 80% of known response regulators (9), which implies that an aromatic amino acid at this position is required for normal function of CheY. Indeed, our earlier paper (18) shows that when position 106 is substituted by a non-aromatic residue (such as Gly, Val, Leu, Ile, and Cys), the mutant molecules lose their signaling, and the cells become smooth-swimming and non-chemoactive. But when Tyr106 is substituted by either Trp or Phe, the mutants retain chemotaxis, although at a reduced level (18).

Involvement of the 106 Rotamer in CheY Activation Is a Post-phosphorylation Event—All three mutants presented here can be easily phosphorylated by CheA, which indicates that neither Thr87 nor Tyr106 are required for phosphorylation. Our data do suggest that Thr87 might be involved in the CheY phosphatase activity, since both the phosphorylated T87I and T87I/Y106W mutants show stability 5 times that of wild-type CheY-P. The Y106W mutant shows a smaller (2-fold) decrease in its phosphorytase activity. This is consistent with the CheY structure: Thr87 is closer to the Asp97 phosphorylation site than Tyr106, and Thr87 connects with Asp97 through two bridging solvent molecules, while Tyr106 is away from the phosphorylation site, closer to the signaling surface defined by suppressor mutations (43, 44).

It is unlikely that the phosphorytase defects in the T87I and T87I/Y106W molecules contribute to the loss of chemotaxis of these mutants. In the signaling flow of wild-type CheY, CheY is phosphorylated by CheA-P, and then CheY-P interacts with the switch motor to generate a tumble motion. Both the T87I and T87I/Y106W mutants have normal phosphorylation activities, but reduced dephosphorylation activities. One would expect a higher concentration of phosphorylated mutant CheY molecules in the cell, which would cause the cell to be more tumbly than wild-type cells. However, our behavioral data show that both mutants are exclusively smooth swimming. This implies that the local structural change of the side chain of residue 106 blocks the signal from phosphorylated CheY. In other words, the defect in signaling ability in these mutants is subsequent to the primary activation event of phosphorylation.

Our earlier paper (18) demonstrates that Tyr106 is not directly involved in switch binding, since mutants with different substitutions at position 106 bind to FliM as well as wild-type CheY. We could not measure the binding affinity of the phosphorylated T87I and T87I/Y106W mutants to FliM, since both mutants could not be phosphorylated by acetyl phosphate (Refs. 18 and 44; acetyl phosphate is the phosphorylating agent used in our in vitro binding assay for CheY-P-FliM association). However, the unphosphorylated forms of T87I and T87I/Y106W do bind to FliM as strongly as the unphosphorylated form of wild-type CheY (data not shown). Thus it is very likely that for wild-type, the conformational heterogeneity of residue 106 does not affect CheY-P’s binding affinity for FliM, but acts to propagate the signal after CheY-P-FliM association. This is supported by the observations that no suppressors of fliG or fliM mutations map to positions 87 or 106 in CheY (43, 44). Further supporting evidence is that the non-phosphorylatable but constitutively active CheY mutant D13K generates a dominant tumbly signal without increased binding to FliM (45).

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