

Binding of the Chemotaxis Response Regulator CheY to the Isolated, Intact Switch Complex of the Bacterial Flagellar Motor

LACK OF COOPERATIVITY*

Received for publication, March 28, 2003, and in revised form, May 6, 2003
Published, JBC Papers in Press, May 7, 2003, DOI 10.1074/jbc.M303201200

Yael Sagi‡, Shahid Khan§, and Michael Eisenbach‡¶

From the ‡Department of Biological Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel and the §Molecular Biology Consortium, Chicago, Illinois 60612

In bacteria, the chemotactic signal is greatly amplified between the chemotaxis receptors and the flagellar motor. In *Escherichia coli*, part of this amplification occurs at the flagellar switch. However, it is not known whether the amplification results from cooperativity of CheY binding to the switch or from a post-binding step. To address this question, we purified the intact switch complex (constituting the switch proteins FliG, FliM, and FliN and the scaffolding protein FliF) in quantities sufficient for biochemical work and used it to investigate whether the binding of CheY to the switch complex is cooperative. As a negative control, we used complexes of switchless basal bodies, formed from the proteins FliF and FliG and similarly isolated. Using double-labeling centrifugation assays for binding, we found that CheY binds to the isolated, intact switch complex in a phosphorylation-dependent manner. We observed no significant phosphorylation-dependent binding to the negative control of the switchless basal body. The dissociation constant for the binding between the switch complex and phosphorylated CheY (CheY~P) was $4.0 \pm 1.1 \mu\text{M}$, well in line with the published range of CheY~P concentrations to which the flagellar motor is responsive. Furthermore, the binding was not cooperative (Hill coefficient ≈ 1). This lack of CheY~P-switch complex binding cooperativity, taken together with earlier *in vivo* studies suggesting that the dependence of the rotational state of the motor on the fraction of occupied sites at the switch is sigmoidal and very steep (Bren, A., and Eisenbach, M. (2001) *J. Mol. Biol.* 312, 699–709), indicates that the chemotactic signal is amplified within the switch, subsequent to the CheY~P binding.

The motility of bacteria such as *Escherichia coli* and *Salmonella enterica* serovar Typhimurium derives from the rotation of their flagella (for reviews, see Refs. 1 and 2). Each flagellum is driven by a bidirectional rotary motor embedded in the cytoplasmic membrane. The direction of flagellar rotation determines the swimming behavior of the cells, enabling bacteria

to approach beneficial environments and escape hostile ones. Thus, the essence of bacterial chemotaxis is modulation of the direction of flagellar rotation (3). This modulation is carried out by a switch that responds to signals received from the chemotaxis receptors (for reviews, see Refs. 4–6). The switch extends from the base of the flagellar motor into the cytoplasm. It is composed of three proteins, FliG (~35 molecules per switch (7, 8)), FliM (~35 molecules per switch (7, 8)), and FliN (~100 molecules per switch (9)), and is mounted on the MS ring of the flagellar motor, formed from the protein FliF (10–12). The signals from the chemotaxis receptors to the switch are transduced by the response regulator CheY. The activity of this protein is modulated by phosphorylation. When phosphorylated in response to chemotactic stimulation, the protein is detached from the histidine kinase CheA, which is a part of the receptor supramolecular complex, and acquires an elevated affinity for the switch protein FliM (13–16). The outcome is an increased probability of shifting the direction of flagellar rotation from the default direction, counterclockwise, to clockwise (for recent reviews, see Refs. 17 and 18).

Bacteria such as *E. coli* and *Salmonella* sense stimuli over a wide concentration range and, despite the wide range, do so with very high sensitivity, suggesting high amplification of the chemotactic signal (19, 20). One of the major questions in bacterial chemotaxis is which of the signal transduction steps amplify the chemotactic signal. One amplification step likely occurs at the receptor level, the amplification being provided by propagation of the excitation signal from the stimulated receptor molecule to neighboring receptor molecules within the receptor cluster (21–24; for a review, see Ref. 17). In view of recent findings that switching from counterclockwise to clockwise upon an increase in the intracellular level of CheY~P¹ is highly cooperative (a Hill coefficient of ~ 10 (25)), another amplification step probably occurs at the switch level (for a review, see Ref. 26). However, it is not known whether this high amplification reflects cooperativity of CheY~P binding to FliM (4, 25, 27, 28) or amplification of post-binding events at the switch (28). This question cannot be addressed by direct *in vitro* assays of CheY binding to purified FliM because it is not known whether purified FliM in solution truly represents the native protein within the switch complex and because the inter-FliM interactions, which normally occur within the switch (29), probably do not occur with soluble FliM molecules.

Recently, Lux *et al.* (30) overexpressed, in a non-flagellated strain, all three switch proteins together with FliF and purified the resulting membrane-associated structure. These structures have now been identified on the basis of immunochemical (30),

* This study was supported by Grant 2000037 from the United States-Israel Binational Science Foundation, Jerusalem, Israel. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Incumbent of the Jack and Simon Djanogly Professorial Chair in Biochemistry. To whom correspondence should be addressed: Dept. of Biological Chemistry, The Weizmann Institute of Science, P. O. Box 26, 76100 Rehovot, Israel. Tel.: 972-8-934-3923; Fax: 972-8-934-4112; E-mail: m.eisenbach@weizmann.ac.il.

¹ The abbreviations used are: CheY~P, phosphorylated CheY; Ni-NTA, nickel-nitrilotriacetic acid.

morphological (31), and *in vivo* CheY-binding criteria (32) as intact functional switch complexes mounted on the transmembrane MS ring. These advances open up the possibility of detailed mechanistic analysis of the cooperative motor response upon binding CheY. Here, we have used this preparation to characterize the phosphorylation-dependent binding of CheY to the intact switch complex *in vitro*.

EXPERIMENTAL PROCEDURES

Preparation of Switch Complex—For the preparation of switch complexes, we overproduced FliG and FliF from the overproducing plasmid pKOT107 and overproduced FliM and FliN from the overproducing plasmid pKLR2 in the nonflagellated *E. coli* strain BL21 (ADE3) (33). The overproduced switch complex, found at the cytoplasmic membrane, was purified according to the procedure of Francis *et al.* (34) for isolating extended basal bodies with several modifications. Cells (21 liters) of strain BL21 (ADE3) containing the plasmids pKOT107 and pKLR2 were grown in Luria broth supplemented with ampicillin (100 μ g/ml) and chloramphenicol (30 μ g/ml). The overproduction of the proteins was induced at $OD_{600} = 0.4$ by isopropyl-1-thio- β -D-galactopyranoside (1 mM). The cells were grown for an additional 3 h and harvested by centrifugation. The pellet was resuspended in 250 ml of ice-cold sucrose solution (0.5 M sucrose, 0.1 M Tris-HCl, pH 7.9), and then lysozyme (final concentration, 1 mg/ml, from a freshly prepared stock solution) and EDTA (final concentration, 10 mM) were added. The mixture was gently stirred at 4 $^{\circ}$ C for about 11 h. The resulting spheroplasts were lysed by two-step addition of Triton X-100: first to a final concentration of 1% in the presence of $MgSO_4$ (10 mM); then, following gentle stirring at 4 $^{\circ}$ C for 3 h, to a final concentration of 2% in the presence of $MgSO_4$ (10 mM). The suspension was further gently stirred at 4 $^{\circ}$ C for 0.5 h. EDTA (20 mM) was added, and the suspension was stirred for 60 min. Unlysed cells and inclusion bodies were removed by centrifugation at $23,000 \times g$ for 30 min, and the pH of the supernatant was raised with NaOH to pH 10 to disintegrate the outer membrane structures. The lysate was spun at $123,000 \times g$ for 60 min, and the pellet was resuspended in a solution containing Tris-HCl (10 mM, pH 7.9), KCl (100 mM), sucrose (10% w/v), and Triton X-100 (0.1%). The suspension was recentrifuged at $23,000 \times g$ for 30 min, and then the supernatant was spun at $200,000 \times g$ for 40 min. The pellet was resuspended in a solution containing Tris-HCl (50 mM, pH 7.9) and Triton X-100 (0.1%). To get rid of residual inclusion bodies that potentially remain in the suspension, the suspension was centrifuged again at $23,000 \times g$ for 10 min. The purified switch complex was stored at 4 $^{\circ}$ C with Complete protease inhibitor (Roche Applied Science).

Preparation of Switchless Basal Body—For the preparation of a switchless basal body, we used the same procedure used for the preparation of the switch complex, with the exception that the cells contained only one plasmid, the plasmid pKOT107 for the overproduction of FliG and FliF alone.

Labeling of CheY—Initially we radiolabeled CheY *in vitro* by methylating the ϵ -amine of its lysine residues and the N terminus with formaldehyde and $NaB^{14}C_4$, as described previously (35). The experiments shown in Fig. 2 were carried out with CheY labeled in this way. However, subsequently we found that high levels of labeling (higher than those used in Fig. 2) inhibited the ability of CheY to undergo *in vitro* phosphorylation by the phosphodonor acetyl phosphate. We, therefore, switched to *in vivo* labeling, which does not involve chemical modification of CheY. *E. coli* strain M15 (36), carrying the plasmid pQE12-CheY-His-tag for the overproduction of His₆-CheY (received from A. Wolfe, Loyola University), was grown to $OD_{590} = 0.5$ in H1 minimal medium of Kaiser and Hogness (37) supplemented with histidine, methionine, and threonine (1 mM each), as well as with leucine (200 μ M), thiamin (5 μ g/ml), ampicillin (100 μ g/ml), and glucose (0.3%, w/v). The overproduction of CheY was induced at $OD_{590} = 0.5$ by isopropyl-1-thio- β -D-galactopyranoside (0.5 mM). For labeling, isopropyl-1-thio- β -D-galactopyranoside was supplemented with [¹⁴C]leucine (final concentration, 0.17 μ Ci/ml). The cells were grown for an additional 4 h, harvested by centrifugation, and sonicated. Non-soluble material (inclusion bodies and cell debris) was removed by centrifugation at $123,000 \times g$ for 30 min. The His₆-tagged CheY protein was purified from the supernatant by Ni-NTA affinity chromatography (Qiagen) according to the manufacturer's instructions, with some modifications. We followed protocol 11 (batch purification of His₆-tagged proteins under native conditions) with the exception that, following the collection of the flow-through, the washing of the lysate-Ni-NTA column was with 80–90 ml of washing buffer (until no protein was eluted) rather than 4 ml, and the elution of CheY from the Ni-NTA column was

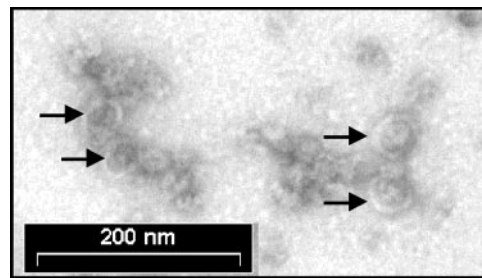


FIG. 1. **Electron micrograph of an isolated switch complex preparation.** Low magnification view of negatively stained structures obtained upon coexpression of the FliF, FliG, FliM, and FliN proteins. Switch complexes are indicated by the black arrows.

with 20–30 ml of elution buffer instead of 2 ml. We concentrated CheY by ultrafiltration through a 10 kDa cut-off membrane in an Amicon chamber (model 52). CheY was stored at -80° C. We verified that the His₆-tag at the C terminus of CheY did not interfere with the function of the protein according to two criteria: (a) we expressed His₆-tagged CheY in the non-chemotactic strain AW546 *eda*⁺ *cheY201* (received from J. Adler) and found that His₆-tagged CheY restored chemotactic responsiveness to this strain, judged by its ability to form typical chemotactic rings on a semisolid agar (0.3% agar) plate containing Tryptone (38); and (b) we measured the ability of His₆-tagged CheY to undergo acetyl phosphate-mediated *in vitro* phosphorylation by measuring the changes in the fluorescence of Trp⁵⁸, known to be strongly reduced upon CheY phosphorylation (39). The fluorescence of CheY was determined using a fluorimeter with excitation and emission wavelengths set at 295 and 345 nm, respectively. Tag-free CheY or His₆-tagged CheY was diluted to a final concentration of 10 μ M into 50 mM Tris-HCl buffer (pH 7.9) containing 5 mM $MgSO_4$, and its fluorescence was recorded. Subsequently, acetyl phosphate (final concentration, 20 mM) was added, and the fluorescence was again recorded. Both His₆-tagged CheY and tag-free CheY were similarly quenched (by ~60%) upon the addition of acetyl phosphate.

Binding of CheY to the Switch Complex and to the Switchless Basal Body—The switch complex or the switchless basal body, at the amount indicated in the figure legends, was incubated with [³H]CheY (2000–7000 dpm/pmol) and [¹⁴C]glucose (final concentration, 3500 dpm/ μ l) or [¹⁴C]CheY (His₆-tagged; 13–17 dpm/pmol) and [³H]glycine (600 dpm/pmol) in a solution of Tris-HCl (50 mM; pH 7.9), $MgSO_4$ (5 mM), and where indicated, bovine serum albumin (10 mg/ml). For phosphorylating conditions, acetyl phosphate (25 mM) was added to the reaction mixture. After a 5-min incubation at room temperature (24 $^{\circ}$ C), the switch complex or the switchless basal body was pelleted by centrifugation at $200,000 \times g$ for 10 min at 4 $^{\circ}$ C. The pellet was resuspended in 100 μ l of Tris-HCl (50 mM, pH 7.9). Aliquots of the reaction mixture prior to centrifugation and of the supernatant and pellet subsequent to centrifugation were counted for ¹⁴C and ³H by a β counter (Tri-Carb liquid scintillation analyzers).

RESULTS

Isolation of the Switch Complex for Biochemical Studies—To produce an intact switch complex in quantities sufficient for biochemical work, we overproduced the three switch proteins (FliG, FliM, and FliN) along with FliF from two plasmids as done by Lux *et al.* (30) and then purified the overproduced switch complex from the cytoplasmic membrane as described under “Experimental Procedures.” In electron micrographs, the purified switch complex (Fig. 1) appeared similar to that obtained by Lux *et al.* (30). As a negative control, we used the host BL21 cells, lacking the overproducing plasmids. These cells, following the same isolation procedure, did not yield the structures shown in Fig. 1.

Demonstration of Specific CheY~P Binding to the Switch Complex—To measure the binding between CheY and the purified switch complex, we equilibrated the complex with [³H]CheY and then separated switch-bound CheY from free CheY by centrifugation. To be able to measure the volume of medium entrapped within the pellet, we also included in the medium an inert radiolabeled compound, [¹⁴C]glucose. The

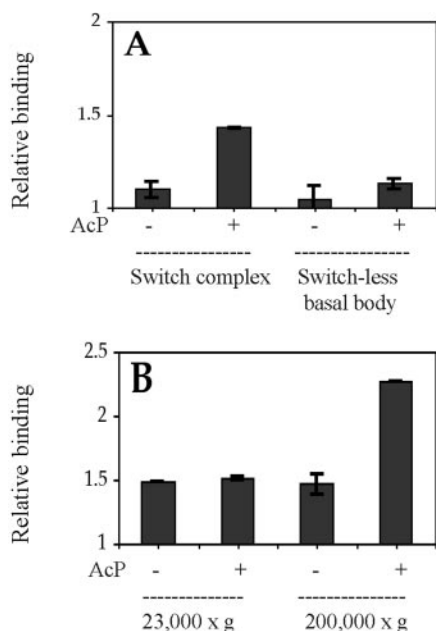


FIG. 2. Specific phosphorylation-dependent binding of CheY to the switch complex. The switch complex and switchless basal body were prepared and assayed for CheY binding as described under "Experimental Procedures," using [^3H]CheY (7000 dpm/pmol) and [^{14}C]glucose (final concentration, 3500 dpm/ μmol). The results, presented as the ratio between the quotient [^3H]CheY/[^{14}C]glucose in the pellet subsequent to centrifugation and the quotient [^3H]CheY/[^{14}C]glucose in the suspension before centrifugation, are the mean \pm S. D. of duplicates in a representative experiment. *A*, comparison between binding of CheY to the switch complex and to the switchless basal body. The amount of switch complex or switchless basal body in each case was 59 μg of total protein. *B*, examination of the potential contribution of FliM-containing inclusion bodies to the observed CheY binding. The amount of the switch complex was 148 μg of total protein. See text for details.

$^3\text{H}/^{14}\text{C}$ ratio in the pellet was higher in the presence of the phosphodonor acetyl phosphate than in its absence (Fig. 2A), suggesting the occurrence of phosphorylation-dependent binding of CheY to the switch complex. Because CheY binds to FliM, we used, as a negative control, a switchless basal body. We isolated it from cells overexpressing FliF and FliG only, using the same isolation procedure as for the intact switch complex. Unlike the case of the intact switch complex, we did not observe a phosphorylation-dependent increase in CheY binding to the switchless basal body (Fig. 2A). These results suggest that the phosphorylation-dependent binding to the intact switch complex is specific and that the isolated switch complex is functional with respect to CheY binding.

A potential source of concern in the above experiments was that traces of FliM-containing inclusion bodies might have remained in the preparation of the switch complex and might have contributed to the observed binding of CheY~P. We verified that this was not the case by the following measures: (a) we did not observe a white fluffy precipitate, typical of inclusion bodies, on top of the precipitate of the switch complex; (b) before precipitating the switch complex at 200,000 $\times g$, we got rid of FliM-containing inclusion bodies by spinning the preparation at 23,000 $\times g$, which is above the force required for their full precipitation (12,500 $\times g$ (40)); and (c) we carried out control experiments in which we equilibrated the switch complex with [^3H]CheY and then separated switch-bound CheY from free CheY by centrifugation either at 23,000 $\times g$ or at 200,000 $\times g$. We observed only a phosphorylation-dependent increase in the $^3\text{H}/^{14}\text{C}$ ratio in the pellet at 200,000 $\times g$ (Fig. 2B), suggesting that the preparation of isolated switch complex did not contain inclusion bodies and that the observed binding

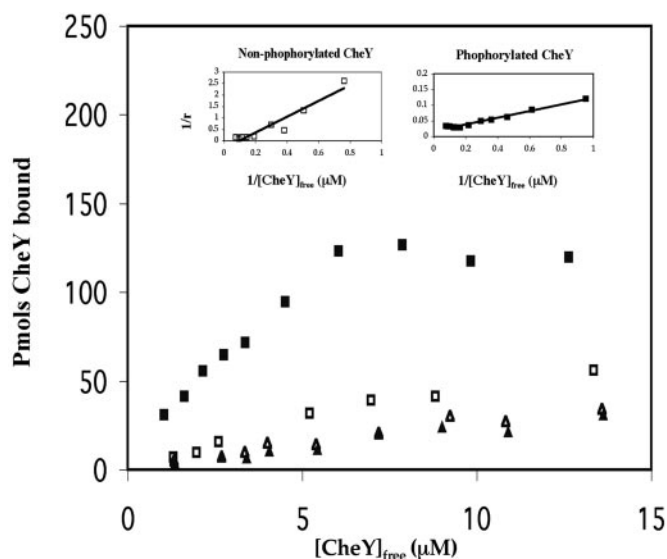


FIG. 3. Concentration-dependent CheY binding to the switch complex. The binding assay was carried out in the presence of bovine serum albumin (10 mg/ml) with switch complex or switchless basal body (115 μg of total protein in each case), [^{14}C]CheY (His₆-tagged; 17 dpm/pmol), and [^3H]glycine (600 dpm/pmol) as described under "Experimental Procedures." Δ , switchless basal body. \blacktriangle , switchless basal body in the presence of acetyl phosphate. \square , intact switch complex. \blacksquare , intact switch complex in the presence of acetyl phosphate. *Left inset*, double reciprocal plot of non-phosphorylated CheY; $K_d = 120 \mu\text{M}$. *Right inset*, double reciprocal plot of CheY in the presence of acetyl phosphate; $K_d = 3.6 \mu\text{M}$. The term r stands for the number of moles of CheY bound to 1 mol of the switch complex. We calculated it from the number of CheY molecules found to be bound (after subtraction of nonspecific binding to the switchless basal body) to a single molecule of FliM and then multiplying it by 35, the estimated number of FliM molecules within a single switch complex (7, 8). The free CheY concentration was calculated by subtracting the concentration of CheY measured to be bound to the switch from the total CheY concentration.

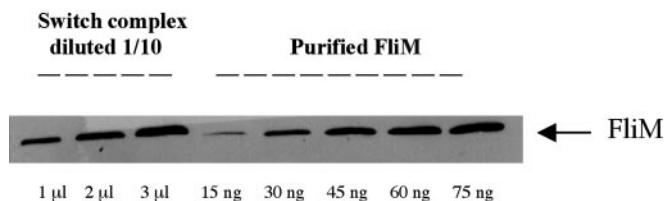


FIG. 4. FliM quantification by Western blots. Aliquots of purified switch complex and of purified FliM (15 ng/ μl) were mixed with sodium dodecyl sulfate buffer, boiled for 10 min, resolved on sodium dodecyl sulfate-12% (w/v) polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes as described previously (46). The blots were probed with anti-FliM antibody (a gift from S. I. Aizawa) and peroxidase-conjugated anti-rabbit antibody (Sigma). Films were scanned with an imaging densitometer (model GS-690; Bio-Rad). The intensities of the bands were quantified by Multi-Analyst software (Bio-Rad). Calculation of the amount of FliM in each preparation of the purified switch complex was made on the basis of a calibration curve prepared according to the band intensities of increasing concentrations of FliM.

of CheY~P was to the switch complex alone.

Quantification of the CheY-Switch Binding—To quantify the binding between CheY and the switch complex, we titrated the complex with increasing amounts of radiolabeled CheY under phosphorylating and nonphosphorylating conditions (presence and absence of acetyl phosphate, respectively). As a control for nonspecific binding, we used the switchless basal body at equal total-protein concentrations. Under nonphosphorylating conditions, CheY bound to the switch complex only slightly better than to the negative control of switchless basal body (Fig. 3). Under phosphorylating conditions, CheY selectively bound to

the switch complex. To quantify the binding, we had to estimate the total amount of FliM within the switch complex. We did this by two independent means: according to the number of CheY molecules bound to the switch complex at saturation and according to a Western blot analysis of the switch complex with anti-FliM antibodies (Fig. 4). Both methods yielded a similar estimate of the FliM amount, 0.95 and 1.1 nmol FliM/mg of total protein, respectively. These values were measured with the same preparation of switch complex used for the results shown in Figs. 3 and 4. The average ratio between these estimations for three different preparations of the switch complex was 0.9 ± 0.2 (mean \pm S.D.), suggesting that the stoichiometry between CheY and FliM is 1:1. Quantitative analysis of the data of this representative experiment revealed a dissociation constant of 3.6 and 120 μM for phosphorylated and nonphosphorylated CheY, respectively (Fig. 3, *insets*). Correcting the CheY~P concentration in the experiment according to the estimate that CheY is 85.5% phosphorylated *in vitro* under these conditions (41), we calculated that the dissociation constant for the CheY~P-switch complex is 2.9 μM . A Hill fit to the binding data yielded a Hill coefficient of 1.0 ($r^2 = 0.99$), suggesting that, within the uncertainties of the experimental error, the binding of CheY~P to the isolated switch complex is not cooperative. This apparent lack of cooperativity was observed in each of the three batches of isolated switch complex assayed for cooperativity (average Hill coefficient = 1.3 ± 0.2 (\pm S.D.)). The dissociation constant varied in the range of 2.9–5.6 μM between different batches of the isolated switch complex, yielding a mean of $4 \pm 1 \mu\text{M}$ (\pm S.D.).

DISCUSSION

In this study we isolated, in quantities sufficient for biochemical work, the flagellar switch complex of *E. coli* and demonstrated that it is functional, at least with respect to CheY binding. We found that this binding ($K_d = 4 \pm 1 \mu\text{M}$) is phosphorylation-dependent, is specific, has a CheY:FliM stoichiometry of 1:1, and is not cooperative. The significance of these findings is discussed below.

Earlier *in vivo* studies found a steep dependence of the probability of clockwise rotation on the intracellular concentration of active CheY (25, 27, 42, 43). Carrying out the measurements in single cells, Cluzel *et al.* (25) found that the increase was over a narrow range of CheY~P concentrations and highly cooperative (Hill coefficient ≈ 10). These observations suggested that the switch acts as some sort of amplifier, translating small changes in the concentration of CheY~P into large changes in clockwise probability. However, these observations could not distinguish between amplification caused by cooperativity of CheY binding to the switch (4, 25, 27, 28) and amplification evolved within the switch (28). Earlier studies, which used *in vitro* binding assays with purified soluble proteins (or peptides thereof), could not address this question either (13, 16, 44, 45). A distinction between both possibilities, although indirect, was later made by Bren and Eisenbach (46), who used FliM proteins almost locked in their clockwise or counterclockwise states as representatives of CheY-bound and CheY-free FliM, respectively. By expressing these FliM proteins to different levels, they found that the dependence of the clockwise probability on the relative level of FliM in the clockwise state is very steep. Because that study bypassed the CheY~P-FliM binding step, it suggested that at least part of the signal amplification occurs at a post-binding step. In the current study, we found no cooperativity of CheY~P binding to the switch, endorsing the notion that the chemotactic signal is amplified within the switch rather than at the preceding binding step of CheY~P to the switch.

While preparing this manuscript for publication, a study by

Sourjik and Berg (47) was published in which resonance energy transfer was used for *in vivo* measurements of CheY binding to the switch. Sourjik and Berg (47) found essentially no cooperativity of binding and a dissociation constant of $\sim 3.7 \mu\text{M}$, very similar to the value measured by us in this study. The similarity between the *in vivo* results of Sourjik and Berg (47) and the *in vitro* results with purified intact switch complex obtained in our current study suggests that the isolated switch complex is functional and reliably represents the function of the switch within the cell. Furthermore, our measured dissociation constant for CheY~P ($4 \pm 1 \mu\text{M}$) is well in line with the range of CheY~P concentrations over which the motor changes its bias from counterclockwise to clockwise (2–6 μM) (25). As might be expected, it is lower than the dissociation constant measured for the binding of an N terminus-containing peptide of FliM (45) to CheY~P ($\sim 27 \mu\text{M}$) (16).

The current study, taken together with the studies of Sourjik and Berg (47), Cluzel *et al.* (25), and Bren and Eisenbach (46), strongly argues that the chemotactic signal is amplified within the switch, subsequent to the CheY~P binding. Such amplification suggests that the switching process can be described as an allosteric transition between the rotational states of the switch, with CheY~P as an allosteric activator. A model for an allosteric transition of switching was recently proposed by Duke *et al.* (48).

Acknowledgment—We thank Dr. S. R. Caplan for insightful discussions and critical reading of the manuscript.

REFERENCES

- Macnab, R. M. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (Neidhardt, F. C., Curtis, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umberger, H. E., eds) 2nd Ed., pp. 123–145, American Society for Microbiology, Washington, D. C.
- Eisenbach, M. (1990) *Mol. Microbiol.* **4**, 161–167
- Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W., and Adler, J. (1974) *Nature* **249**, 74–77
- Macnab, R. M. (1995) in *Two-component Signal Transduction* (Hoch, J. A., and Silhavy, T. J., eds) pp. 181–199, American Society for Microbiology, Washington, D. C.
- Eisenbach, M., and Caplan, S. R. (1998) *Curr. Biol.* **8**, R444–R446
- Silversmith, R. E., and Bourret, R. B. (1999) *Trends Microbiol.* **7**, 16–22
- Zhao, R. B., Amsler, C. D., Matsumura, P., and Khan, S. (1996) *J. Bacteriol.* **178**, 258–265
- Thomas, D. R., Morgan, D. G., and DeRosier, D. J. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 10134–10139
- Zhao, R. H., Pathak, N., Jaffe, H., Reese, T. S., and Khan, S. (1996) *J. Mol. Biol.* **261**, 195–208
- Ueno, T., Oosawa, K., and Aizawa, S.-I. (1992) *J. Mol. Biol.* **227**, 672–677
- Oosawa, K., Ueno, T., and Aizawa, S. (1994) *J. Bacteriol.* **176**, 3683–3691
- Kubori, T., Yamaguchi, S., and Aizawa, S.-I. (1997) *J. Bacteriol.* **179**, 813–817
- Welch, M., Oosawa, K., Aizawa, S.-I., and Eisenbach, M. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 8787–8791
- Schuster, S. C., Swanson, R. V., Alex, L. A., Bourret, R. B., and Simon, M. I. (1993) *Nature* **365**, 343–347
- Li, J. Y., Swanson, R. V., Simon, M. I., and Weis, R. M. (1995) *Biochemistry* **34**, 14626–14636
- McEvoy, M., Bren, A., Eisenbach, M., and Dahlquist, F. W. (1999) *J. Mol. Biol.* **289**, 1423–1433
- Bren, A., and Eisenbach, M. (2000) *J. Bacteriol.* **182**, 6865–6873
- Bourret, R. B., and Stock, A. M. (2002) *J. Biol. Chem.* **277**, 9625–9628
- Segall, J. E., Block, S. M., and Berg, H. C. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 8987–8991
- Jasuja, R., Yu-Lin, Trentham, D. R., and Khan, S. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 11346–11351
- Bray, D., Levin, M. D., and Morton-Firth, C. J. (1998) *Nature* **393**, 85–88
- Duke, T. A. J., and Bray, D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 10104–10108
- Ames, P., Studdert, C. A., Reiser, R. H., and Parkinson, J. S. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 7060–7065
- Kim, S.-H., Wang, W. R., and Kim, K. K. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 11611–11615
- Cluzel, P., Surette, M., and Leibler, S. (2000) *Science* **287**, 1652–1655
- Bray, D. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 7–9
- Kuo, S. C., and Koshland, D. E. (1989) *J. Bacteriol.* **171**, 6279–6287
- Spiro, P. A., Parkinson, J. S., and Othmer, H. G. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 7263–7268
- Marykwas, D. L., Schmidt, S. A., and Berg, H. C. (1996) *J. Mol. Biol.* **256**, 564–576
- Lux, R., Kar, N., and Khan, S. (2000) *J. Mol. Biol.* **298**, 577–583
- Young, H. S., Dang, H., Lai, Y., DeRosier, D. J., and Khan, S. (2003) *Biophys. J.* **84**, 571–577

32. Khan, S., Pierce, D., and Vale, R. D. (2000) *Curr. Biol.* **10**, 927–930
33. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol.* **185**, 60–89
34. Francis, N. R., Sosinsky, G. E., Thomas, D., and DeRosier, D. J. (1994) *J. Mol. Biol.* **235**, 1261–1270
35. Blat, Y., and Eisenbach, M. (1996) *J. Biol. Chem.* **271**, 1232–1236
36. Zamenhof, P. J., and Villarejo, M. (1972) *J. Bacteriol.* **110**, 171–178
37. Kaiser, A. D., and Hogness, D. S. (1960) *J. Mol. Biol.* **2**, 392–415
38. Adler, J. (1966) *Science* **153**, 708–716
39. Lukat, G. S., McCleary, W. R., Stock, A. M., and Stock, J. B. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 718–722
40. Zhao, R. B., Schuster, S. C., and Khan, S. (1995) *J. Mol. Biol.* **251**, 400–412
41. Blat, Y., Gillespie, B., Bren, A., Dahlquist, F. W., and Eisenbach, M. (1998) *J. Mol. Biol.* **284**, 1191–1199
42. Scharf, B. E., Fahrner, K. A., Turner, L., and Berg, H. C. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 201–206
43. Alon, U., Camarena, L., Surette, M. G., Arcas, B. A. Y., Liu, Y., Leibler, S., and Stock, J. B. (1998) *EMBO J.* **17**, 4238–4248
44. Welch, M., Oosawa, K., Aizawa, S.-I., and Eisenbach, M. (1994) *Biochemistry* **33**, 10470–10476
45. Bren, A., and Eisenbach, M. (1998) *J. Mol. Biol.* **278**, 507–514
46. Bren, A., and Eisenbach, M. (2001) *J. Mol. Biol.* **312**, 699–709
47. Sourjik, V., and Berg, H. C. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 12669–12674
48. Duke, T. A. J., Le Novère, N., and Bray, D. (2001) *J. Mol. Biol.* **308**, 541–553