Modulation of the Thermosensing Profile of the Escherichia coli Aspartate Receptor Tar by Covalent Modification of Its Methyl-accepting Sites*  

(Received for publication, May 31, 1995, and in revised form, April 30, 1996)  

Toshifumi Nara†, Ikuro Kawagishi§, So-ichiro Nishiyama, Michio Homma, and Yasuo Imae†  

From the Department of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan  

The Escherichia coli aspartate receptor Tar is involved in the thermotactic response. We have studied how its thermosensing function is affected by the modification of the four methyl-accepting residues (Gln295, Glu302, Gln309, and Glu491), which play essential roles in adaptation. We found that the primary translational product of tar mediates a chemoresponse, but not a thermoresponse, and that Tar comes to function as a thermoreceptor, once Gln295 or Gln309 is deamidated. This is the first identification of a thermosensing-specific mutant form, suggesting that the methylation sites of Tar constitute at least a part of the region required for thermoreception, signaling, or both. We have also investigated the inverted thermoresponse mediated by Tar in the presence of aspartate. We found that, whereas the deamidated-and-unmethylated form functions as a warm receptor, eliciting a smooth-swimming signal upon increase of temperature, the heavily methylated form functions as a cold receptor, eliciting a smooth-swimming signal upon decrease of temperature. Thus, it is suggested that Tar exists in at least three distinct states, each of which allows it to function as a warm, cold, or null thermoreceptor, depending on the modification patterns of its methylation sites.

Temperature is one of the crucial environmental parameters that restrict growth and other biological activities in any organism. Therefore, virtually all organisms show some kind of response to an increase or decrease in temperature. It is, however, generally very difficult to investigate how an organism senses temperature shifts, since most higher organisms have no specialized thermosensing organ, and temperature affects a wide variety of cell functions. Thermotaxis in Escherichia coli, however, has been well characterized in terms of physiology, genetics, and biochemistry (1). Thermal stimuli are sensed by the four closely related transmembrane receptors (2–6). These receptors, also known as methyl-accepting chemotaxis proteins (MCPs),1 were originally identified as chemosensory receptor/transducers (7–13). They are reversibly methylated at four or five glutamate residues within their C-terminal cytoplasmic domains (14–16). Methylation and demethylation are the basis of adaptation in chemotaxis and are catalyzed by CheR and CheB, respectively (17, 18). An attractant stimulus, whether chemical or thermal, causes elevated methylation of the receptors, which shuts off the attractant signal output. In contrast, a repellent stimulus brings about lowered levels of methylation to counteract the repellent signal (15). Increases and decreases in methylation levels correspond to the magnitudes of attractant and repellent stimuli, respectively. It should be noted that some of the methyl-accepting glutamate residues are derived from glutamine residues in the primary translational products (19, 20). CheB is also responsible for the specific and irreversible deamidation of these groups.

Extensive genetic and biochemical studies on bacterial chemotaxis have identified all of the protein components involved in the chemotactic signal transduction pathway from the detection of attractants and repellents to the control of swimming behavior (for review, see Refs. 10–13). In brief, when a chemoreceptor binds to a repellent, it activates the cytoplasmic autophosphorylating protein kinase CheA that can transfer phosphate group to the cytoplasmic signaling protein CheY and the receptor methyltransferase CheB. Phospho-CheY interacts with switch component(s) of the flagellar motor to cause its clockwise rotation; phospho-CheB demethylates chemoreceptor molecules to shut off the tumbling signal. On the other hand, binding of an attractant to a receptor causes inhibition of CheA kinase, which results in a decrease in CheY and CheB phosphorylation. The same signaling pathway (from the receptor to the flagellar motor) is utilized in thermotaxis, but the mechanism underlying thermosensing has not been elucidated.

Each receptor forms a ternary complex with CheA and the coupling protein CheW (21). Since the complex is stable in vitro (21, 22) and in vivo (23), receptor-mediated control of CheA activity is thought to occur through structural changes within this receptor-CheW-CheA complex. Therefore, it is reasonable to assume that the thermosensing mechanism involves similar structural changes in the ternary complex to those caused by increases and decreases in temperature. It should be stressed that temperature dependence of autophosphorylation, phosphotransfer, or dephosphorylation cannot simply explain thermotaxis. Rather, the receptors seem to be primary thermosensors (2–6): they fall into two classes, namely warm receptors (Tsr, Tar, and Trg) and cold receptors (Tar in the presence of attractants [see below] and Tap). A warm receptor produces attractant and repellent signals upon increases and...

---

1 The abbreviation used is: MCP, methyl-accepting chemotaxis protein.

---

† Professor Yasuo Imae, who was a pioneering researcher in the fields such as bacterial thermotaxis and sodium-driven flagellar motor, died unexpectedly on July 2, 1993. This article is dedicated to him by the rest of the authors with deep sorrow, respect, and affection.

‡ Present address: Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060, Japan.

§ To whom all correspondence should be addressed: Dept. of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan. Tel.: 81-52-789-2993; Fax: 81-52-789-3001; E-mail: i45406a@nucc.cc.nagoya-u.ac.jp.
Modulation of Bacterial Thermosensor

17933

decreases in temperature, respectively, whereas a cold receptor mediates the opposite responses to the same stimuli (6).

Interestingly, two lines of evidence suggest that covalent modification of the methyl-accepting sites might influence the thermosensing properties of the receptors. First, cheB-defective strains, which can respond to but cannot adapt to chemotactic stimuli, show no thermostresponse, whereas cheR-defective strains, which cannot adapt either, retain thermosensing abilities (2, 24). The absence of a thermostresponse in cheB mutants might be due to the absence of deamination and/or demethylation of the receptors. Alternatively, CheB might be involved in the thermosensing/signaling process: e.g., it might constitute a thermosensor complex with the receptor, CheW and CheA. Second, Tar produces inverted thermotactic signals in the presence of any of its specific attractants, such as aspartate and maltose (3, 5). Whereas it usually functions as a warm receptor, Tar appears to be converted into a cold receptor after adapting to an attractant. Enhanced methylation of Tar has been proposed to cause this attractant-dependent conversion of thermoreceptor function.

In this study, we have studied the roles of the methyl-accepting residues (Gln295, Glu302, Gln309, and Glu491: the set of the four residues will be referred to as [QEQE] throughout this paper) in the thermosensing function of Tar, by examining thermosensing profiles in the various genetic background.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains**—All of the bacterial strains used in this study are derivatives of E. coli K-12. Strains HCB339 (Δtar·ΔcheB)2234 (25), CP553 (Δtar·ΔcheB)2234 Δtar-10thr·ΔcheB)2234 Δtar-7028) (26), and RP1245 (cheR) were provided by H. C. Berg of Harvard University, C. Park of Korea Advanced Institute of Science and Technology, and J. S. Parkinson of University of Utah, respectively.

Plasmids—A pBR322-based plasmid pAK101 (27), which carries tar and cheW, was provided by M. I. Simon of California Institute of Technology. Plasmids pRA130, pRA131, and pRA132, coding for the mutant Tar proteins whose Gin295, Gin309, Gin309, and Gin309, respectively, were replaced by Gln, were constructed from pAK101 in this study as follows. The codons for residues 295 and 309 were changed from CAG (Gln) to GAG (Glu) using site-directed mutagenesis. The mutations were verified by nucleotide sequencing. Plasmid pNI130, which was constructed from pRA101 and pLAN931 (28), is the pBR322-based plasmid that carries the same mutant tar gene as pRA130 but lacks most of the cheW sequence.

A cheR-carrying plasmid pRA1 was constructed as follows. The 2.3 kb NruI-PvuI fragment of plasmid pDV2, provided by P. Matsumura of University of Illinois, Chicago, was blunt-ended and ligated with EcoRV-digested pACYC184. The ligation mixture was introduced into the cheR mutant RP1245, and chloramphenicol-resistant CheR-transformants were selected. Plasmid DNA was extracted from the clones and examined by restriction enzyme digestion.

**DNA Manipulations**—Routine DNA manipulations were carried out according to standard procedures (29). Restriction endonucleases and other enzymes for DNA manipulations were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). Site-directed mutagenesis was performed by the method of Kunkel et al. (30). Oligonucleotides for mutagenesis and DNA sequencing were synthesized at the Center for Gene Research at Nagoya University.

Measurement of Chemoreceptor—Temporal stimulation assays were carried out as described previously (5). Cells were grown at 30°C with vigorous shaking in tryptone-glycerol broth (1% Bacto-tryptone, Difco Laboratories, Detroit, MI), 0.5% NaCl, and 0.5% glycerol) supplemented with ampicillin (50 µg/ml) and, if necessary, chloramphenicol (25 µg/ml). After 5 h of cultivation, cells were harvested by centrifugation at room temperature and washed with motility medium (10 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM sodium DL-lactate (pH 7.0), and 0.1 mM l-methionine). Cells were resuspended in motility medium and kept at room temperature. Immediately after adding an attractant or a repellent to the cell suspension, the swimming pattern of the cells was observed with a dark-field microscope and recorded on videotape. The smooth-swimming fraction was measured photogaphically as described previously (31). The threshold concentrations of chemicals for tactic responses were estimated from their dose-response curves. Synthetic sodium L-aspartate obtained from ICN Pharmaceuticals, Inc. (Plainview, NY) was used throughout the measurement of thermoreponse.

Measurement of Thermoreponse—A cell suspension was prepared as described in the previous section. If necessary, an appropriate concentration of aspartate or glyceral was added to cells suspended in motility medium. A drop of the suspension was placed on a glass slide mounted on a temperature control apparatus as described previously (2). The temperature was changed from 20 to 30°C and returned to 20°C. Temperature changes were monitored by a constantan-chromel thermocouple inserted into the suspension. Changes in the swimming pattern of the odf's were measured quantitatively as described in the previous section.

**RESULTS**

Inhibition of the Thermosensing Ability of Tar by the Remaining Glutamine Residues—To address the role of cheB in thermosensing, we first examined whether Tar with [QEQE] functions as a theremoreceptor, rather than [QEmQEm] (Em standing for a methylated glutamate residue), which is expressed in cheB-defective strains and does not mediate thermoreponse. The plasmid pAK101 carrying wild-type tar gene, A, swimming behavior of the cells with (closed circles) or without (open circles) 10 µM aspartate. B, the time course of the temperature.

FIG. 1. Absence of theromotic response of the CP553 (CheB-CheR) cells carrying pAK101 which contains the wild-type tar gene. A, swimming behavior of the cells with (closed circles) or without (open circles) 10 µM aspartate. B, the time course of the temperature.

2 J. S. Parkinson, personal communication.

3 P. Matsumura, personal communication.
ability, failure of demethylation of the receptors in cheB mutants cannot explain their inability to respond to temperature shift.

We then examined whether the absence of deamidating activity in cheB mutants is responsible for this phenomenon. We constructed a tar gene coding with two glutamine codons in the methylation sites replaced by gluta- marate and expressed the resulting "genetically deamidated" receptor ([EEEE]) in the cheB cheR background (CP553). The resulting cells (pRA130/CP553) swam extremely smoothly regardless of temperature change (Fig. 2), but showed a normal thermoresponse when the smooth-swimming fraction was decreased by adding glycerol, a repellent recognized by Tar [31, 32] (Fig. 2). It should be noted that CP553 cells which do not have any receptor swim always smoothly and did not respond to temperature change even in the presence of glycerol (data not shown). Moreover, CheB+, CheR+ cells expressing wild-type Tsr, Tar, Trg, or Tap show thermoresponses in the absence of glycerol (2–6). Therefore, temperature-dependent changes in the intracellular concentration of acetyl phosphate, which can donate a phosphate group to CheY (33), is not likely to be the cause of thermotaxis. The fact that even a cheB defective strain can respond to temperature changes indicates that CheB is not a thermosensor nor a part of a thermosensor complex. Rather, GinP95, GinP99, or both seems to prevent thermosensing function of Tar.

To examine which glutamine residue has the inhibitory effect on thermosensing ability, GlnP95 and GlnP99 of Tar were replaced by Glu singly: i.e. [EEQE] and [QEEE]. In the cheB cheR background, both of them mediated normal thermoresponses when smooth-swimming fraction of the cells was decreased by adding glycerol (Table I). These results suggest that the presence of both GinP95 and GinP99 in the methyl-accepting sites prevents Tar from thermosensing in the cheB-defective strains. We therefore concluded that in the wild-type strain, the primary translational product of Tar ([QQEQ]) cannot function as a thermoreceptor, although it has the thermosensing ability, and that Tar acquires thermoreceptor function, once either of its glutamine residues is deamidated by CheB.

Methylation of Tar in the Presence of Aspartate Results in Its Conversion from a Warm to a Cold Receptor—We now turn to the question how Tar is converted to a cold receptor in the presence of a specific attractant such as aspartate. We first examined whether the genetically deamidated mutant Tar receptor ([EEEE]) has the same thermosensing characteristics as the wild-type Tar ([QEQE]) in the CheR− CheB− background. Plasmid pNI130 coding for Tar with [EEEE] was introduced into E. coli strain HCB339, which lacks all four chemoreceptors. As shown in Fig. 3, the resultant cells showed an essentially similar aspartate dependence of thermoresponses as the wild-type Tar. In the absence of aspartate, the cells showed attractant and repellent responses to increases and decreases in temperature, respectively. Even after adapting to 1 μM aspartate (for 30 min), the cells showed similar responses to thermal stimuli. However, when aspartate was added at a concentration of 10 μM, the cells showed a repellent response to increased temperature (Fig. 3). They did not seem to respond to decreased temperature, since they swam too smoothly even after prolonged incubation with aspartate. However, when glycerol was added further to adjust their swimming bias toward tumbling, they showed a clear inverted thermoresponse (data not shown).

The concentration of aspartate required for the inverted thermoresponses of pNI130/HCB339 is 1 order of magnitude lower than that of the tsr-defective mutants (3). However, this should not be due to the mutations of tar (GlnP95 to Glu and GinP99 to Glu) on pNI130, since the HCB339 cells carrying the wild-type tar-containing plasmid pAK101 also require 10 μM aspartate for the inverted thermoresponse (5). Thus, Tar with [EEEE] can be used to assess the role of methylation in the inversion of thermoresponses. It has been considered that the attractant-induced methylation of Tar might be responsible for the inverted thermoresp- onse (1, 5). Therefore, we next compared thermosensing abilities of the methylated and unmethylated forms of Tar as follows. As described previously, CP553 (cheB cheR) cells carrying pBR322-based plasmid pRA130 encoding Tar with [EEEE] showed extremely smooth-biased basal swimming pattern, but normal thermoresponse in the presence of glycerol (Fig. 2). The cells never showed a clear thermoresponse under the conditions tested. We now constructed the pACYC184-based plasmid pRAR1 carrying the methyltransferase gene (cheR). The pRA130/CP553 cells were further transformed with pRAR1. In the transformant (pRA130/pRAR1/ CP553) cells, Tar is expected to be heavily methylated, since the cells retain methyltransferase but lack methylesterase. In fact, Borkovich et al. (34) have demonstrated that Tar is fully methylated in the cheB-defective but cheR-overexpressing cells. Since we did not examine experimentally whether Tar was fully methylated in the transformant cells, we can only state that Tar must be heavily methylated (this can be predicted from the cells’ tumbly biased swimming behavior). For simplicity, we will hereafter describe Tar as fully methylated ([EMeEMeEMe]) in these cells. The cells showed inverted thermoresponses in the presence of 10 μM aspartate (Fig. 4). These results indicate that receptor methylation causes the inverted thermoresponse mediated by Tar in the presence of aspartate.

Temperature Dependencies of the Steady-state Signaling Biases Produced by the Heavily Methylated and the Unmethylated Forms of Tar—To further characterize the different thermosensing profiles induced by the unmethylated and the heavily
methylated forms of Tar, we then examined the steady-state swimming patterns of cells with either of these two forms of Tar at various temperatures (Fig. 5).

The cells expressing Tar with [EEEE] (pNI130/CP553; CheB−CheR−) showed exclusively smooth swimming at all temperatures tested (from 15 to 35°C). In the presence of 10% glycerol, however, they showed a temperature-dependent swimming pattern: almost all cells were tumbling at 15°C, and as the temperature increased, the smooth-swimming fraction of the cells gradually increased and finally became almost 100% at about 30°C or above. This profile of the unmethylated form of Tar is consistent with its warm receptor function. Moreover, the cells swam extremely smoothly in the presence of 1 or 10 μM aspartate at all temperatures tested (data not shown). This indicates that in the absence of methylation, aspartate cannot convert Tar from a warm to a cold receptor.

The cells expressing Tar with [EmEmEmEm] (pNI130/pRAR1/CP553; CheB−CheR+) showed exclusively tumbling behavior at all temperatures tested (from 15 to 35°C). In the presence of 0.1 mM aspartate, however, they showed a temperature-dependent swimming pattern: almost all cells tumbled at 30°C or above and as the temperature decreased, the smooth-swimming fraction of the cells increased and became about 75% at 15°C. This profile is consistent with a cold receptor function.

Thus, we conclude that the methyl esterification of the specific glutamate residues is required for the conversion of Tar from a warm to a cold receptor in the presence of aspartate.

**DISCUSSION**

In this study, we have demonstrated that the primary translational product of tar (Tar with [QEQE]) does not have thermosensing ability (Fig. 1), but acquires it by the deamidation of either Gln295 or Gln309 (Table I). The multifunctional nature of bacterial chemoreceptors has been exploited to isolate mutants defective only in the response for a specific chemoeffector, leading to the identification of the amino acid residues responsible for its recognition (for Tar and Tsr, see Refs. 5 and 35–41). This is the first identification of a thermosensing-specific mutant form of the receptor, since it has been reported for Tar (35, 42) and Tsr (43) that covalent modification of a receptor does not severely affect its ligand binding affinity.

One interpretation of the results presented here is that the methylation sites of Tar constitute at least a part of the region required for the recognition of temperature or thermotactic signal production. In this regard, it would be interesting to test whether a cytoplasmic fragment of Tar can mediate thermoresponse at all, since some cytoplasmic fragments of Tar and Tsr have been shown to retain abilities to produce chemotactic signals (44, 45).

We have also shown that the unmethylated ([EEEE]) and the heavily methylated ([EmEmEmEm]) forms of Tar function as a warm receptor and a cold receptor, respectively. The unmethylated form of Tar mediated a normal thermoresponse in the presence of glycerol (Fig. 2). However, under no conditions...
tested, did it show any characteristics of a cold receptor in the cheB cheR background. In contrast, the heavily methylated form (i.e. the genetically deamidated Tar expressed in the cheB background) mediated an inverted thermoresponse in the presence of aspartate (Fig. 4). Under no conditions tested did it show any characteristics of a warm receptor. Thus, the specific methylation, and possibly the presence of aspartate or any of the other Tar-mediated attractants also, are required for the conversion of Tar from a warm to a cold receptor.

In summary, covalent modification of the four methyl-accepting residues of Tar modulates thermosensing properties of the receptor. These findings reinforce the notion that thermotaxis is resulted from a temperature-dependent shift in an equilibrium between the two signaling states (corresponding to smooth swimming and tumbling) of the receptor itself. Further investigation of the effects of covalent modification would provide an important clue to elucidate what kind of structural change of the receptor plays an essential role in thermosensation.

Acknowledgments—We thank Drs. H. C. Berg, P. Matsumura, C. Park, J. S. Parkinson, and M. I. Simon for providing us with bacterial strains and plasmids and N. Nishioka for drawing. We especially thank Dr. R. M. Macnab of Yale University for critically reading the manuscript.

REFERENCES

1. Imae, Y. (1985) in Sensing and Response in Microorganisms (Eisenbach, M., and Balaban, M., eds) pp. 73–81, Elsevier Science Publishing Co., Inc., New York.

2. Maeda, K., and Imae, Y. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 91–95

3. Mizuno, T., and Imae, Y. (1984) J. Bacteriol. 159, 360–367

4. Lee, L., Mizuno, T., and Imae, Y. (1988) J. Bacteriol. 170, 4769–4774

5. Lee, L., and Imae, Y. (1990) J. Bacteriol. 172, 377–382

6. Nara, T., Lee, L., and Imae, Y. (1991) J. Bacteriol. 173, 1120–1124

7. Macnab, R. M. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) pp. 732–759, American Society for Microbiology, Washington, D. C.

8. Stewart, R. C., and Dahlquist, F. W. (1987) Chem. Rev. 87, 997–1025

9. Hazelbauer, G. L., Yaghmai, R., Burrows, G. G., Baumgartner, J. W., Dutton, D. P., and Morgan, D. G. (1990) in Biology of the Chemotactic Response (Armitage, J. P., and Lackie, J. M., eds) pp. 107–134, Society for General Microbiology Symposium Vol. 46, Cambridge University Press, Cambridge, UK.

10. Bourret, R. B., Borkovich, K. A., and Simon, M. I. (1991) Annu. Rev. Biochem. 60, 403–441

11. Stock, J. B., Lukat, G. S., and Stock, A. M. (1991) Annu. Rev. Biophys. Biophys. Chem. 20, 109–136

12. Manson, M. D. (1992) Adv. Microb. Physiol. 33, 277–346

13. Parkinson, J. S. (1993) Cell 73, 857–871

14. Springer, M. S., Goy, M. F., and Adler, J. (1979) Nature 280, 279–284

15. Keilty, M. R., and Dahlquist, F. W. (1962) J. Biol. Chem. 237, 10378–10386

16. Terwilliger, T. C., and Koshland, D. E., Jr. (1986) J. Biol. Chem. 259, 7719–7725

17. Springer, W. R., and Koshland, D. E., Jr. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 533–537

18. Stock, J. B., and Koshland, D. E., Jr. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3659–3663

19. Keilty, M. R., Bond, M. W., Hunkapiller, M. W., and Dahlquist, F. W. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 3599–3603

20. Sherris, D., and Parkinson, J. S. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6051–6055

21. Egger, J. A., Graham, D. R., Roth, A. F., and Dahlquist, F. W. (1992) Cell 70, 975–982

22. Schuster, S. C., Swanson, R. V., Alex, L. A., Bourret, R. B., and Simon, M. I. (1993) Nature 365, 343–347

23. Maddock, J., and Shapiro, L. (1993) Science 259, 1717–1723

24. Imae, Y., Mizuno, T., and Maeda, K. (1994) J. Bacteriol. 159, 368–374

25. Wolff, A. J., Conley, M. P., Kramer, T. J., and Berg, H. C. (1987) J. Bacteriol. 159, 1989–1992

26. Burrows, G. G., Newcomer, M. E., and Hazelbauer, G. L. (1989) J. Biol. Chem. 264, 17309–17315

27. Krikos, A., Conley, M. P., Boyd, A., Berg, H. C., and Simon, M. I. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1326–1330

28. Tatsuno, I., Lee, L., Kawagishi, I., Homma, M., and Imae, Y. (1994) Mol. Microbiol. 14, 755–762

29. Samuel, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

30. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 367–382

31. Oosawa, K., and Imae, Y. (1983) J. Bacteriol. 154, 104–112

32. Oosawa, K., and Imae, Y. (1984) J. Bacteriol. 157, 576–581

33. McCleary, W. R., Stock, J. B., and Ninfa, A. J. (1993) J. Bacteriol. 175, 2793–2798

34. Borkovich, K. A., Alex, L. A., and Simon, M. I. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6756–6760

35. Hedblom, M. L., and Adler, J. (1980) J. Bacteriol. 144, 1048–1060

36. Kosmann, M., Wolff, C., and Manson, M. D. (1988) J. Bacteriol. 150, 4516–4521

37. Wolff, C., and Parkinson, J. S. (1988) J. Bacteriol. 170, 4509–4515

38. Gardina, P., Conway, C., Kossmann, M., and Manson, M. (1992) J. Bacteriol. 174, 1528–1536

39. Gomi, S., Lee, L., Iwama, T., and Imae, Y. (1993) J. Biochem. (Tokyo) 113, 208–213

40. Gomi, S., Lee, L., Iwama, T., Imae, Y., and Kawagishi, I. (1994) J. Olfaction and Taste XI (Kurihara, K., Suzuki, N., and Ogawa, H., eds) pp. 210–214, Springer-Verlag Tokyo, Tokyo

41. Iwama, T., Kawagishi, I., Gomi, S., Homma, H., and Imae, Y. (1995) J. Bacteriol. 177, 2218–2221

42. Dunten, P., and Koshland, D. E., Jr. (1991) J. Biol. Chem. 266, 1491–1496

43. Lin, L.-N., Li, J., Brandts, J. F., and Weis, R. M. (1994) Biochemistry 33, 6564–6570

44. Oosawa, K., Mutoh, N., and Simon, M. I. (1988) J. Bacteriol. 170, 2521–2526

45. Arnes, P., and Parkinson, J. S. (1994) J. Bacteriol. 176, 6340–6348