JOURNAL OF BACTERIOLOGY, Oct. 1988, p. 4769–4774
0021-9193/88/104769-06$02.00/0

Vol. 170, No. 10

Thermosensing Properties of *Escherichia coli tsr* Mutants Defective in Serine Chemoreception

LAN LEE, TAKAFUMI MIZUNO† AND YASUO IMAE*

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

Received 13 June 1988/Accepted 18 July 1988

Tsr, a chemoreceptor for serine and repellents in *Escherichia coli*, also functions as a thermoreceptor. The relationship between the chemoreceptor and thermoreceptor functions of Tsr was examined in five *tsr* mutants with altered serine detection thresholds. The thermosensing abilities of the mutant Tsr proteins were not affected by the alterations in their affinities to serine. In contrast, the ability of serine to inactivate thermoreceptor function was altered in these mutants. The minimal serine concentration required for thermoreceptor inactivation was directly related to the decreased affinity of the mutant Tsr for serine. The amino acid replacements in the mutant receptors were deduced from DNA sequence analyses and occurred at two different locations in the presumed periplasmic domain of Tsr. Two mutations caused histidine or cysteine replacements at arginine 64, whereas three others caused isoleucine or proline replacements at threonine 156.

Escherichia coli cells can sense changes in their environment and respond by modulating their swimming pattern; attractant stimuli cause smooth swimming, and repellent stimuli cause tumbling. Genetic and biochemical studies of chemotaxis have shown that *E. coli* has four transmembrane chemoreceptors, called Tsr, Tar, Trg, and Tap (for reviews, see references 8 and 19). Many chemical stimuli are detected by these receptors, which then transmit sensory information into the cell, generating signals that modulate the swimming pattern of the organism. Adaptation to stimuli is brought about through the reversible methylation of specific glutamic acid residues in the cytoplasmic domain of these receptors.

We found that *E. coli* can also sense thermal stimuli (10). Cells show smooth swimming upon a temperature increase and tumbling upon a temperature decrease. Further studies showed that two major chemoreceptors, Tsr and Tar, which detect serine and aspartate, respectively, also function as thermoreceptors (4, 9, 14). Thus, these receptors are able to detect two quite different stimuli, one chemical and the other physical.

Competition experiments between chemical and thermal stimuli demonstrated that the thermosensing ability of these receptors was altered by first adapting the cells to serine or aspartate (4, 9, 14). Tsr in serine-adapted cells loses its ability to detect thermal stimuli, and therefore, cells with only Tsr show no thermoresponse after adapting to serine. In contrast, Tar in cells which have been adapted to aspartate retains its ability to detect thermal stimuli but generates an inverted sensory signal. Thus, after adapting to aspartate, cells with only Tar tumble in response to a temperature increase and swim smoothly in response to a temperature decrease. In wild-type cells in which Tsr is present at approximately twice the level of Tar, aspartate reduces, but does not invert, thermoresponses. Since adaptation to attractants is caused by methylation of these receptors (8, 19), the increase in receptor methylation levels may be responsible for attractant-induced alterations of thermoreceptor function in Tsr and Tar (4, 14).

In this paper, we compare the chemosensing and thermosensing abilities of *tsr* mutants isolated by Hedblom and Adler (3). These mutants were reported to have specific defects in serine sensing and were classified into two groups on the basis of their residual responses to serine stimuli. Class A mutants exhibited reduced affinity for serine, whereas class B mutants showed no affinity for serine. Tsr receptors from both classes of mutants were still able to sense thermal stimuli, indicating that their receptor defects are probably confined to the serine-binding site.

MATERIALS AND METHODS

Bacterial strains and plasmids. All strains used in this work are derivatives of *E. coli* K-12. The serine taxis mutants, AW679, AW680, AW681, AW682, and AW683, which carry the A1, A2, B1, B2, and B3 mutations, respectively, in the *tsr* gene (3), were provided by J. Adler of the University of Wisconsin. They have a common genetic background (F$^-$ *thr leu his metF ara gal lac xyl eda tonA tsx rpsL*). A *tsr* deletion mutant with a similar genetic background, RP5968 (Δ*tsr-7028 metF*) (6) was obtained from J. S. Parkinson of the University of Utah. A mutant with multiple defects in chemoreceptors, AB1200 [F$^-$ *thr leu his met eda rpsL* Δ(*tar-tap*)*5201* Δ*tsr-7028*] (7), was obtained from M. Simon of the California Institute of Technology. Plasmid pACYC184 for cloning and phages M13mp10 and M13mp11 and their host JM101 for all sequencing procedures were obtained from N. Mutoh of Aichi Prefectural Colony Research Institute. A *tsr*-carrying plasmid, pJFG5 (12), was obtained from J. Gebert of the University of Konstanz.

Cloning of mutated *tsr* genes. Routine DNA manipulations were carried out as described by Maniatis et al. (11). A Takara ligation kit (Takara Shuzo Co., Japan) was used for all ligation reactions. Extraction of DNA fragments from agarose gels was carried out with a Gene Clean Kit (Bio 101 Inc., La Jolla, Calif.).

For the cloning of the mutant *tsr* genes, chromosomal DNA was isolated from each of the serine taxis mutants, digested with *Eco*RI restriction enzyme, and separated by agarose gel electrophoresis. Since the *tsr* gene is known to lie on a 6-kilobase (kb) *Eco*RI fragment (2), DNA fragments of this size were isolated from the gel and ligated into the *Eco*RI site of pACYC184. The resultant hybrid plasmids were then transferred into AB1200. When necessary, colony

* Corresponding author.
† Present address: Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo 186, Japan.

hybridization was carried out by using a ^{32}P-labeled 1.4-kb *Pvu*II fragment of the wild-type *tsr* gene.

DNA sequencing. DNA sequencing was carried out by the dideoxy method (13) as described in the manual of the dideoxy M13 sequencing kit (Amersham-Japan). [α-^{32}P] dATP (3,000 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.) was used for sequencing.

Measurement of chemoresponse and thermoresponse. Cells were grown at 30°C in tryptone broth supplemented with 0.5% glycerol. Ampicillin (50 μg/ml) or tetracycline (12.5 μg/ml) was added for the growth of the cells with plasmids. At the late logarithmic phase of growth, cells were harvested, washed twice with motility medium (pH 7.0) consisting of 10 mM potassium phosphate buffer, 0.1 mM EDTA, 0.1 mM methionine, and 10 mM DL-lactate, and then suspended in the same medium. When necessary, medium pH was adjusted to 6.0.

For the temporal assay of chemoresponse, washed cells in a test tube were mixed with various chemoeffectors, and then their changes in swimming pattern were observed under a microscope as described previously (5, 14). For the quantitative measurement of chemoresponse to serine, various concentrations of serine were added to the cells together with 0.5 M glycerol, which is a Tsr-mediated repellent (16, 17). The addition of glycerol also induced a 1-min tumbling response, and therefore any increase in smooth swimming caused by simultaneous presentation of serine could be easily quantitated by photographic methods (5, 16). The thermoresponse of the cells was measured as described previously (14).

To measure the swarming ability of the cells, tryptone swarm plates with tryptone broth and 0.35% Bacto-Agar (Difco Laboratories, Detroit, Mich.) were used, and plates were incubated at 30°C.

Analysis of methylation. Cells in motility medium without methionine were incubated with 3.3 μM [^{3}H]methionine (0.5 Ci/mmol; Dupont, NEN) for 30 min at 30°C. Serine was added to a final concentration of 20 mM, or an equal volume of distilled water was added, and the cells were incubated for another 30 min. Methylation banding patterns were visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Slonczewski et al. (18).

RESULTS

Thermosensing phenotype of the *tsr* mutants. It has been shown that the serine chemoreceptor, Tsr, also functions as a thermoreceptor (4, 9, 14). To determine the relationship between serine sensing and thermosensing by Tsr, *tsr* mutants with altered serine sensing were examined for thermosensing. The *tsr* mutants isolated by Hedblom and Adler (3) were used; the class A mutants with the A1 or A2 mutation showed reduced serine sensing, and the class B mutants with the B1, B2, or B3 mutation showed almost no serine sensing. Because all the mutants showed normal responses to Tsr-specific repellents, such as leucine and acetate, these mutations were suggested to cause only limited functional alterations in Tsr receptor activity.

The thermoresponse of these mutants was measured after adapting the cells to 0.1 mM aspartate, since the mutants have normal Tar, which is also active for thermosensing (4, 14). As shown in Fig. 1, both class A and class B mutants showed a wild-type thermoresponse, namely, a brief period of smooth swimming upon a temperature increase. In contrast, a *tsr* deletion mutant showed an inverted thermoresponse, mediated through its aspartate-adapted Tar receptor

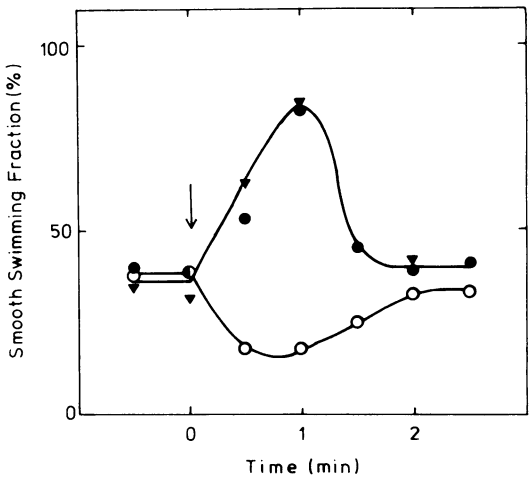

FIG. 1. Thermoresponse in various *tsr* mutants in the presence of 0.1 mM aspartate. Cells were adapted to 0.1 mM aspartate by being incubated for 20 min; at the time indicated by the arrow, the temperature was shifted from 20 to 30°C. Changes in the fraction of smooth-swimming cells were analyzed by the photographic method as described in Materials and Methods. Symbols: ○, RP5968 (Δ*tsr*); ●, AW679 (*tsr* with A1 mutation); ▼, AW682 (*tsr* with B2 mutation).

(14). From these results, it appeared that both class A and class B *tsr* mutants retained normal thermosensing ability.

To further characterize the thermoreceptor function of these mutant Tsr proteins, their structural genes were cloned on a plasmid vector and transferred into a host with no Tar to avoid possible interference by Tar with the analysis.

Cloning of mutant *tsr* genes. The *tsr* coding region is known to lie on a 6-kb *Eco*RI fragment of *E. coli* chromosomal DNA (2). *Eco*RI fragments of this size were therefore isolated from the chromosomal DNA of each mutant and cloned into pACYC184 (Fig. 2). The plasmid DNA was then introduced into strain AB1200, which has all the genes necessary for chemotaxis but lacks the *tsr*, *tar*, and *tap* genes. Transformants carrying mutant *tsr* genes were identified by swarming ability or by colony hybridization and then tested individually for responsiveness to temporal stimulation with serine, aspartate, or acetate, a Tsr-specific repellent. The parental strain AB1200 showed no response to any of these stimulants. The transformants with the class A mutant *tsr* gene responded to 1 mM serine and 33 mM acetate at pH 6.0, but not to 1 mM aspartate. The transformants with class B mutant *tsr* genes responded only to acetate. The plasmids carrying class A or class B mutant *tsr* genes were designated pLYA and pLYB, respectively (Fig. 2).

Since the wild-type *tsr* coding region (1.6 kb) has *Pvu*II cleavage sites near both ends (1, 2), the 1.4-kb *Pvu*II fragment of each of the mutant *tsr* genes was subcloned into pJFG5, replacing the corresponding fragment of the wild-type *tsr* gene (Fig. 2). The resultant plasmids exhibited the same behavioral phenotypes as did the parental plasmids and were designated pLBA1, pLBA2, pLBB1, pLBB2, and pLBB3 to indicate the *tsr* mutation they carried.

Chemosensing phenotype of the cloned mutant *tsr* genes. The serine-sensing abilities of the cloned mutant Tsr receptors were measured quantitatively to determine the extent of their chemoreception defects. Figure 3 shows the relationship between serine concentration and the fraction of responding cells for the various *tsr* mutants. AB1200(pJFG5), the wild-type control, showed a low-threshold attractant response to serine; about 2 μM serine was sufficient to

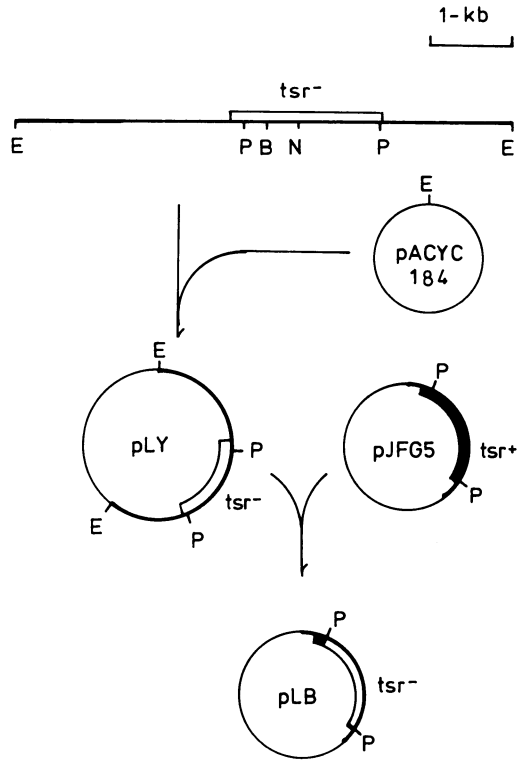

FIG. 2. Strategy for the cloning of mutant *tsr* genes. Thin lines represent vector DNA, and thick lines represent inserted chromosomal DNA. The wild-type *tsr* gene (1.6 kb) is represented by the thick solid bar, and the mutant *tsr* gene is represented by the open bar. Restriction endonuclease cleavage sites are abbreviated as follows: E, *Eco*RI; B, *Bcl*I; N, *Nde*I; P, *Pvu*II.

induce a response in about 50% of the cells. The value is close to the reported K_d value of 5 μM for the high-affinity serine-binding site of wild-type Tsr (3). AB1200(pLBA1) carrying the A1 mutation in the *tsr* gene also responded to serine, but with a higher threshold; about 100 μM serine was required to induce a response in 50% of the cells. AB1200(pLBA2) carrying the A2 mutation showed similar behavior. Thus, compared with wild-type Tsr, the receptors with class A mutations seem to have about a 50-fold reduction in serine affinity.

AB1200 with class B mutant plasmids showed various levels of serine affinity (Fig. 3). AB1200(pLBB1) carrying the B1 mutation in the *tsr* gene showed a small response to 100 mM serine, but AB1200(pLBB2) with the B2 mutation showed no response to 100 mM serine. AB1200(pLBB3) carrying the B3 mutation showed a definite response to 10 mM serine. Thus, the class B Tsr receptors showed drastic reductions in their affinities for serine, although each had a different residual level of serine sensitivity.

All of these mutant Tsr strains showed a tumbling response to 33 mM acetate at pH 6.0, indicating that their defect is mainly in serine sensing. Thus, the properties of the cloned mutant *tsr* genes were essentially the same as those of the mutant genes in the original isolates (3). As described in a later section, the A1 and A2 mutations were found to be identical; therefore, only the cells with pLBA1 were used for further experiments.

Thermosensing phenotype of the cloned mutant *tsr* genes. The thermosensing ability of the mutant Tsr receptors was investigated by quantitative measurement of their thermoresponses. Upon a temperature decrease from 35 to 20°C, AB1200 with wild-type Tsr showed tumbling for about 2 min. AB1200 with any of the mutant Tsr receptors showed essentially the same response (Fig. 4). In contrast, AB1200 with no plasmid or with a plasmid carrying no *tsr* gene always swam smoothly and showed no change in swimming pattern upon temperature decrease (data not shown). These results clearly indicate that the mutant Tsr receptors retain the ability to sense thermal stimuli. It is noteworthy that the time course of the thermoresponse in the cells with mutant Tsr was essentially the same as that in the cells with wild-type Tsr. This indicates that all the mutant Tsr receptors have normal abilities not only for excitation but also for adaptation to thermal stimuli.

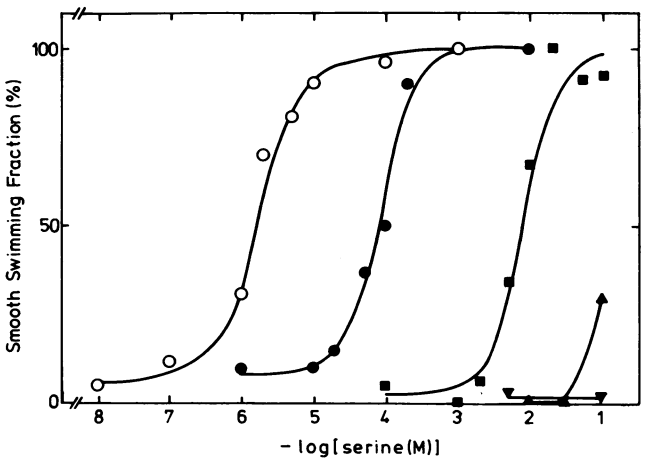

FIG. 3. Serine-sensing abilities of various mutant Tsr receptors. AB1200 with the plasmid carrying wild-type or mutant *tsr* genes was stimulated at 25°C with various concentrations of serine, and the changes in the fraction of smooth-swimming cells after 30 s were measured. The plasmids (and *tsr* genes) used were pJFG5 (wild-type *tsr*) (○), pLBA1 (A1 mutant *tsr*) (●), pLBB1 (B1 mutant *tsr*) (▲), pLBB2 (B2 mutant *tsr*) (▼), and pLBB3 (B3 mutant *tsr*) (■).

It has been shown that the thermoreceptor function of wild-type Tsr is inactivated by adapting the cells to 0.1 mM

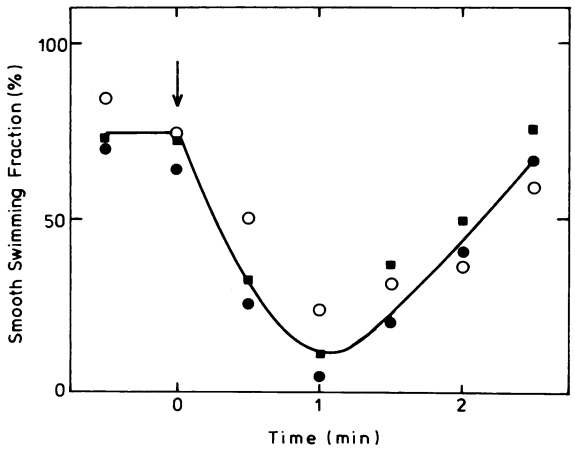

FIG. 4. Thermoresponse in AB1200 with plasmids carrying wild-type or mutant *tsr* genes. Cells in motility medium were incubated at 35°C; at the time indicated by the arrow, temperature was shifted to 20°C. The plasmids (and *tsr* genes) used were pJFG5 (wild-type *tsr*) (○), pLBA1 (A1 mutant *tsr*) (●), and pLBB3 (B3 mutant *tsr*) (■).

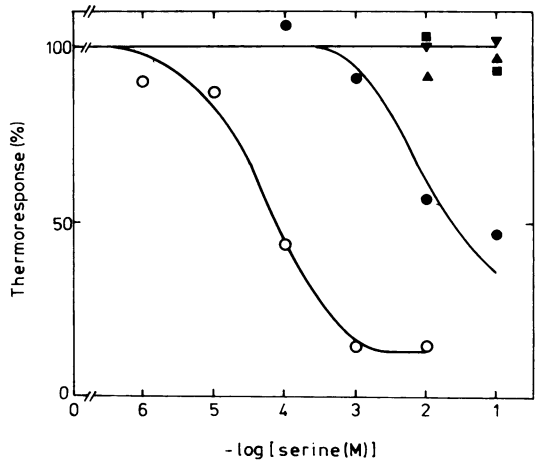

FIG. 5. The inactivation of thermoreceptor function of Tsr proteins by serine. AB1200 with the plasmids carrying wild-type or mutant *tsr* genes was adapted to various concentrations of serine for 20 min, and then the temperature was decreased from 35 to 25°C. The decrease in the smooth-swimming fraction 1 min after the temperature change was measured, and the values were expressed as a percentage of that obtained in the absence of serine. The plasmids (and *tsr* genes) used were pJFG5 (wild-type *tsr*) (○), pLBA1 (A1 mutant *tsr*) (●), pLBB1 (B1 mutant *tsr*) (▲), pLBB2 (B2 mutant *tsr*) (▼), and pLBB3 (B3 mutant *tsr*) (■).

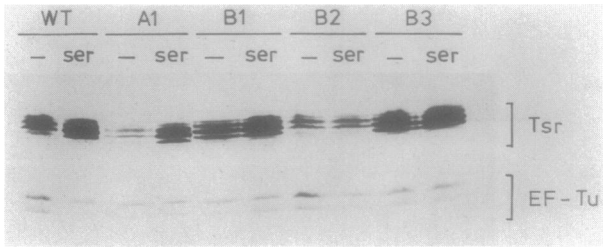

FIG. 6. Methylation banding pattern of wild-type and mutant Tsr. AB1200 with plasmids carrying wild-type or mutant *tsr* genes was incubated with [^{3}H]methionine at 30°C for 30 min, and then serine (ser) was added to a final concentration of 20 mM or an equal volume of distilled water (—) was added. The samples were withdrawn after another 30 min of incubation and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. The plasmids (for each pair of lanes from left to right) were pJFG5, pLBA1, pLBB1, pLBB2, and pLBB3, respectively. The lower migration bands (EF-Tu) are elongation factor Tu.

or higher concentrations of serine (9, 14). Figure 5 shows the effect of serine on the thermoresponses of cells with various mutant Tsr proteins. The thermoresponse in AB1200 with wild-type Tsr was clearly inhibited by adapting the cells to 0.1 mM serine. In the case of AB1200 with class A mutant Tsr, 10 mM or higher concentrations of serine were required for inhibition of the thermoresponse. In contrast, the thermoresponse in AB1200 with any of the class B mutant receptors was almost normal, even after adapting the cells to 100 mM serine. These results indicate that the concentration of serine required for inactivation of thermoreceptor function in the mutant receptors is roughly parallel to their affinity for serine.

Methylation levels of the mutant Tsr proteins. On sodium dodecyl sulfate-polyacrylamide gels, Tsr migrates as a characteristic set of bands reflecting molecules with different numbers of methyl groups (8, 19). As shown in Fig. 6, the methylation patterns of both A1 and B2 mutant Tsr in the absence of serine stimuli were quite similar to that of wild-type Tsr. The Tsr with the B1 or B3 mutation showed some increase in unstimulated methylation level (Fig. 6), but overmethylation does not appear to be directly related to the alterations in their serine-sensing abilities.

The addition of serine to a final concentration of 20 mM caused clear increases in the methylation levels of wild-type and A1 mutant Tsr but caused no detectable change in the methylation levels of B1 and B2 mutant Tsr (Fig. 6). In the case of B3 mutant Tsr, a slight increase in methylation was detected. The addition of 33 mM acetate at pH 6.0 caused clear decreases in the methylation levels of all the mutant Tsr receptors (data not shown). These results are consistent with the repellent-sensing ability and the serine detection thresholds of the mutant receptors.

Identification of the mutation sites. As described in the previous section, all the mutations were localized within the 1.4-kb *Pvu*II fragment of the *tsr* coding region (Fig. 2). In vitro recombination between the wild-type *tsr* gene and the mutant *tsr* genes by using *Pvu*II, *Nde*I and *Bcl*I cleavage sites further localized the mutation sites to either the *Pvu*II-*Bcl*I fragment or the *Bcl*I-*Nde*I fragment. B1 and B3 mutations were in the former fragment and other mutations were in the latter fragment.

By cloning the appropriate fragment from each mutant to an M13 vector, the nucleotide changes in the mutations could be determined by DNA sequencing. As summarized in Fig. 7, all the mutations resulted from a single-base change at various sites, although the A1 and A2 mutations were found to be identical. It is noteworthy that the DNA sequence corresponding to amino acid residues 148 to 160 of the *tsr* gene in pJFG5 has recently been reported to differ from the published sequence (1, 12). Our sequence data on the cloned mutant *tsr* genes were consistent with the sequence found in pJFG5, indicating that the amino acid sequence of residues 148 to 161 in wild-type Tsr protein in *E. coli* deduced from the DNA sequence should be Ile-148–Asn–Glu–Phe–Phe–Asp–Gln–Pro–Thr-156–Gln–Gly–Tyr–Gln–Asp-161. Hence, the deduced amino acid replacements of mutant Tsr proteins indicate that the alterations involve only two amino acid residues, arginine 64 and threonine 156, both of which are in the presumed periplasmic domain of Tsr.

DISCUSSION

We have shown that the thermosensing ability of Tsr is not affected by alterations that reduce or eliminate its affinity for serine. This indicates that thermosensing by Tsr is not a secondary effect of temperature-induced alterations of the serine-binding site of Tsr which might alter its affinity for serine or related chemoeffectors. We have previously suggested that thermosensing by Tsr is mediated by a temperature-induced conformational change in the molecule (4, 14). If this is the case, the structural distortions associated with these mutations are evidently too minor to interfere with these conformational changes.

Although thermosensing ability was not affected by the mutations, the inactivation of Tsr thermoreceptor function by serine was strongly affected by the alterations in serine affinity. In the case of wild-type Tsr, significant inactivation is caused by about 0.1 mM serine, which is about 20-fold or more higher than the K_d of Tsr for serine. The inactivation of class A mutant Tsr, which has about 100-fold-reduced serine affinity, is caused by 100 mM or higher concentrations of serine. Furthermore, all the class B mutant Tsr receptors,

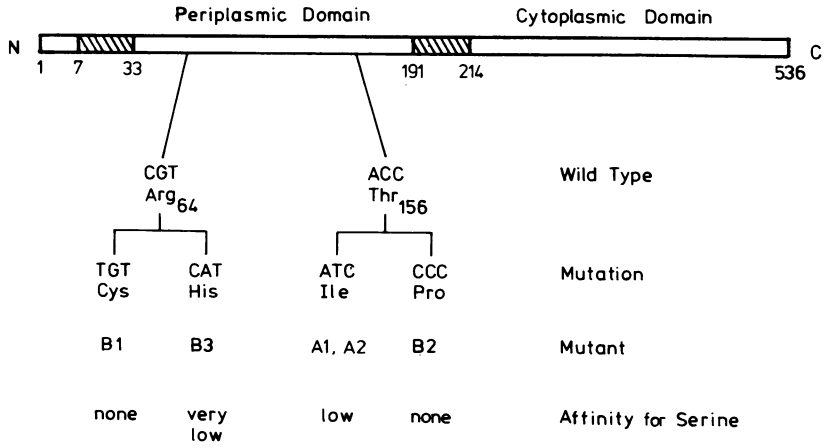

FIG. 7. Changes in the nucleotide sequence and deduced amino acid sequence of mutant *tsr*. Two putative membrane-spanning regions of Tsr are indicated by hatched bars. Amino acids are numbered from the N-terminal residue.

which have no or very little affinity for serine, are not inactivated by serine concentrations up to 100 mM. Thus, the minimal serine concentrations required for inactivation of thermoreceptor function in Tsr increase roughly in proportion to the decrease in serine affinity of the mutant receptors and are always about 100-fold higher than the K_d concentrations. This may indicate that, if the inactivation is caused by an increase in the methylation level of Tsr, as suggested previously (4, 14), only the highly methylated species of Tsr lose their ability to undergo the temperature-induced conformational change required for thermosensing.

Hedblom and Adler (3) classified the *tsr* mutants with altered serine affinity into two classes: class A, with low affinity for serine, and class B, with no affinity for serine. Furthermore, binding experiments with radioactive serine indicated two serine-binding sites in wild-type Tsr, one with a K_d of 5 μM and one with a K_d of greater than 0.2 mM (3). Therefore, it was thought that Tsr had high- and low-affinity serine-binding sites. However, our quantitative analysis with cells with plasmid-borne mutant *tsr* genes revealed graded rather than discrete levels of affinities to serine. Furthermore, different amino acid replacements at the same residue (threonine 156) were found in a class A and a class B mutant, suggesting that Tsr has only one serine-binding site and that the different mutations in Tsr produce various levels of serine affinity. The high and low serine affinities observed in wild-type Tsr may have some relation to the methylation-induced alterations of serine affinity in Tsr (21). In the case of the *tsr* mutants, however, we showed that their methylation levels had no relation to their affinities for serine, indicating that the altered serine affinity could not be a consequence of the mutation-induced alterations in the methylation level of Tsr.

According to a recent model of the structure of the periplasmic domain of Tar proposed by Moe and Koshland (15), a pair of peptide loops extrudes from the cytoplasmic membrane. Because of the high homologies between the amino acid sequences in Tar and Tsr, it is quite reasonable to speculate that the periplasmic domain of Tsr has similar structure to that of Tar. If this was the case, the identified mutation sites of the *tsr* mutants, arginine 64 and threonine 156, would be located near the top of each loop. This suggests that the recognition of serine by Tsr might be carried out by interposing serine between these two loops. It is noted that both amino acid residues in question, arginine 64 and threonine 156 of Tsr, are found at the same or very close position in Tar. Furthermore, Wolff and Parkinson (20) have found that arginine 64 in Tar is essential for aspartate sensing. Hence, it is likely that arginine 64 is involved in the recognition of a common part, such as the α-carboxyl group of serine and aspartate.

ACKNOWLEDGMENTS

We thank J. Adler, J. Gebert, N. Mutoh, J. S. Parkinson, and M. I. Simon for sending us bacterial strains, plasmids, and phages. We especially thank J. S. Parkinson for critically reading the manuscript, and O. Koiwai and N. Mutoh for their kind help and useful advice on recombinant DNA techniques and DNA sequencing.

This work was supported in part by a Grant-in-Aid for Scientific Research to Y.I. from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Boyd, A., K. Kendall, and M. I. Simon.** 1983. Structure of the serine chemoreceptor in *Escherichia coli*. Nature (London) **301:** 623–626.
2. **Callahan, A. M., B. L. Frazier, and J. S. Parkinson.** 1987. Chemotaxis in *Escherichia coli*: construction and properties of λtsr transducing phage. J. Bacteriol. **169:**1246–1253.
3. **Hedblom, M. L., and J. Adler.** 1980. Genetic and biochemical properties of *Escherichia coli* mutants with defects in serine chemotaxis. J. Bacteriol. **144:**1048–1060.
4. **Imae, Y.** 1985. Molecular mechanism of thermosensing in bacteria, p. 73–81. *In* M. Eisenbach and M. Balaban (ed.), Sensing and response in microorganisms. Elsevier Science Publishing, Inc., New York.
5. **Imae, Y., T. Mizuno, and K. Maeda.** 1984. Chemosensory and thermosensory excitation in adaptation-deficient mutants of *Escherichia coli*. J. Bacteriol. **159:**368–374.
6. **Imae, Y., K. Oosawa, T. Mizuno, M. Kihara, and R. M. Macnab.** 1987. Phenol: a complex chemoeffector in bacterial chemotaxis. J. Bacteriol. **169:**371–379.
7. **Krikos, A., M. P. Conley, A. Boyd, H. C. Berg, and M. I. Simon.** 1985. Chimeric chemosensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**1326–1330.
8. **Macnab, R. M.** 1987. Motility and chemotaxis, p. 732–759. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
9. **Maeda, K., and Y. Imae.** 1979. Thermosensory transduction in *Escherichia coli*: inhibition of the thermoresponse by L-serine. Proc. Natl. Acad. Sci. USA **76:**91–95.

10. **Maeda, K., Y. Imae, J. Shioi, and F Oosawa.** 1976. Effect of temperature on motility and chemotaxis of *Escherichia coli*. J. Bacteriol. **127**:1039–1046.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Manoil, C., and J. Bechwith.** 1986. A genetic approach to analyzing membrane protein topology. Science **233**:1403–1408.
13. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
14. **Mizuno, T., and Y. Imae.** 1984. Conditional inversion of the thermoresponse in *Escherichia coli*. J. Bacteriol. **159**:360–367.
15. **Moe, G. R., and D. E. Koshland, Jr.** 1986. Transmembrane signaling through the aspartate receptor, p. 163–168. *In* D. C. Youvan and F. Daldal (ed.), Microbial energy transduction: genetics, structure, and function of membrane proteins. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Oosawa, K., and Y. Imae.** 1983. Glycerol and ethylene glycol: members of a new class of repellents of *Escherichia coli* chemotaxis. J. Bacteriol. **154**:104–112.
17. **Oosawa, K., and Y. Imae.** 1984. Demethylation of methyl-accepting chemotaxis proteins in *Escherichia coli* induced by the repellents glycerol and ethylene glycol. J. Bacteriol. **157**: 576–581.
18. **Slonczewski, J. L., R. M. Macnab, J. R. Alger, and A. M. Castle.** 1982. Effect of pH and repellent tactic stimuli on protein methylation levels in *Escherichia coli*. J. Bacteriol. **152**:384–399.
19. **Stewart, R. C., and F. W. Dahlquist.** 1987. Molecular components of bacterial chemotaxis. Chem. Rev. **87**:997–1025.
20. **Wolff, C., and J. S. Parkinson.** 1988. Aspartate taxis mutants of the *Escherichia coli* Tar chemoreceptor. J. Bacteriol. **170**:4509–4515.
21. **Yonekawa, H., and H. Hayashi.** 1986. Desensitization by covalent modification of the chemoreceptor of *Escherichia coli*. FEBS Lett. **198**:21–24.