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Conditional Inversion of the Thermoresponse in *Escherichia coli*

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Mutants in *Escherichia coli* having defects in one of the methyl-accepting chemotaxis proteins, Tsr protein, which is the chemoreceptor and transducer for L-serine, showed a reduced but similar type of thermoresponse compared with wild-type strains; the cells showed smooth swimming upon temperature increase and tumbling upon temperature decrease. However, when the mutant cells were adapted to attractants such as L-aspartate and maltose, which are specific to another methyl-accepting chemotaxis protein, Tar protein, the direction of the thermoresponse was found to be inverted; a temperature increase induced tumbling and a temperature decrease induced smooth swimming. Consistent with this, the mutant cells showed inverted changes in the methylation level of Tar protein upon temperature changes. Wild-type strains but not Tar protein-deficient mutants exhibited the inverted thermoresponse when the cells were simultaneously adapted to L-aspartate and L-serine, indicating that Tar protein has a key role in the inversion of the thermoresponse. Thus, besides Tsr protein, Tar protein has a certain role in thermoreception. A simple model for thermoreception and inversion of the thermoresponse is also discussed.

Escherichia coli as well as higher organisms can sense a change in temperature as a thermal stimulus and respond to it by changing their swimming pattern (1, 17). Analysis of the thermosensory system of *E. coli* might therefore clarify the molecular mechanism of thermoreception not only in bacteria but also in higher organisms.

In a previous paper (16), we showed by using various chemotaxis mutants of *E. coli* that the thermoresponse was clearly reduced only in mutants having a defect in the pathway specific for L-serine. Furthermore, the thermoresponse was severely inhibited only by L-serine. We therefore assumed that the L-serine chemoreceptor is the main thermoreceptor. Recent studies showed that Tsr protein, which is a methyl-accepting chemotaxis protein (MCP) and the sensory transducer for L-serine, is itself the chemoreceptor for L-serine (2, 8, 32). Therefore, Tsr protein is now considered to be a main thermoreceptor.

During a study of the residual thermoresponse in Tsr protein-deficient mutants, we found that the direction of the mutant thermoresponse could be inverted if the mutants were adapted to the attractants specific to Tar protein, another MCP in the cell. In this paper, we describe conditions to invert the thermoresponse not only in the Tsr protein-deficient mutants but also in wild-type strains of *E. coli*. The results show that, although the thermal stimulus is mainly processed by Tsr protein, Tar protein has a certain role in the thermoreception and plays a key role in the inversion of the thermoresponse.

MATERIALS AND METHODS

Bacterial strains. All of the bacterial strains used in this work were *E. coli* K-12 derivatives. Strains AW405 (wild type), AW518 (*tsr-1*), AW655 (*tsr-12*), AW539 (*tar-1*), AW656 [*tar*(Am)], AW701 (*trg-1*), AW702 (*trg-2*), AW569 (*tsr-1 tar-1*), AW659 [*tsr-12tar*(Am)], AW657 (*tsr-12 trg-1*), and AW658 [*tar*(Am) *trg-1*] were obtained from J. Adler of the University of Wisconsin (13). Strain TH403 (*trg*::Tn*5*) was obtained from S. Harayama of the University of Tokyo (6). Strains RP487 (wild type), RP4790 (*tsr-14*), RP5698 (Δ*tsr*), RP4324 (*tar-52*Δ1), RP3525 (Δ*tap*-m365-4), and RP3544 (*tsr*::Tn*5*-1a Δ*tap*-m365-4) were obtained from J. S. Parkinson of the University of Utah (23, 25). RP5698 lacks most of the *tsr* gene (21), and RP4324 lacks most of both the *tar* and *tap* genes (3). All strains obtained from J. S. Parkinson have a *metF* allele.

Growth conditions. Cells were grown at 35°C with shaking in tryptone broth consisting of 1% tryptone (Difco Laboratories, Detroit, Mich.) and 0.5% NaCl supplemented with 0.5% glycerol. At the late log phase of growth (absorbance at 590 nm = 0.7), cells were harvested by centrifugation at room temperature.

Chemicals. L-[*methyl*-^{3}H]methionine (12.0 Ci/mmol) was obtained from New England Nuclear (Boston, Mass.). Chloramphenicol was purchased from P-L Biochemicals, Inc. (Milwaukee, Wis.). Synthetic L-serine and L-aspartate were the products of ICN Pharmaceuticals, Inc. (Plainview, N.Y.). α-Methyl-DL-aspartic acid was purchased from Sigma Chemical Co. (St. Louis, Mo.). ACSII was obtained from Amersham Corp. (Arlington Heights, Ill.).

Measurement of thermoresponse. Cells were washed and suspended in motility medium consisting of 10 mM potassium phosphate buffer (pH 7), 0.1 mM potassium EDTA, and 10 mM sodium lactate. In the case of methionine auxotrophic strains, the motility medium was supplemented with 0.1 mM L-methionine. The cell concentration was adjusted to about 5×10^7 cells per ml. A drop of the cell suspension was put on a glass slide which was placed on a temperature control apparatus as described previously (17). Thermal stimulation was given to the cells by changing the temperature of the apparatus. The maximum changing rate was ca. 0.2°C/s. The temperature of the cell suspension on a glass slide was directly monitored by inserting a thin constantan-chromel thermocouple. Swimming cells were observed with a dark-field microscope and recorded on videotape. Swimming tracks were obtained from the videoscenes by a photographic method with an exposure time of 1 s (18). The thermoresponse was quantitated by measuring the smooth swimming fraction, which was defined as a percentage of the smooth swimming tracks at a time point in the total swimming tracks obtained before temperature change. More than

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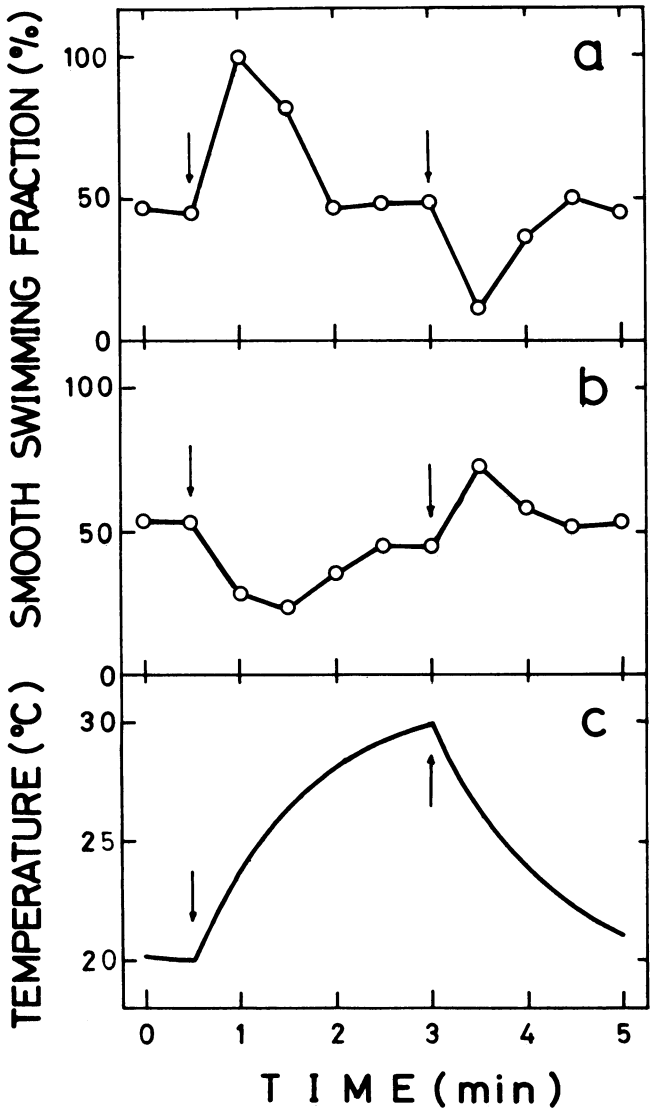

FIG. 1. Inverted thermoresponse in AW518 (*tsr-1*) induced by L-aspartate. Cells in motility medium were incubated at 35°C for 40 min with or without 0.1 mM L-aspartate. Then, the temperature of the cell suspension was changed at the time point indicated by arrows, and the smooth swimming fraction was measured at intervals. (a) No aspartate; (b) plus 0.1 mM L-aspartate; (c) time course of the temperature change.

100 swimming tracks were measured. Only the swimming tracks having no sharp bend were counted as smooth swimming tracks (21). This method was suitable to quantitate the small thermoresponse observed in the *tsr* mutants compared with the previously used one in which the thermoresponse was quantitated by counting the swimming tracks having two or more tumbles (16). By the present method, the difference in the thermoresponse between wild-type strains and various *tsr* mutants was represented in the response time; wild-type strains showed a clearly longer thermoresponse than did the *tsr* mutants.

To analyze the effect of various chemoeffectors on the thermoresponse, cells were mixed with a chemoeffector and incubated at 35°C for 40 min for adaptation. Then, the thermoresponse of the cells were measured as above.

Measurement of methylation level of MCPs. Changes in the methylation level of MCPs were measured by the method of Springer et al. (30) with a minor modification. Cells were washed and suspended in motility medium supplemented with 10 μM L-methionine and 125 μg of chloramphenicol per ml to a cell density of 1.0 at 590 nm. Cells were mixed with various attractants and incubated for 10 min at 30°C. L-[*Methyl*-^{3}H]-methionine (12 Ci/mmol) was then added to a final concentration of 3 μCi/ml, and the methyl groups in the MCPs were selectively labeled by incubating the cells for 30 min more. The cells were divided into two parts. One was kept standing at 30°C and the other was transferred to 20°C. At intervals, 0.5 ml each of samples was withdrawn and poured into a tube containing 0.5 ml of 10% trichloroacetic acid, which was incubated at 20 or 30°C. Care was taken to avoid unnecessary changes in temperature during the experiments, except for the temperature shift step. When the changes in the methylation level of MCPs upon temperature increase were measured, the cells were incubated at 20°C with radioactive methionine for 30 min and then a part of the cell suspension was transferred to 30°C. After terminating the methylation-demethylation reaction of MCPs with trichloroacetic acid, tubes were chilled in ice. Precipitates were collected by centrifugation and suspended in 45 μl of a sample buffer consisting of 62.5 mM Tris-hydrochloride (pH 6.8), 12% sodium dodecyl sulfate, 10% glycerol, 14.5% β-mercaptoethanol, 20% sucrose, and 0.01% bromophenol blue. Samples were neutralized by the addition of 10 μl of 2 M Tris, boiled for 1 min, and then transferred on a 10% polyacrylamide slab gel prepared by the method of Laemmli (15). After electrophoresis, the gel was stained with 0.1% Coomassie brilliant blue R in 50% trichloroacetic acid. The region corresponding to MCPs (apparent molecular weights of 45,000 to 65,000) was cut out and treated with 2 N NaOH in 20% methanol for 6 h in a sealed vial (19). Radioactivity was counted with a scintillation counter after the addition of 1 ml of water and 6 ml of ACSII.

RESULTS

Conditions to induce the inverted thermoresponse in *tsr* mutants. The direction of the residual thermoresponse in AW518 (*tsr-1*), a Tsr protein-deficient mutant, in motility medium was the same as that of wild-type strains; a temperature increase induced a transient smooth swimming and a temperature decrease induced a transient tumbling (Fig. 1a). However, when the mutant cells were adapted to L-aspartate, which is an attractant specific to Tar protein, the direction of the residual thermoresponse was found to be completely inverted; a temperature increase induced a transient tumbling and a temperature decrease induced a transient smooth swimming (Fig. 1b).

Since Tar protein is intact in AW518, the addition of L-aspartate to the cells caused a transient smooth swimming. The thermoresponse in the mutant was therefore masked for a while by this smooth swimming response, and the inverted thermoresponse became clear after adaptation of the cells to L-aspartate (Table 1). In addition to L-aspartate, attractants such as L-glutamate, α-methyl-DL-aspartate, and maltose, which are all known to be processed by Tar protein, were almost equally effective in inducing the inverted thermoresponse at a concentration of 1 mM. In contrast, other attractants such as L-serine, D-glucose, and D-ribose, which are not processed by Tar protein, were ineffective (Table 2). It is noteworthy that adaptation of the mutant cells to Ni^{2+}, which is specifically processed by Tar protein (30), did not induce the inverted thermoresponse (Table 2). Glycerol, a

TABLE 1. Time course of the appearance of the inverted thermoresponse in AW518 (*tsr-1*)

Time after L-aspartate addition (min)	Thermoresponse[a]
−5	Normal
1	None
9	Inverted
21	Inverted
40	Inverted

[a] Cells were mixed with 1 mM L-aspartate at time zero and incubated at 35°C. At the time indicated, a drop of the cell suspension was placed on a glass slide which was incubated at 30°C, and 2 min later the temperature of the glass slide was decreased to 20°C.

repellent which is partly mediated by Tar protein (21), also showed no inversion.

The direction of the thermoresponse in AW518 cells was changed depending upon the concentration of L-aspartate in the medium. A 1 μM or lower concentration of L-aspartate caused the normal type of thermoresponse, whereas 0.1 mM or higher concentrations induced the inverted thermoresponse (Fig. 2a). A non-metabolizable analog of L-aspartate, α-methyl-DL-aspartate, showed similar results (Fig. 2b). Since the dissociation constant of L-aspartate to Tar protein was reported to be about 5 μM (5), a concentration of L-aspartate higher than the K_d to Tar protein is required to invert the thermoresponse.

Inversion of the thermoresponse was also observed in other *tsr* mutants such as AW655 (*tsr-12*), RP4790 (*tsr-14*), and RP5698 (Δ*tsr*) when the cells were adapted to 0.1 mM L-aspartate. Furthermore, similar results were obtained by using tethered cells of *tsr* mutants (data not shown). Thus, the inverted thermoresponse is related neither to a special mutation in the *tsr* gene nor to the method used to measure the thermoresponse.

Effect of L-serine or α-aminoisobutyrate on the thermoresponse in *tar* mutants. The results presented in the preceding section show that the inverted thermoresponse is induced when Tsr protein is altered by mutations and Tar protein is altered by adaptation to its specific attractants. Therefore, the opposite combination, namely Tsr protein altered by adaptation to its specific attractants and Tar protein altered by mutations, might be expected to induce the inverted thermoresponse.

TABLE 2. Effect of various chemoeffectors on the thermoresponse of AW518 (*tsr-1*)

Chemoeffector	Thermoresponse[a]
None	Normal
L-Serine, 1 mM	Normal
L-Aspartate, 1 mM	Inverted
L-Glutamate, 1 mM	Inverted
α-Methyl-DL-aspartate, 1 mM	Inverted
Maltose, 1 mM	Inverted
D-Glucose, 1 mM	Normal
D-Galactose, 1 mM	Normal
D-Ribose, 1 mM	Normal
L-Leucine, 50 mM	Normal
Glycerol, 1 M	Normal
$NiSO_4$, 0.1 mM	Normal

[a] Cells were mixed with the chemoeffector at the concentration indicated. After 40 min at 35°C, the thermoresponse was measured as in the legend to Fig. 1.

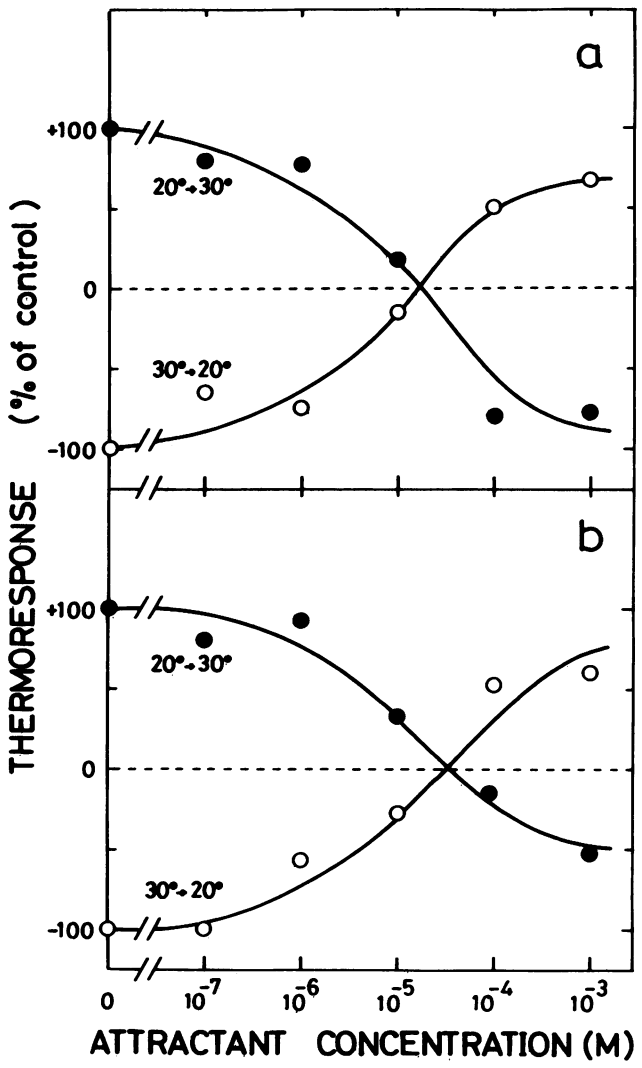

FIG. 2. Relationship between the concentration of L-aspartate or α-methyl-DL-aspartate and the direction of the thermoresponse in AW518 (*tsr-1*). Cells were incubated at 35°C for 40 min with various concentrations of L-aspartate (a) or α-methyl-DL-aspartate (b), and then the temperature was changed as described in the legend to Fig. 1. The increase in the smooth swimming fraction at the peak point of the thermoresponse was measured, and the values were expressed as a percentage of that obtained in the absence of L-aspartate. Symbols: (●) temperature increased from 20 to 30°C; (○) temperature decreased from 30 to 20°C.

AW539 (*tar-1*), a Tar protein-deficient mutant, exhibited the normal type of thermoresponse in the absence of any attractant (Fig. 3a). Adaptation of the mutant cells to L-serine, which is a Tsr protein-specific attractant, up to 10 μM had almost no effect on the thermoresponse. Further increase in L-serine concentrations caused a gradual decrease in the thermoresponse, and at 1 mM or higher concentrations the thermoresponse was completely inhibited. The presence of L-aspartate together with L-serine did not affect the results, indicating that the mutant is surely Tar protein deficient. Similar results were obtained by using AW656 [*tar*(Am)] and RP4324 (*tar-52*Δ1).

The addition of α-aminoisobutyrate, which is also known to interact with Tsr protein, did not have any effect on the

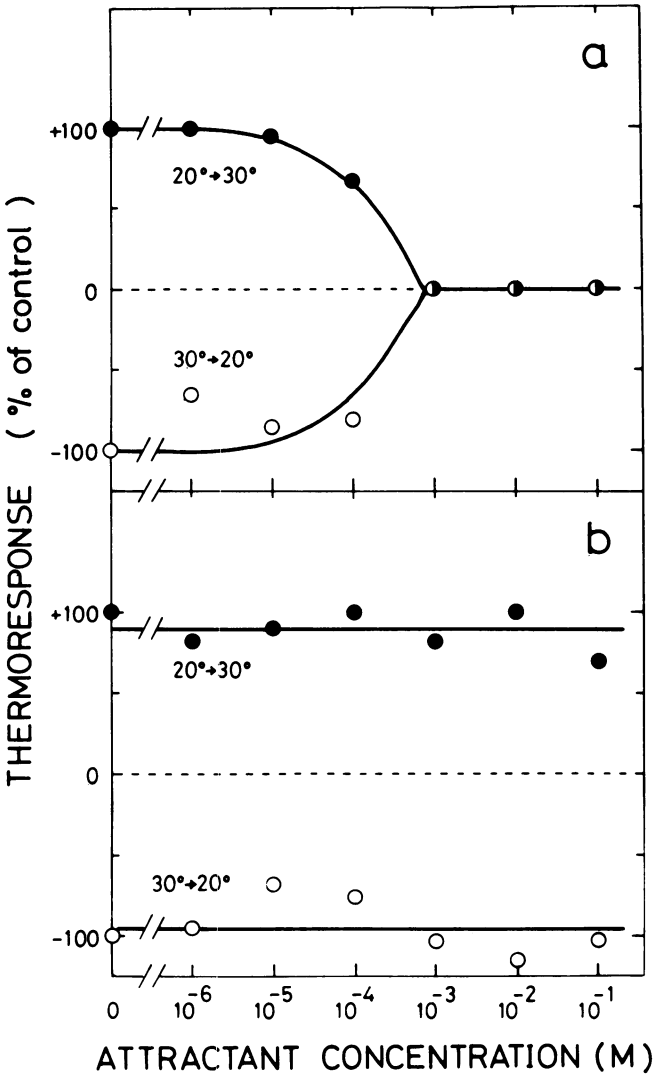

FIG. 3. Effect of various concentrations of L-serine (a) or α-aminoisobutyrate (b) on the thermoresponse in AW539 (*tar-1*). Experimental procedures and the data presentation are similar to those given in the legend to Fig. 2. Temperature changes: 20 to 30°C (●); 30 to 20°C (○).

thermoresponse in AW539 up to 0.1 M (Fig. 3b). L-Homoserine, a Tsr protein-specific attractant, up to 0.1 M also showed no effect. Similar results were obtained with RP4324. These results are consistent with our previous report, in which L-serine but not α-aminoisobutyrate caused a severe inhibition of the thermoresponse in wild-type strains (16). Thus, no inversion of the thermoresponse was observed in *tar* mutants under the conditions tested.

Conditions to induce the inverted thermoresponse in wild-type strains. The direction of the thermoresponse in a wild-type strain, AW405, was not affected by adaptation of the cells to 0.1 mM L-aspartate or L-serine, although L-serine at this concentration caused some inhibition of the thermoresponse as described before. However, when the cells were adapted to both attractants at the same time, the direction of the thermoresponse was completely inverted (Fig. 4). Similar results were obtained by using another wild-type strain, RP487. Thus, the simultaneous alterations in Tsr and Tar proteins by adaptation to L-serine and L-aspartate at the same time caused the inversion of the thermoresponse in wild-type strains.

In the presence of 0.1 mM L-serine, the concentration of L-aspartate necessary to induce the inverted thermoresponse in wild-type strains was 0.1 mM or higher. α-Methyl-DL-aspartate or maltose at a concentration of 0.1 mM or higher showed an effect similar to that of L-aspartate. In the presence of 0.1 mM L-aspartate, a critical concentration of L-serine, approximately 0.1 mM, was effective in inducing the inversion; 10 μM or lower concentrations were not effective, whereas 1 mM or higher concentrations caused a complete inhibition of the thermoresponse. The replacement of L-serine with α-aminoisobutyrate (up to 0.01 M) or L-homoserine (up to 0.1 M), both of which are known to interact with

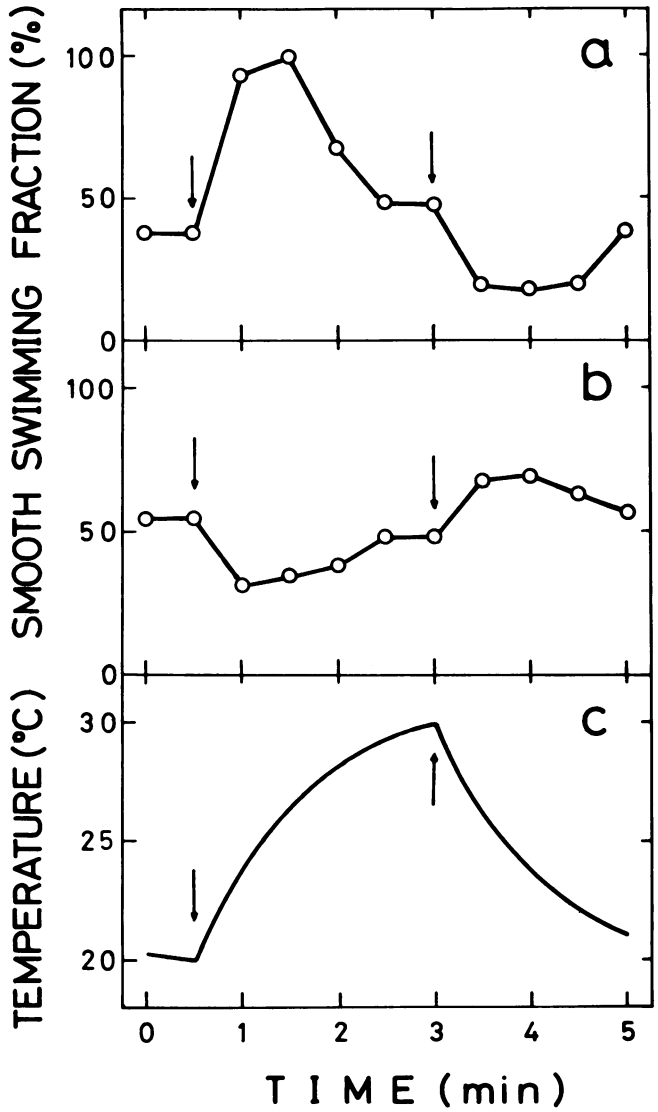

FIG. 4. Inverted thermoresponse in a wild-type strain, AW405, induced by the simultaneous addition of L-aspartate and L-serine. Cells were incubated at 35°C for 40 min with or without 0.1 mM each L-aspartate and L-serine, and then the temperature of the cell suspension was changed at the time points indicated by arrows. (a) No addition; (b) plus 0.1 mM each L-aspartate and L-serine; (c) time course of the temperature change.

Tsr protein, was not successful; the cells showed the normal type of thermoresponse irrespective of the concentration of these attractants. Thus, to induce the inverted thermoresponse in wild-type strains, the alteration of Tsr protein is caused only by L-serine at a concentration of around 0.1 mM, whereas the alteration of Tar protein is caused by any Tar protein-specific attractant at a concentration of 0.1 mM or higher.

Inverted thermoresponse in other MCP mutants. In addition to Tsr and Tar proteins, *E. coli* has two more MCPs, which are the products of *trg* and *tap* genes; the Trg protein is used for D-galactose and D-ribose sensing but the *tap* gene product seems to have no definite function in chemotaxis (3, 33). The role of these MCPs in the thermoresponse under various conditions was examined, and the results are summarized in Table 3. Various *trg* mutants including a deletion mutant showed the inverted thermoresponse when the cells were simultaneously adapted to L-serine and L-aspartate. Similarly, a *tap* deletion mutant showed the inverted thermoresponse in the presence of both L-serine and L-aspartate. Thus, neither Trg protein nor the *tap* gene product is required for the inverted thermoresponse. Consistent with this, the combination of a *tsr* or *tar* mutation with a *trg* or *tap* mutation did not alter the ability of the *tsr* or *tar* mutants to show the inverted thermoresponse.

Consistent with previous data (16), the *tsr-tar* double mutants did not show any thermoresponse even in the presence of both L-serine and L-aspartate.

Changes in the methylation level of MCPs upon temperature changes. Much evidence shows that adaptation to chemical stimuli is caused by changes in the methylation level of the relevant MCPs (30). In either the normal or the inverted type of thermoresponse, the response upon temperature changes was observed only temporarily, indicating that the cells have the ability to adapt to the thermal stimuli under either condition. Since MCPs are involved in thermosensing, it is suggested that adaptation to thermal stimuli is caused by changes in the methylation level of MCPs.

To detect changes in the methylation level of MCPs upon temperature changes, the methyl groups in MCPs were selectively labeled by incubating the cells with L-[*methyl*-^{3}H]methionine for about 30 min in the presence of an inhibitor of protein synthesis, and then the temperature of the cell suspension was changed. Figure 5 shows changes in the methylation level of MCPs in a *tsr* deletion mutant, RP5698, upon a temperature decrease. Under the conditions where the cells showed the normal type of thermoresponse, temperature decrease induced a clear decrease in the methylation level of MCPs (Fig. 5a). Since Tar protein is a major MCP remaining in the mutant cells (7), this result indicates that temperature decrease caused a clear demethylation of Tar protein in the mutant cells. In contrast, when the mutant cells were adapted to 1 mM L-aspartate to invert the direction of their thermoresponse, the methylation level of Tar protein was clearly increased upon temperature decrease

TABLE 3. Effect of L-serine and L-aspartate on the thermoresponse in various MCP mutants

Strain	Thermoresponse[a]			
	No addition	+ L-Serine	+ L-Aspartate	+ L-Serine and L-aspartate
AW405 (wild type)	N	N	N	Inv
AW655 (*tsr-12*)	N	N	Inv	Inv
AW656 [*tar*(Am)]	N	N	N	N
AW701 (*trg-1*)	N	N	N	Inv
AW702 (*trg-2*)	N	N	N	Inv
TH403 (*trg*::Tn*5*)	N	N	N	Inv
RP3525 (Δ*tap*-m365-4)	N	N	N	Inv
AW657 (*tsr-12 trg-1*)	N	N	Inv	Inv
AW658 [*tar*(Am) *trg-1*]	N	N	N	N
RP3544 (*tsr*::Tn*5*-1a Δ*tap*-m365-4)	N	N	Inv	Inv
AW569 (*tsr-1 tar-1*)	0	0	0	0
AW659 [*tsr-12 tar*(Am)]	0	0	0	0

[a] Cells were adapted to 0.1 mM L-serine or L-aspartate or both as indicated, and then the thermoresponse was measured as in the legend to Fig. 1. N, Normal type of thermoresponse; Inv, inverted type of thermoresponse; 0, no thermoresponse.

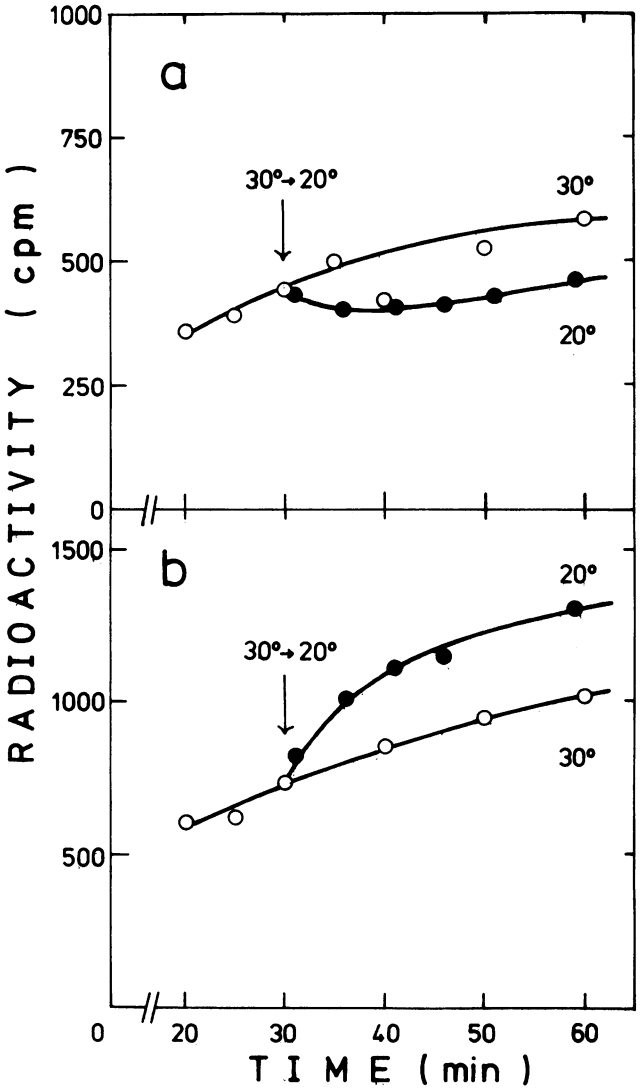

FIG. 5. Changes in the methylation level of Tar protein in RP5698 (Δ*tsr*) upon temperature decrease. Cells were incubated with L-[*methyl*-^{3}H]methionine in the presence of 1 μM (a) or 1 mM (b) L-aspartate. After 30 min of incubation at 30°C, half of the cell suspension was transferred to 20°C. Samples were withdrawn at intervals, and the methylation level of Tar protein was measured. Symbols: (○) cells incubated at 30°C; (●) cells transferred to 20°C.

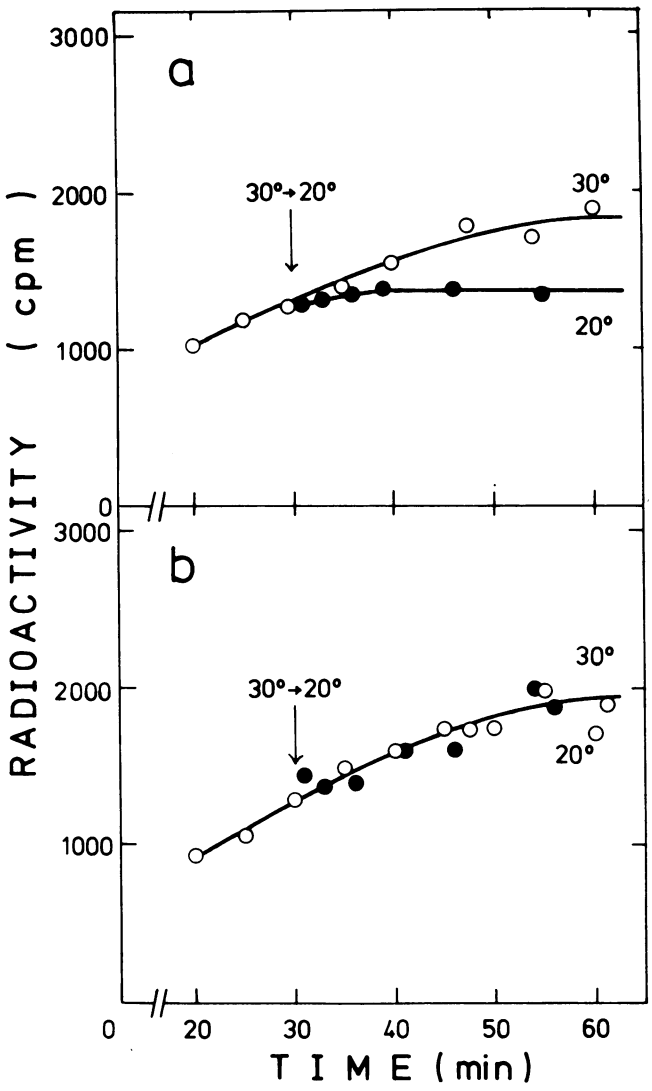

FIG. 6. Changes in the methylation level of Tsr protein in RP4324 (*tar-52Δ1*) upon temperature decrease. Cells were incubated at 30°C with 1 μM (a) or 1 mM (b) L-serine. Other procedures were the same as described in the legend to Fig. 5. Symbols: (○) cells incubated at 30°C; (●) cells transferred to 20°C.

(Fig. 5b). In the case of temperature increase, the methylation level of Tar protein under either condition was changed in the opposite direction compared with the above results (data not shown). Thus, the direction of changes in the methylation level of Tar protein is in parallel with that of the thermoresponse, indicating that Tar protein is involved not only in thermosensing but also in adaptation of the Tsr protein-deficient mutant to thermal stimuli.

When a *tar* deletion mutant, RP4324, was adapted to 1 μM L-serine, a temperature decrease induced a clear decrease in the methylation level of MCPs (in this case, Tsr protein) (Fig. 6a). Increasing the L-serine concentration to 1 mM did not cause any inversion of changes in the methylation level of Tsr protein but caused a complete inhibition of the change (Fig. 6b). These results are consistent with the data shown in Fig. 3a, in which the thermoresponse of the *tar* mutant was not inverted but completely inhibited by adaptation to 1 mM L-serine. Thus, it is concluded that, in Tar protein-deficient mutants, Tsr protein is concerned with both thermosensing and adaptation to thermal stimuli and that high concentrations of L-serine inhibit the function of Tsr protein.

In the case of wild-type strains, the direction of changes in the methylation level of MCPs upon temperature changes was also in parallel with the direction of the thermoresponse, although the changes in the methylation level of MCPs were small (data not shown). Thus, irrespective of the direction of temperature change, the smooth swimming response is always accompanied by an increase in the methylation level of MCPs and the tumbling response is always accompanied by a decrease in the methylation level of MCPs.

DISCUSSION

It has been assumed that the L-serine chemoreceptor is the main thermoreceptor in *E. coli* (16). Since Tsr protein is identified as the L-serine chemoreceptor (8, 32), Tsr protein is now assumed to be a main thermoreceptor. However, Tsr protein-deficient mutants, including a deletion mutant, showed weak but significant thermoresponse, indicating that *E. coli* also has, besides Tsr protein, a minor thermoreceptor. We found that the direction of the residual thermoresponse observed in Tsr protein-deficient mutants could be inverted if the mutants were adapted to the attractants specific to Tar protein. Consistent with this, the mutants under these conditions showed an inverted change in the methylation level of Tar protein. The inverted thermoresponse was also observed in wild-type strains, but only when the cells were simultaneously adapated to L-aspartate and L-serine. Under these conditions, Tsr protein-deficient mutants showed inversion of the thermoresponse but Tar protein-deficient mutants did not. Furthermore, the elimination of Trg protein and the *tap* gene product had no effect on the thermoresponse of either type. Thus, only Tar protein is responsible for inversion of the thermoresponse. Therefore, it is concluded that, in addition to Tsr protein, Tar protein also has a certain role in thermoreception. A structural similarlity between Tsr and Tar proteins (2, 14) gives strong support to this conclusion.

Adaptation of the cells to the attractants specific to Tar protein was essential to induce the inverted thermoresponse. This implies that the methylation of Tar protein plays a key role in the inversion of the thermoresponse, since adaptation to attractants is associated with an increase in the methylation level of MCPs (31). Consistent with this, Tar protein-specific repellents, which cause the demethylation in Tar protein, did not induce the inverted thermoresponse. Thus, upon temperature changes, the signals produced by Tar protein with high methylation levels is considered to have opposite polarity compared with that produced by Tar protein with low methylation levels. Based on this idea, a simple model is proposed to explain the mechanism of the thermoreception and of the inverted thermoresponse in *E. coli* (Fig. 7). Conformational changes of Tsr and Tar proteins are assumed to be essential for thermoreception. Upon temperature increase, Tsr and Tar proteins with low methylation levels produce the smooth swimming signals; Tsr protein produces larger signals than does Tar protein. When the cells are adapted to the attractants, Tsr protein with high methylation levels produces no signal upon temperature increase, whereas Tar protein with high methylation levels produces the tumbling signals. As a result, the adapted cells show tumbling upon temperature increase, namely the inverted thermoresponse. When Tsr protein is deficient, the single addition of the attractants specific to Tar protein is

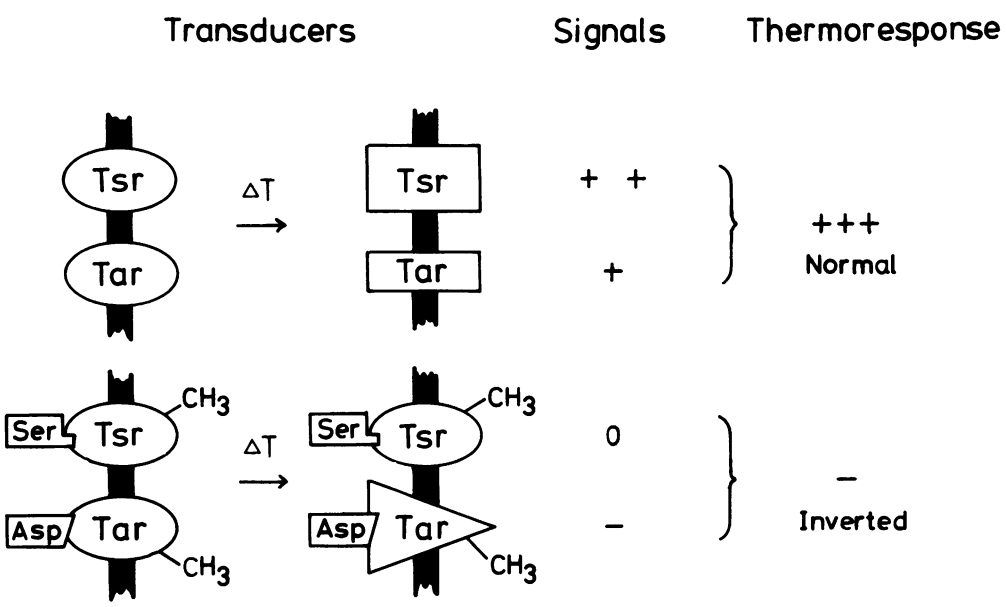

FIG. 7. Simple model to explain the mechanism of thermoreception and the inverted thermoresponse in *E. coli*. See the text for details. Symbols: (+) positive signal; (−) negative signal; (0) no signal.

enough to induce the inverted thermoresponse. In contrast, Tar protein-deficient mutants never show the inverted thermoresponse and only show inhibition of the thermoresponse as increases in the methylation levels of Tsr protein.

It is interesting that among various attractants specific to Tsr protein only L-serine at 1 mM or higher concentrations can completely inhibit the thermoresponse of Tar protein-deficient mutants, suggesting that the low-affinity L-serine site of Tsr protein (K_d = ca. 0.2 mM; 8) has an essential role in the inhibition. To induce the inverted thermoresponse in wild-type cells, the presence of ca. 0.1 mM L-serine, besides Tar protein-specific attractants, was essential and other Tsr protein-specific attractants had no effect. Thus, the partial interaction of L-serine with the low-affinity L-serine site of Tsr protein to reduce the thermosensory transduction through Tsr protein seems to be an important factor in visualizing the inverted thermoresponse in wild-type cells. In a previous paper (16), we assumed that the high-affinity L-serine site was involved in inhibition of the thermoresponse, since rather low concentrations of L-serime were enough to show the inhibition. The apparent discrepancy in the L-serine concentration necessary for inhibition of the thermo-response between these experiments may, besides the difference in experimental conditions, be due to the source of L-serine; in the previous experiments, we used L-serine isolated from protein hydrolysates, whereas here we used chemically synthesized L-serine. The contamination of L-aspartate in L-serine isolated from protein hydrolysates has been claimed (8, 9).

As a related phenomenon to the inverted thermoresponse, the inversion of the chemoresponse in *E. coli* has been reported from several laboratories. Muskavitch et al. (20) showed that Tsr protein-deficient mutants were attracted by weak acid-type repellents such as acetate and benzoate, which had been known to produce tumbling signals by interacting with Tsr protein (30). Kihara and Macnab (12) also observed a similar phenomenon. To explain this phenomenon, Muskavitch et al. (20) assumed that the information of these repellents was processed by both Tsr and Tar proteins and had small effects of opposite polarity on Tar proteins. Thus, the inverted chemoresponse observed in Tsr protein-deficient mutants seems to have a mechanism related to the inverted thermoresponse, although the methylation of Tar protein is not required for the inverted chemoresponse.

MCP-methylesterase-deficient mutants (*cheB* mutants) also showed the inverted chemoresponse to acetate and benzoate (12, 24, 26). However, the methylation reaction of MCPs and also the presence of Tar protein were not required for this inversion (24). This phenomenon might therefore have a different mechanism.

Rubik and Koshland (28) reported that *cheC* mutants in *Salmonella typhimurium* showed the inverted chemoresponse to both attractants and repellents. Khan et al. (11) showed that the inversion was only observed in free-swimming cells and not in tethered cells and that this inversion was caused by the mutation in the switch of the flagellar rotation system rather than the information-processing system; the mutation produced an abnormality in the conformational and hydrodynamic properties of the flagella. Since both the inverted thermoresponse and the inverted chemoresponse in *E. coli* are also detected in tethered cells, the mechanisms of the inverted responses observed in *E. coli* is definitely different from that in *cheC* mutants of *S. typhimurium*.

In higher organisms, there are two different types of thermoreceptor cells, warm receptor and cold receptor (10). Since the methylation of Tar protein in *E. coli* seems to alter the polarity of the signals produced by a thermal stimulus, the difference between the thermoreceptors in these different receptor cells might be small.

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