

Thermosensory transduction in *Escherichia coli*: Inhibition of the thermoresponse by L-serine

(thermotaxis/chemoreceptor/thermoreceptor)

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ABSTRACT Information processing of the thermoresponse in *Escherichia coli* was compared with that of the chemoresponse. Competition experiments between various chemical stimuli and the thermal stimulus showed that only L-serine was a potent inhibitor of the thermosensory transduction. The concentration of L-serine necessary for complete inhibition of the thermoresponse was about 0.1 mM. L-Serine at this concentration did not inhibit chemoresponses to many amino acids. Pleiotropic aspartate-taxis mutants (*tar*) showed normal thermoresponse but pleiotropic serine-taxis mutants (*tsr*) showed decreased or almost no thermoresponse. These results suggest that the thermosensory transducing system in *E. coli* has an intimate interaction with the chemosensory transducing pathway specific for L-serine. A simple model for the thermosensory transduction is discussed.

Like higher organisms, bacteria can sense various stimuli such as chemical stimuli, thermal stimuli, and photostimuli (1-4). Recent studies on the analysis of the chemosensory transducing system in *Escherichia coli* (1, 5) and *Salmonella typhimurium* (6) have revealed that chemical stimuli are first detected by specific chemoreceptors on the cell surface and then transmitted and processed by various proteins known as *che* gene products. Springer *et al.* (7) and Silverman and Simon (8) have shown that in *E. coli* there are two complementary pathways of information processing for most chemical stimuli: one is the Tar pathway for chemical stimuli such as L-aspartate and maltose, and the other is the Tsr pathway for stimuli such as L-serine and α -aminoisobutyrate (AiBu). The processed information regulates the rotation mode of flagella to promote bacterial movement toward attractants or away from repellents; an increase in an attractant concentration causes smooth, translational swimming, and an increase in repellent concentration induces tumbling. Thus, the information flow in *E. coli* starts at chemoreceptors, passes through *tar* or *tsr* proteins, and finally reaches the flagellar rotation system.

In our previous paper (2), we showed that *E. coli* has the ability to sense a thermal stimulus and that the response to a temperature increase is analogous to that for an increase in chemical attractants: a temperature increase suppresses tumbling, and a temperature decrease induces tumbling. Because generally nonchemotactic mutants of *E. coli* have a defect in responding to thermal stimulus, there must be some overlapping between pathways of the information processing for chemoresponse and thermoresponse.

To understand the thermosensory transducing pathway in *E. coli*, we have investigated the participation of the chemosensory transducing pathway in thermosensory transduction. In this paper, we will show that the thermoresponse is specifically inhibited by L-serine. This result suggests the existence of an interaction between the information processing pathway for thermal stimuli and that for the L-serine stimulus.

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MATERIALS AND METHODS

Bacteria. Wild-type and all the chemotaxis-negative mutants used in this study were derivatives of *E. coli* K-12. RP 477 (*che*⁺), *cheB* 277, *cheB* 287, *cheB* 294, *cheZ* 278, *cheZ* 292, and *tsr* 192-2 (isolated from *cheD* 192) were supplied by J. S. Parkinson (University of Utah) (9). AW 518 (*tsr*), AW 539 (*tar*), AW 569 (*tsr-tar*), and *cheA* 457 were obtained from J. Adler (University of Wisconsin) (7, 10), and MS 5235 (*tar* 52 Δ 1) was from M. Silverman (University of California at San Diego) (8).

Cells were grown in 2.6% Tryptose broth (Difco) containing 0.5% glycerol at 35°C until the cell concentration reached about 4×10^8 cells per ml. Cells were harvested by filtration through a membrane filter (pore size, 0.45 μ m; Sartorius-Membranefilter GmbH, Göttingen) and washed with motility medium (0.01 M potassium phosphate buffer, pH 7.0/0.1 mM potassium EDTA/0.5% glycerol). Cells were resuspended in the same medium to a cell concentration of 1×10^7 cells per ml.

Temperature Control and Measurement of Thermoresponse. The procedures for temperature control, temperature measurement, and recording of swimming tracks of bacteria by photography were as described (2). All pictures of the swimming tracks were taken at an exposure time of 1.5 sec.

For the simple and quantitative measurement of the thermoresponse in the wild type upon a sudden temperature drop, maximal increase in the fraction of swimming tracks having two or more tumbles per 1.5 sec was measured as an expression of the increase in tumbling frequency. The definition of a tumble on the swimming tracks has been described (2); detailed procedures will be described elsewhere.

In the case of constantly tumbling mutants, the quantitative method of Spudich and Koshland (11) was applied and maximal increase in the fraction of smooth swimming tracks upon a sudden temperature increase was measured.

For competition experiments between thermal stimuli and various chemoeffectors, cells were incubated with various concentrations of attractants or repellents for more than 20 min to adapt the cells to the chemoeffector so that the swimming behavior of the cells was returned to the original state. Cells were incubated for 5 min at 30°C for temperature drop experiments and at 20°C for temperature increase experiments. Then, the temperature change was carried out and the thermoresponse values were measured as described above.

Time Course of Stimulation by Attractants. Constantly tumbling mutants in the motility medium at 30°C were exposed to an abrupt change in attractant concentration to induce smooth swimming. The time for 50% of the responding cells to begin tumbling once again was measured directly as described by Parkinson (12).

Abbreviations: AiBu, α -aminoisobutyrate; *tsr*, pleiotropic serine-taxis mutant; *tar*, pleiotropic aspartate-taxis mutant.

RESULTS

Competition between Thermoresponse and Chemoreponse. Bacterial cells that have become adapted to one attractant show no or decreased response when exposed to another attractant that shares portions of the same pathway for information processing (1, 13, 14). This observation was applied to analyze the pathway of thermosensory transduction.

Wild-type cells (RP 477) were mixed with various chemoeffectors at different concentrations and incubated for more than 20 min to adapt the cells to the chemoeffector. Then, the temperature of the cell suspension was quickly decreased from 30 to 20°C in order to induce tumbling. As shown in Fig. 1, the induction of tumbling was greatly inhibited by adaptation of the cells to L-serine. The concentration of L-serine that produced a 50% inhibition of the thermoresponse was only 4 μ M. Other attractants, including L-aspartate, AiBu, L-methionine, L-alanine, and galactose, required concentrations higher than 0.01 M to produce a 50% inhibition; in addition, L-glutamate, L-threonine, glycine, and a repellent, L-leucine, showed a weak inhibition of the thermoresponse and required a concentration of 0.01 M or more for 50% inhibition (data not shown).

Inhibition of the thermoresponse by L-serine was also detected by utilizing a constantly tumbling mutant (*cheZ 278*). Similar to a sudden increase in attractant concentration (11), exposing this mutant to a sudden temperature increase from 20 to 34°C caused transient smooth swimming (see Fig. 4, discussed below). This induction of smooth swimming by a temperature increase was strongly suppressed by adaptation of the cells to L-serine (Fig. 2). The concentration of L-serine necessary for 50% inhibition was low, about 1 μ M. L-Aspartate, AiBu, L-methionine, L-alanine, and galactose produced less than 50% inhibition at 0.01 M. In addition, L-glutamate, L-threonine, and glycine showed less than 50% inhibition at 0.01 M (data not shown).

These results clearly indicate that L-serine is a potent and specific inhibitor of the thermoresponse.

Effect of *tsr* and *tar* Mutations on Thermoresponse. From the experiments on chemoresponses in a pleiotropic serine-taxis mutant (*tsr*) and in a pleiotropic aspartate-taxis mutant (*tar*),

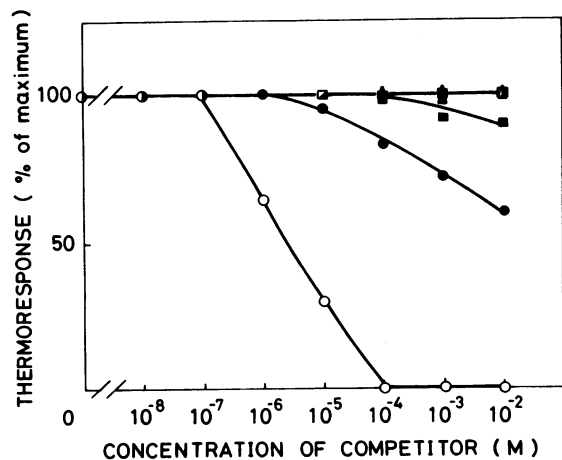


FIG. 1. Competition between various chemoeffectors and the thermal stimulus in *E. coli* wild-type strain RP 477. Cells were incubated in motility medium containing different concentrations of various attractants for 20 min. The temperature was quickly decreased from 30 to 20°C at a maximal rate of 0.10°C/sec. The response values are expressed as percentage of the value obtained in the absence of competitor. To determine the effect of galactose, cells were grown for 1 hr in the presence of 10 mM galactose before harvesting. O, L-Serine; ●, L-aspartate; □, AiBu; ■, L-alanine; ▲, L-methionine; △, galactose.

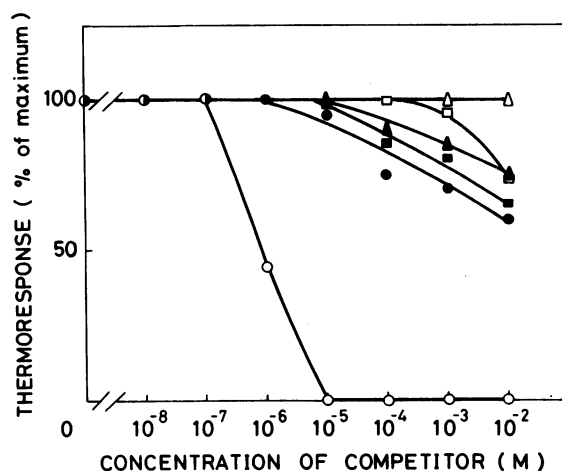


FIG. 2. Competition between various chemoeffectors and the thermal stimulus in a constantly tumbling mutant, *cheZ 278*. Experimental conditions were as in Fig. 1, except that the temperature change was from 20 to 34°C at a maximal rate of 0.15°C/sec. The response values were expressed as percentage of the value obtained in the absence of the competitor. O, L-Serine; ●, L-aspartate; □, AiBu; ▲, L-methionine; ■, L-alanine; △, galactose.

the pathway of various chemosensory transductions was divided into two complementary pathways: the Tsr pathway and the Tar pathway (7, 8). Because the information for L-serine was reported to be processed mainly by the Tsr pathway (7), the role of the Tsr pathway in thermosensory transduction was investigated to clarify the relationship between the pathways for the thermal stimulus and for the L-serine stimulus.

Fig. 3 shows the transient thermoresponse in a wild type and various mutants after a sudden temperature decrease from 30 to 20°C. A wild-type strain and two *tar* mutants showed almost the same pattern; namely, the tumbling frequency greatly increased upon a temperature decrease, reached a maximum at about 30 sec after the decrease, and then returned to the normal level. Thus, the thermoresponse to a temperature decrease was not affected by the *tar* mutation. However, in the case of *tsr* mutants, AW 518 showed a weak response and *tsr* 192-2 showed no response. A *tsr-tar* double mutant (AW 569) also showed no thermoresponse, and the pattern was almost the same as that of a generally nonchemotactic smooth-swimming mutant (Fig. 3f).

Thus, the data indicate that the thermosensory transduction, like L-serine, mainly utilizes the Tsr pathway.

Thermoresponse and Chemoreponse in *cheB* Mutants. As described before, one group of constantly tumbling mutants (*cheZ* mutants) showed a transient thermoresponse upon a sudden temperature increase (Fig. 4b). However, another group of constantly tumbling mutants (*cheB* mutants) showed almost no thermoresponse upon a temperature increase (Fig. 4c). Therefore, the *cheB* gene product seems to be required for the thermoresponse. However, as shown in Table 1, this group of mutants showed a level of chemoreponse similar to that of *cheZ* mutants only when the L-serine concentration was increased from 0.1 to 10 mM. The concentration increase of the other attractants from 0 to 10 mM and of L-serine from 0 to 0.1 mM resulted in no induction or a weak transient response. Thus, the *cheB* gene mutants seem to be generally defective in all their responses except the response to the high concentration of L-serine.

Inhibition of Thermoresponse by a Combination of AiBu and L-Aspartate. AiBu alone and L-aspartate alone only weakly affected the thermoresponse in a wild-type strain and also in *cheZ 278*. The addition of both chemicals together at a total concentration of 0.1 mM produced more than a 50% inhibition

Table 1. Chemoresponses of various constantly tumbling mutants to attractants

Attractant*	Response time, † sec				
	<i>cheZ</i> 278	<i>cheZ</i> 292	<i>cheB</i> 277	<i>cheB</i> 287	<i>cheB</i> 294
L-Serine					
0 → 0.1 mM	77	91	<15	<15	33
0.1 → 10 mM	81	95	85	81	106
L-Alanine	76	80	No	No	No
Glycine	75	60	No	No	No
L-Aspartate	60	82	(No)‡	(No)‡	(No)‡
L-Methionine	47	24	No	No	No
L-Glutamate	50	64	No	No	No
L-Threonine	35	36	No	No	No
AiBu	45	42	No	No	No
Galactose§	43	59	No	No	No
Maltose§	41	67	No	No	No

* Attractant jump: 10-fold from 0.1 to 1 mM at 30°C, except L-serine as indicated.

† The time for 50% of the responding cells to begin tumbling once again, shown as means ($n > 5$). No, response not detected. Response time was <15 sec even when the attractant was jumped from 0 to 10 mM.

‡ Response not detected, but cells responded for about 30 sec when the attractant was jumped from 0 to 10 mM.

§ Cells were cultured in the presence of 10 mM galactose and 10 mM maltose for 1 hr before harvesting.

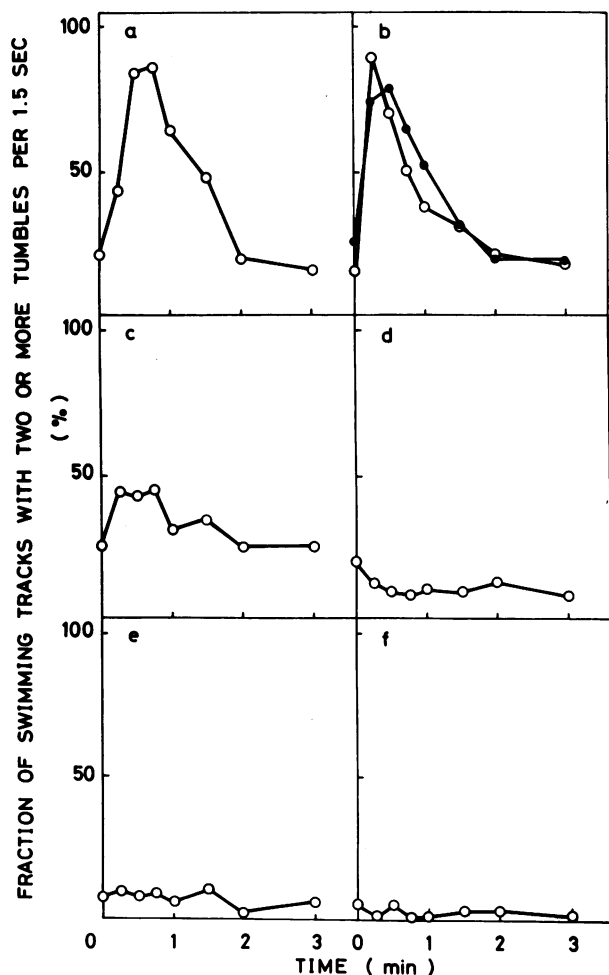


FIG. 3. Thermoresponse in a wild type and various chemotaxis mutants. The temperature was decreased from 30 to 20°C at a maximal rate of 0.10°C/sec. The thermoresponse value was determined from the fraction of swimming tracks having two or more tumbles per 1.5 sec. (a) Wild-type RP 477; (b) *tar* mutants AW 539 (O) and MS 5235 (●); (c) *tsr* mutant AW 518; (d) *tsr* mutant *tsr* 192-2; (e) *tsr-tar* double mutant AW 569; (f) *cheA* mutant *cheA* 457.

of the thermoresponse in both strains. However, this mixture gave 40–60% inhibition of the chemoresponses upon 0 to 10 mM jump of L-serine, L-alanine, glycine, L-aspartate, L-methionine, galactose, or maltose. Thus, the mixture of these two chemicals showed nonspecific inhibition of all the responses in *E. coli*.

Effect of L-Serine on the Chemoresponses to Other Attractants. An L-serine concentration higher than 10 mM generally suppresses all chemoresponses (13, 15). However, 0.1 mM L-serine, which is sufficient to suppress the thermoresponse completely, did not inhibit the chemoresponses to L-aspartate, L-methionine, or L-glutamate (Table 2). Chemoresponses to some other attractants such as L-alanine, L-threonine, and

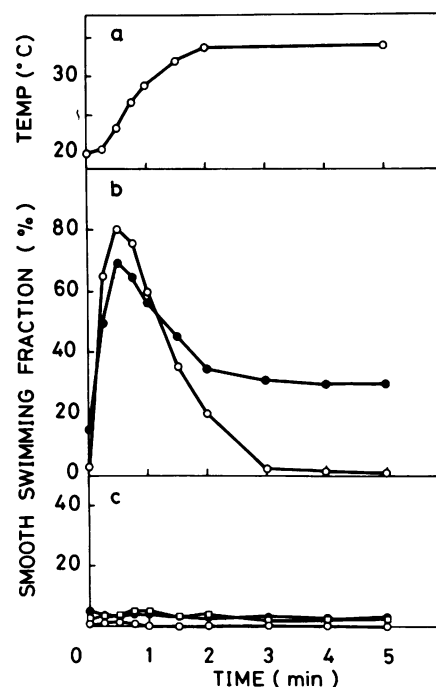


FIG. 4. Thermoresponse in constantly tumbling mutants. Temperature was increased from 20 to 34°C at a maximal rate of 0.15°C/sec. The smooth swimming fraction of cells was measured. (a) Time course of temperature change. (b) *cheZ* mutants *cheZ* 278 (O) and *cheZ* 292 (●). (c) *cheB* mutants *cheB* 277 (□), *cheB* 287 (●), and *cheB* 294 (○).

Table 2. Effect of L-serine on the chemoresponse of *cheZ* 278 to various attractants

Attractant*	Response time,† sec		Ratio,‡ %
	No serine	With serine	
L-Serine	120	72	60 ± 4
L-Alanine	110	60	55 ± 14
Glycine	92	61	66 ± 9
L-Aspartate	100	90	90 ± 6
L-Methionine	65	60	92 ± 11
L-Glutamate	92	77	84 ± 6
L-Threonine	77	49	64 ± 10
AiBu	106	59	56 ± 7
Galactose§	86	46	55 ± 11
Maltose§	68	46	68 ± 14

* Attractant jump: 0 to 10 mM.

† The time for half of the responding cells to begin tumbling once again, shown as means ($n > 5$). Cells were incubated with or without 0.1 mM L-serine for more than 20 min at 30°C prior to attractant jump.

‡ Calculated as (with serine/no serine) × 100 and shown as means ± SD.

§ Cells were cultured in the presence of 10 mM galactose and 10 mM maltose for 1 hr before harvesting.

glycine were partially inhibited by 0.1 mM L-serine, but these attractants are known to share a common pathway of information processing with L-serine at least partly (13).

L-Serine at 0.1 mM partially suppressed chemoresponses to galactose and maltose, although these chemoeffectors apparently have different pathways of information processing from the pathway of L-serine (7). However, this suppression effect was not limited to L-serine because L-aspartate at 0.1 mM showed almost the same effect on the galactose and maltose chemoresponses. Furthermore, the plot of inhibition of the galactose response by L-serine and by L-aspartate showed a more gradual slope compared to that of the inhibition of the thermoresponse by L-serine (data not shown). L-Alanine and AiBu showed almost no inhibitory effect on galactose response at a concentration of 0.1 mM.

DISCUSSION

Thermosensory Transducing Pathway in *E. coli*. The results presented in this paper show that (i) L-serine at a concentration of about 0.1 mM is a potent inhibitor of the thermoresponse (either an increase or a decrease in temperature), (ii) all other chemoeffectors tested have only a weak effect on the thermoresponse, and (iii) the Tsr pathway is utilized by the thermosensory transduction.

According to Springer *et al.* (7), there are two chemoreceptor systems for L-serine in *E. coli*; one is a high-affinity system that has a K_m of 3×10^{-6} M for L-serine and utilizes the Tsr pathway for information processing; the other is a low-affinity system with a K_m of 3×10^{-4} M. The L-serine concentration necessary for 50% inhibition of the thermoresponse was 1–4 μ M (Figs. 1 and 2). The similarity of this value to the K_m of the high-affinity chemoreceptor system for L-serine suggests that the inhibitory effect of L-serine on the thermoresponse occurs at the level of this chemoreceptor. The involvement of the Tsr pathway in the thermosensory transduction supports this idea.

CheZ mutants showed almost the same level of chemoresponse to an increase in L-serine concentration either from 0 to 0.1 mM or from 0.1 to 10 mM. However, in *cheB* mutants, the response to an increase in L-serine concentration from 0 to 0.1 mM was much weaker than that from 0.1 to 10 mM (Table

1). These results strongly support the idea that the L-serine chemoreceptor system in *E. coli* consists of a high-affinity and a low-affinity system. Furthermore, the finding that *cheB* mutants lack both the thermoresponse and the chemoresponse to a low concentration of L-serine is consistent with the idea that the high-affinity chemoreceptor system of L-serine is utilized in thermosensory transduction.

It is noteworthy that the *cheB* mutants showed little or no response to each of the other chemoeffectors tested. Therefore, it is likely that the *cheB* gene product is required for all sensory transductions in *E. coli* and that only the chemosensory transduction involving the low-affinity chemoreceptor system of L-serine can escape the *cheB* lesion. The reason for this is not clear.

Fig. 5 shows a simple model for thermosensory transduction in *E. coli*. In this model, the high-affinity L-serine chemoreceptor, with a K_m of about 10^{-6} M, is assumed to be the thermoreceptor. L-Serine binds to the receptor and induces a change in the conformation of it. The conformationally altered receptor can interact with Tsr proteins to transmit the information from the L-serine stimulus. A temperature increase induces the same or a similar change in the conformation of the L-serine receptor, so that the receptors having a thermally induced conformational change can also interact with the Tsr proteins. Thus, the thermal stimulus can be processed and transmitted as an L-serine stimulus would be. Because the K_m of the receptor for L-serine is very low, the presence of about 0.1 mM L-serine completely suppresses the thermoresponse. Much more complicated models could be drawn for the thermosensory transducing pathway because the change in temperature can influence various cell constituents. A feature of this model is the introduction of a specific receptor for thermal stimuli. The isolation of L-serine receptor mutants is suggested as an important test for the operation of this model.

In the case of higher organisms, various stimuli such as chemical stimuli, thermal stimuli, and photostimuli are detected and transmitted by different nerve cells specific for each of these stimuli. However, single-cell organisms such as bacteria must detect and treat all stimuli in the same cell. Therefore, an overlapping of the sensory transducing systems for various stimuli would be economical for the cell. In fact, for photosensory transduction in *E. coli* and *S. typhimurium*, at least some elements of the chemosensory transducing system are utilized (16).

Interaction among Chemosensory Transducing Pathways. L-Serine and L-aspartate inhibited galactose response in a tumble mutant with almost the same efficiency, although other

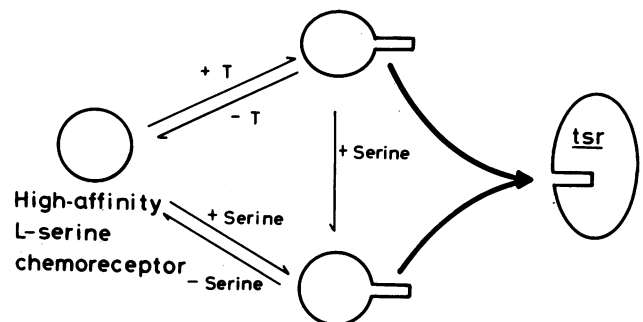


FIG. 5. Suggested role of a high-affinity L-serine chemoreceptor system in thermosensory transduction in *E. coli*. L-Serine and thermal stimuli induce the same or a similar conformational change in the high-affinity chemoreceptor system of L-serine [$K_m \approx 10^{-6}$ M (7)]. The receptor then interacts with Tsr gene product to transmit the information. + T, temperature increase; - T, temperature decrease; Tsr, Tsr gene products.

amino acids such as L-alanine and AiBu required a very high concentration to give the same effect. For galactose chemoreception there is a well-characterized galactose-binding protein (17). Therefore, the interference with the galactose response by L-serine or L-aspartate could not occur at the level of the receptor.

Furthermore, although AiBu alone or L-aspartate alone did not show a severe effect on many of the chemoresponses or on the thermoresponse, the combination of these two chemicals at a total concentration of 0.1 mM showed 40–60% inhibition of most of the chemoresponses and the thermoresponse. These results also indicate that the inhibition does not occur at the level of receptors. Because all the information flow from the receptors should pass the *tsr* or *tar* proteins or both, it is very likely that the interference occurs at the level of *tsr* or *tar* proteins. These two proteins are known as methyl-accepting chemotaxis proteins (7, 8), and therefore, the methylation system of them may be involved in this interference.

The thermoresponse was completely inhibited by L-serine at about 0.1 mM, but this concentration of L-serine inhibited the chemoresponses to L-alanine, AiBu, and glycine by only about 40%. It is known that all these amino acids utilize the Tsr pathway for information processing (7, 13). Therefore, the competition between the thermal stimulus and the L-serine stimulus at the methyl-accepting chemotaxis protein level is negligible compared to their competition at the receptor level.

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