THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 273, No. 46, Issue of November 13, pp. 30110–30115, 1998

Printed in U.S.A.

Intersubunit Interaction between Transmembrane Helices of the Bacterial Aspartate Chemoreceptor Homodimer*

(Received for publication, March 30, 1998, and in revised form, June 1, 1998)

Tohru Umemura, Ichiro Tatsuno‡, Manabu Shibasaki§, Michio Homma, and Ikuro Kawagishi¶

From the Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan

The transmembrane domain that connects the extracellular and intracellular domains of cell-surface receptors must play a critical role in signal transduction. Here, we report studies of the interaction between the transmembrane helices (TM1 and TM2) of the *Escherichia coli* aspartate chemoreceptor (Tar). Tar exists as a homodimer regardless of its state of ligand occupancy. A particular residue substitution in TM1 (A19K) abolishes the signaling ability of Tar. This signaling defect can be suppressed by single residue substitutions in TM2 (W192R, A198E, V201E, and V202L). We have found that these suppressors can be divided into two groups. A198E and V201E (class 1) almost completely suppress the defects caused by A19K, and this suppression occurs between two subunits of the Tar dimer. In contrast, W192R and V202L (class 2) fail to suppress some signaling defects, and their suppression does not occur between subunits. Because disulfide-crosslinking studies predict that residues 198 and 201 point toward residue 19 of the partner subunit, we propose that the class 1 suppressors form an intersubunit salt bridge with Lys-19. Indeed, A19K was suppressed by several additional aspartate or glutamate substitutions on the same face of TM2 occupied by residues 198 and 201. None of these intersubunit salt bridges perturb signaling function, suggesting that the mechanism of transmembrane signal propagation does not involve large displacements (such as extensive rotation) of the TM1 and TM2 helices relative to each other.

Cell-surface receptors detect extracellular signals and convert them into intracellular signals. Their extracellular and intracellular domains are connected by transmembrane (TM)[1] domains, which typically consist of α-helices. In addition to supporting the molecular architecture of the proteins, these TM domains must also play a critical role in signal transduction across the cytoplasmic membrane.

The aspartate chemoreceptor (Tar) of enteric bacteria is well suited for studying the function of TM domains (for reviews, see Refs. 1–4). *Escherichia coli* Tar also mediates responses to maltose by interacting with liganded maltose-binding protein. Tar is a homodimeric protein (5) with a subunit molecular mass of about 60 kDa. It has two TM helices, TM1 and TM2. Unlike some homodimeric eukaryotic receptors with tyrosine-kinase activity, Tar apparently does not undergo monomer-dimer transitions during signaling, because some disulfide--crosslinked dimers are fully active (5, 6). Therefore, binding of ligands to Tar is thought to trigger a conformational change within the receptor dimer, which must include some displacement of the TM domains with respect to each other.

The Tar dimer forms a stable ternary complex with a homodimer of the autophosphorylating histidine kinase CheA and two molecules of the coupling protein CheW (7, 8). When aspartate or another attractant ligand binds to Tar, CheA activity is inhibited. Otherwise, phosphorylated CheA transfers the phosphoryl group of the response regulator CheY, and phospho-CheY promotes clockwise rotation of the flagellar motor and thereby causes the cell to tumble. When phospho-CheY is not bound to it, the motor rotates counterclockwise, and the cell swims smoothly.

The periplasmic, ligand-binding domain of Tar has been crystallized both in the presence and absence of aspartate (9). Each monomer contains four α-helices (α1, α2, α3, and α4), which form a four-helix bundle. The longer helices, α1 and α4, are contiguous with TM1 and TM2, respectively, and they form a quasi four-helix bundle with the α1′ and α4′ helices of their partner subunit. Recent studies suggest that the α-helical pair TM1/α1–TM1′/α1′, which constitutes an interface between the two subunits of the receptor dimer, is rather static (10–15). In contrast, binding of ligand to the receptor dimer is thought to cause a displacement (a tilt, rotation or vertical slide) of α4/TM2 relative to the TM1/α1–TM1′/α1′ pair.

In this context, it is relevant to note that a single amino acid substitution (A19K) in TM1 abolishes the signaling ability of Tar without impairing the aspartate-binding ability of the receptor (16). Many intragenic suppressors of A19K were isolated, and four of them caused residue substitutions in TM2 (W192R, A198E, V201E, and V202L). None of these suppressors abolish receptor function in the absence of the original A19K mutation. Consistent with this observation, TM2 (17–19) is relatively tolerant for substitutions. TM2 of Tar can be replaced by that of the related serine chemoreceptor (Tsr), or vice versa, without destroying receptor function (17). On the other hand, certain substitutions at position 204 in TM2 (I204F, I204Y, and I204W) impair the signaling ability of Tar (18), and several other mutations causing defects in signaling were identified in TM2 of the related ribose-galactose chemoreceptor (Trg) (19).

In this study, we examined how mutations in TM2 suppress the TM1 mutation A19K. The original TM2 suppressors could be divided into two groups: A198E and V201E almost com-

* This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan (to I. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Bacteriology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-0071, Japan.

§ Present address: Kowa Research Institute, Kowa Co., Ltd., 1-25-5 Kannon-dai, Tsukuba, Ibaraki 305-0856, Japan.

¶ To whom correspondence should be addressed. Tel.: 81-52-789-2993; Fax: 81-52-789-3001; E-mail: i45406a@nucc.cc.nagoya-u.ac.jp.

[1] The abbreviations used are: TM, transmembrane; MSA, minimal semisolid agar; TM1, transmembrane region 1; TM2, transmembrane region 2; TSA, tryptone semisolid agar.

pletely suppress the defects caused by A19K, whereas W192R and V202L fail to suppress some of the defects. Moreover, the former two mutations, but not the latter two, can suppress A19K even if they are present in the partner subunit of the dimer. A19K can also be suppressed by the introduction of negatively charged residues (Asp or Glu) at position 205 or Asp at position 201, suggesting that intersubunit suppression by A198E or A201E results from formation of a salt bridge between TM1 and TM2′. This finding places some clear constraints on the possible mechanisms of TM signaling.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—All strains used in this study are derivatives of *Escherichia coli* K-12. Strains RP4372*recA* (F⁻ *thi thr leu met eda rpsL* Δ(*tar-tap*)*5201 tsr-1 recA*) (20) and KO607 (Δ*tsr-7021* Δ(*tar-tap*)*5201* Δ*trg-100 recA*) (21), both of which were provided by K. Oosawa of Nagoya University, were used as the plasmid hosts in chemotaxis assays. Strain DH5α (F⁻ λ⁻ *recA1 hsdR17 endA1 gyrA96 supE44 relA1 thi-1* Δ(*argF-lacZYA*)*U169* φ80d*lacZ*Δ*M15*) (22) was used for plasmid construction. A pBR322-based plasmid, pAK101, carries the wild-type *tar* gene (20). Its derivatives carrying *tar* genes encoding Tar-A19K or Tar-A19K with suppressors (W192R, A198E, V201E, V202L) were provided by K. Oosawa. Plasmid pIT6 was constructed by subcloning a *tar*-containing DNA fragment into the plasmid vector pSU18 (23), which contains the P15A replicon and the chloramphenicol acetyltransferase gene.

Mutagenesis of Tar—DNA manipulations were carried out using standard methods. Site-directed mutagenesis was performed by a two-step polymerase chain reaction (24) using plasmid pAK101 as the template and primers synthesized by Sawadi Technology (Tokyo). Polymerase chain reaction was carried out using ExTaq polymerase (Takara Shuzo, Kyoto) with 25 cycles of denaturing at 96 °C for 5 min, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min. The polymerase chain reaction products were cloned between the *Xba*I and *Kpn*I sites of pAK101. *Eco*RV fragments (1.2 kilobases) containing the desired mutations were introduced into pAK101 or pIT6. The DNA sequences were verified by the dideoxy chain termination method.

Swarm Assay of Chemotaxis—Chemotaxis was examined in tryptone semisolid agar (TSA) (1% tryptone, 0.5% NaCl, 0.25% agar) or minimal semisolid agar (MSA) (0.05 M potassium phosphate buffer (pH 7.0), 1 mM $MgSO_4$, 1 mM glycerol, 0.1 mM each of threonine, leucine, histidine, methionine, 1 μg/ml thiamine, 1 mM $(NH_4)_2SO_4$, and 0.25% agar), supplemented with 0.1 mM aspartic acid or maltose as needed. Ampicillin and/or chloramphenicol were added as required. Semisolid agar was inoculated with aliquots of exponential-phase cultures (about 4×10^6 cells) and incubated at 30 °C.

Temporal Assay of Chemotactic Response—Temporal-stimulation assays were carried out essentially as described previously (25). Cells were grown at 30 °C in TG broth (1% tryptone, 0.5% NaCl, 0.5% (w/v) glycerol) with ampicillin and/or chloramphenicol. When necessary, 0.2% maltose was added. Cells were harvested in late exponential phase, washed twice with motility medium (10 mM potassium phosphate buffer (pH7.0), 0.1 mM EDTA, 0.1 mM methionine, 10 mM sodium DL-lactate), and resuspended in motility medium at room temperature. The swimming pattern of the cells was observed with a dark-field microscope and recorded on videotape. For time-course assays, the fraction of smooth-swimming cells was determined every 30 s after the addition of an attractant. Video images were analyzed using an Argus-10 image processor (Hamamatsu Photonics K. K., Shizuoka). In an image integrated for 1 s, smooth-swimming and tumbling cells gave linear and blurred-dot traces, respectively. The smooth-swimming fraction was defined as the percent fraction of smooth-swimming cells per total swimming cells.

Analysis of Methylation Patterns—Receptor methylation was assayed as described previously (26). Cells expressing wild-type or mutant Tar proteins were grown, harvested, and washed as described above. A chemoeffector was added to cells suspended in motility medium, and the suspension was then incubated at room temperature for 30 min. The cells were collected by centrifugation and suspended in SDS-loading buffer (67 mM Tris-HCl (pH 6.8), 8% glycerol, 1% SDS, 0.003% bromphenol blue) supplemented with 7.7% 2-mercaptoethanol. Samples were boiled for 3 min and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto a polyvinylidene difluoride membrane (Millipore Japan, Tokyo) using a semidry blotting apparatus (Biocraft, Tokyo). Anti-Tsr-T156C serum (27), which cross-reacts with Tar, and alkaline phosphatase-conjugated goat anti-rabbit IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) were used as the first and the second antibodies, respectively. Protein-antibody complexes were visualized in 5 ml of AP buffer (0.1 M Tris-HCl (pH 9.5), 1 M NaCl, 5 mM $MgCl_2$) supplemented with 33 μl of nitroblue tetrazolium solution (50 mg/ml in 70% (v/v) dimethylformamide) and 16.5 μl of 5-bromo-4-chloro-3-indolylphosphate solution (50 mg/ml).

TABLE I
Properties of the Tar-A19K · Sup homodimers

Wild-type and mutant Tar receptors were expressed in KO607, which lacks Tar and the related chemoreceptors Tsr, Trg, and Tap. Swarming ability was examined in MSA containing 0.1 mM aspartate (Asp) or 0.1 mM maltose (Mal). In temporal-stimulation assays, cells expressing any receptor other than Tar-A19K responded to aspartate (Asp), maltose (Mal), and glycerol (Glyc). After prolonged incubation with these chemoeffectors, the cells were examined to see if they had adapted to the stimuli. Significant (+), weak (±), or no (−) adaptation was observed.

Tar	Swarming ability		Adaptation ability		
	Asp	Mal	Asp	Mal	Glyc
Wild type	+	+	+	+	−
A19K	−	−	NA[a]	NA	NA
A19K · W192R	+	±	+	±	+
A19K · A198E	+	+	+	+	±
A19K · V201E	+	+	+	+	+
A19K · V202L	+	±	+	±	+

[a] NA, not applicable due to lack of an initial response.

RESULTS

Identification of Two Classes of TM2 Suppressors of A19K—We characterized the Tar proteins having the A19K substitution and a TM2 suppressor on the same subunit (collectively referred to as Tar-A19K·Sup) (Table I). These proteins were expressed in strain KO607, which lacks all of the methyl-accepting chemoreceptors except Aer, which mediates aerotactic responses (28, 29). The resulting transformants were tested for their swarming ability. In TSA and MSA-aspartate (MSA-Asp), cells expressing any Tar-A19K·Sup protein formed swarms like those of cells expressing wild-type Tar. In MSA-maltose (MSA-Mal), however, cells expressing Tar-A19K·W192R or V202L did not swarm well, whereas the swarms made by cells expressing Tar-A19K·A198E or V201E were comparable with those of cells expressing wild-type Tar.

In temporal-stimulation assays, responses to aspartate or maltose of cells expressing any Tar-A19K·Sup were similar to those of cells expressing wild-type Tar, and the threshold attractant concentrations (apparent sensitivities to the attractants) were similar. However, adaptation to maltose was impaired in cells expressing Tar-A19K·W192R or V202L, whereas cells expressing Tar-A19K·A198E or V201E adapted normally to maltose. In contrast, cells expressing any Tar-A19K·Sup adapted to aspartate indistinguishably from cells expressing wild-type Tar.

These results suggest that the TM2 suppressors can be classified into two groups. Class 1 suppressors (A198E and V201E) almost completely suppressed the signaling and adaptation defects caused by A19K. Class 2 suppressors (W192R and V202L) did not suppress some of the defects, notably those in adaptation to maltose.

Trans Suppression of A19K by the Class 1 Suppressors—To address how the TM2 substitutions suppress defects caused by A19K, we tested their ability to suppress an A19K substitution in the partner subunit. We reported previously that A198E suppresses A19K *in trans* and used this system to ask whether a receptor dimer with only one intact signaling domain can mediate an attractant response to aspartate (30). Subsequently, we constructed a series of mutant Tar proteins having one of the TM2 suppressors and the aspartate-binding site alteration T154P. These mutant proteins (collectively referred to as Tar-T154P·Sup) were co-expressed with Tar-A19K in strain RP4372*recA*, which lacks Tar and the related chemore-

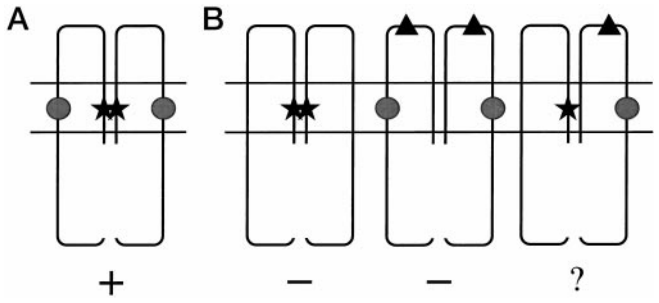

FIG. 1. **The strategy for intersubunit suppression.** *A*, a functional (+) homodimer of Tar with A19K (*star*) and its suppressor (Sup) (*circle*); *B*, co-expression of Tar-A19K and Tar-T154P·Sup. Tar-Sup is functional, and so the mutation T154P (*triangle*), which causes a severe defect in aspartate sensing, was introduced into Tar-Sup. The function of the heterodimer of Tar-A19K and Tar-T154P·Sup (*right*) can be monitored, because neither of the homodimers (*left* and *center*) can mediate an aspartate response (−).

ceptors Tsr and Tap (Fig. 1). Immunoblots verified that all of the mutant proteins were expressed (data not shown). Essentially similar results were obtained when the mutant proteins were expressed in strain KO607.

These cells were then tested for their swarming ability in MSA-Asp (Fig. 2) and TSA (not shown). In TSA or MSA-Asp, neither of these Tar derivatives alone supported formation of swarm rings, indicating that the homodimers of these Tar proteins do not mediate an attractant response to aspartate, as expected. However, cells expressing both Tar-A19K and Tar-T154P·A198E or V201E swarmed well in both TSA and MSA-Asp. In contrast, neither W192R nor V202L *in trans* could suppress the defect in swarming caused by A19K.

We then examined the attractant responses of these cells to aspartate directly, using the temporal-stimulation assay (Fig. 3). Again, cells expressing Tar-A19K or any Tar-T154P·Sup protein alone did not respond to aspartate (data not shown). However, cells expressing Tar-T154P·A198E or V201E with Tar-A19K, did respond to aspartate. The concentration of aspartate required for a half-maximal response was similar to that of cells expressing wild-type Tar. However, cells co-expressing Tar-A19K and Tar-T154P·W192R or V202L did not respond, even to 0.01 M aspartate. These results indicate that the class 1 suppressors, but not the class 2 suppressors, can suppress the defect in signaling caused by the A19K substitution on the partner subunit (intersubunit suppression).

Identification of Suppressors of A19K among Asp or Glu Substitutions at Residues Near A198 and V201—The class 1 (intersubunit) suppressors A198E and V201E introduce a negatively charged residue into TM2, whereas the other suppressors introduce a positively charged or uncharged residue. Furthermore, disulfide-crosslinking studies of TM1 and TM2 (10, 11, 31, 32) predict that residues 198 and 201 of one subunit face residue 19 of the partner subunit (Fig. 4). Therefore, we propose that intersubunit suppression results from formation of a salt bridge between the ϵ-amino group of Lys-19 and the γ-carboxyl group of the suppressing Glu residue (Glu-198 or Glu-201).

To test this hypothesis, we introduced Asp or Glu into positions on the face of TM2 helix predicted to be facing the partner subunit (Fig. 4). Immunoblots demonstrated that the mutant proteins were expressed (data not shown). Cells expressing these proteins were tested for their swarming ability (Fig. 5). In MSA-Asp, they produced three types of swarms (Fig. 5). (i) Cells expressing Tar-A19K·A198E, V201D, V201E, L205D, or L205E produced swarm rings that were as sharp as although smaller than that of cells expressing wild-type Tar. (ii) Cells expressing Tar-A19K·I204D or I204E produced small and diffuse swarms. (iii) Cells expressing Tar-A19K·A198D, A208D, or A208E produced no swarm ring. Essentially, similar swarming patterns were observed in TSA (data not shown).

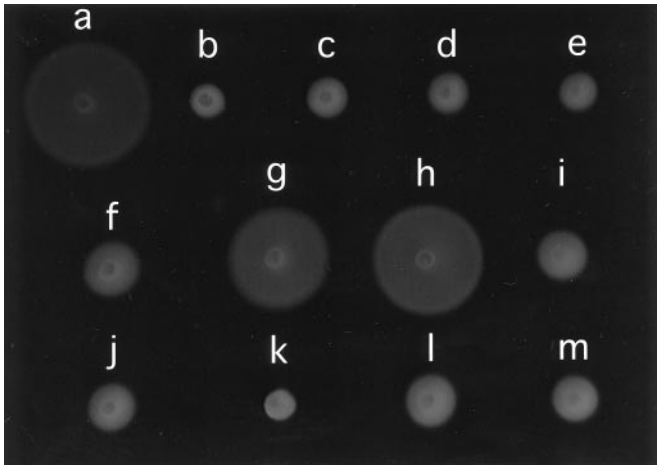

FIG. 2. **Swarming abilities of RP4372*recA* cells co-expressing Tar-A19K and Tar-T154P·Sup from compatible plasmids.** Overnight cultures were spotted onto MSA containing ampicillin, chloramphenicol, and 0.1 mM aspartate. The plate was incubated at 30 °C. The Tar proteins present are: *a*, wild-type Tar; *b*, no Tar; *c*, Tar-A19K; *d*, Tar-T154P; *e*, Tar-A19K + Tar-T154P; *f*, Tar-A19K + Tar-T154P·W192R; *g*, Tar-A19K + Tar-T154P·A198E; *h*, Tar-A19K + Tar-T154P·V201E; *i*, Tar-A19K + Tar-T154P·V202L; *j*, Tar-T154P·W192R; *k*, Tar-T154P·A198E; *l*, Tar-T154P·V201E; *m*, Tar-T154P·V202L.

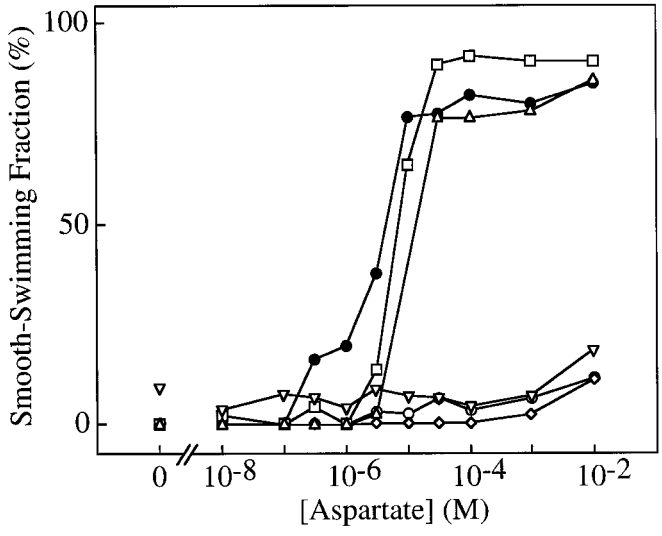

FIG. 3. **Aspartate-sensing abilities of heterodimers containing Tar-A19K and Tar-T154P·Sup.** Various concentrations of aspartate were added to a suspension of cells expressing wild-type Tar (●) or co-expressing Tar-A19K with Tar-T154P (○), Tar-T154P·W192R (◇), Tar-T154P·A198E (△), Tar-T154P·V201E (□), or Tar-T154P·V202L (▽). After 20 s, the smooth-swimming fractions of the cells were measured.

In temporal-stimulation assays, the first group of cells showed almost the same threshold for aspartate as cells expressing wild-type Tar (Fig. 6*A*), despite the varied diameters of the swarms formed by the mutants. The second group of cells did not give a significant response immediately after the addition of aspartate (Fig. 6*B*). However, their smooth-swimming fractions increased up to 30% within 30 s (data not shown). In contrast, the third group of cells did not respond to aspartate at all (Fig. 6*C*). These results demonstrate that A19K can be suppressed by Asp or Glu substitutions for Ala-198, Val-201, and Leu-205 (except A198D), suggesting that the basis of suppression really is the formation of a salt bridge between Lys-19 and an introduced negatively charged residue.

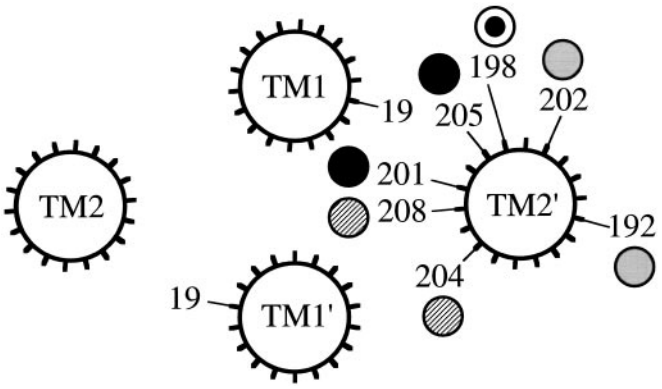

FIG. 4. **Schematic representation of the TM domains of the Tar homodimer.** The relative orientation of the putative α-helices (TM1, TM1′, TM2, and TM2′) is based on disulfide-crosslinking studies (10, 11, 31, 32). The view is from the periplasmic surface (after Ref. 10). *Closed circles*, positions at which substitutions created class 1 suppressors; *gray circles*, positions at which substitutions generated class 2 suppressors; *hatched circles*, positions at which Asp or Glu substitutions did not fully suppress A19K. The *circle* with a *black center* at position 198 indicates that A198E suppressed, whereas A198D did not.

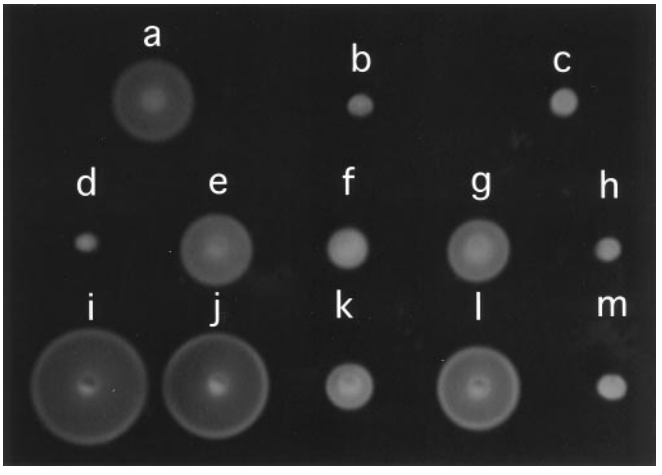

FIG. 5. **Suppression of A19K by Asp or Glu substitutions in TM2.** RP4372*recA* cells expressing each mutant Tar were examined for their swarming ability in MSA containing ampicillin and 0.1 mM aspartate as described in the legend to Fig. 2. The Tar proteins present are: *a*, wild-type Tar; *b*, no Tar; *c*, Tar-A19K; *d*, Tar-A19K·A198D; *e*, Tar-A19K·V201D; *f*, Tar-A19K·I204D; *g*, Tar-A19K·L205D; *h*, Tar-A19K·A208D; *i*, Tar-A19K·A198E; *j*, Tar-A19K·V201E; *k*, Tar-A19K·I204E; *l*, Tar-A19K·L205E; *m*, Tar-A19K·A208E.

We also examined the expression levels and methylation patterns of these mutant Tar proteins by immunoblotting (Fig. 7). Multiple methylation of a chemoreceptor by CheR causes stepwise increases in its mobility in SDS-polyacrylamide gel electrophoresis (33–36). All of the mutant receptors were detected in whole cell lysates, although their amounts and levels of methylation varied substantially. Stimulation of methylation by the addition of aspartate was observed with receptors that mediated responses to aspartate (Tar-A19K·A198E, V201D, V201E, L205D, and L205E) but not with those that mediated little or no responses to aspartate (Tar-A19K·A198D, I204D, I204E, A208D, and A208E).

Trans Suppression of A19K by Asp or Glu Substitutions in TM2—We also tested for *trans* suppression of A19K by the Asp or Glu substitutions. In MSA-Asp, RP4372*recA* cells co-expressing Tar-A19K with Tar-T154P·V201D, L205D, or L205E produced swarm rings comparable with those of cells expressing wild-type Tar (Fig. 8). In contrast, cells co-expressing Tar-A19K with Tar-T154P·A198D, I204D, I204E, A208D, or A208E produced little or no swarm ring. Essentially similar swarming

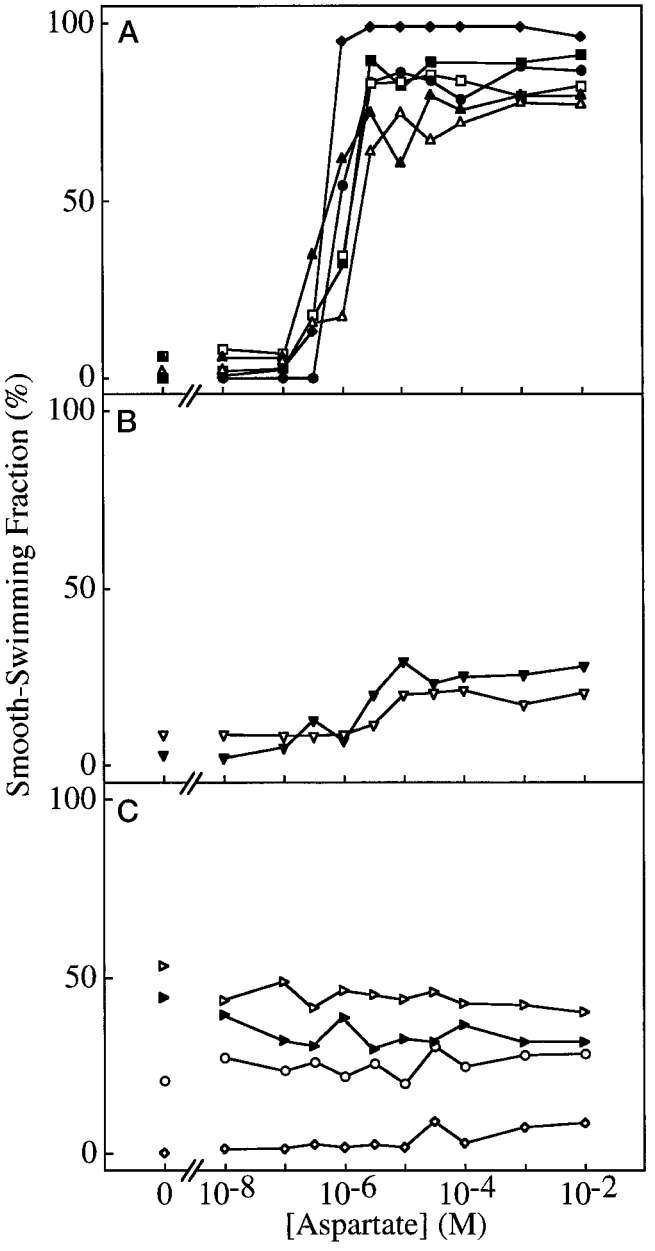

FIG. 6. **Aspartate-sensing ability of homodimers of Asp- or Glu-substituted Tar-A19K.** Responses to aspartate mediated by wild-type Tar (●), Tar-A19K (○), or Tar-A19K with A198D (◇), A198E (◆), V201D (△), V201E (▲), I204D (▽), I204E (▼), L205D (□), L205E (■), A208D (▷), or A208D (▶) were determined as described in the legend to Fig. 3. *A*, cells that showed aspartate responses comparable with that mediated by wild-type Tar; *B*, cells that showed marginal responses to aspartate; *C*, cells that did not respond to aspartate.

patterns were observed in TSA (data not shown). These results demonstrate that V201D, L205D, and L205E can suppress A19K *in trans*. Thus, V201D, L205D, and L205E are also class 1 suppressors.

DISCUSSION

In this study, we examined how single amino acid substitutions in TM2 suppress the detrimental substitution A19K in TM1. A19K does not affect the ligand binding, but it does abolish signaling ability (16). Our results divide the four suppressors in TM2 into two groups. The A198E and V201E substitutions (class 1 suppressors) almost completely reverse the defects caused by A19K, and this effect can be exerted between the two subunits of the Tar dimer. In contrast, the W192R and

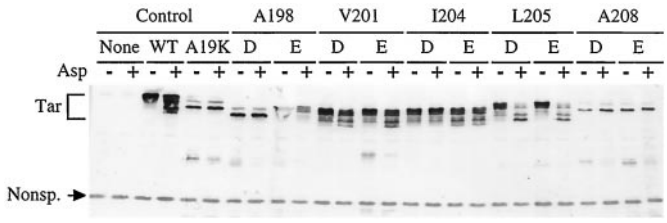

FIG. 7. **Expression levels and methylation patterns of Asp- or Glu-substituted Tar-A19K proteins.** RP4372*recA* cells expressing wild-type or mutant Tar receptors were incubated with (+) or without (−) 10 mM aspartate (*Asp*), and whole cell lysates were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with anti-Tsr serum. *None*, no Tar; *WT*, wild-type Tar; *A19K*, Tar-A19K; *A198D etc.*, Tar-A19K·A198D *etc.*; *Nonsp.*, a band cross-reacting nonspecifically with the serum.

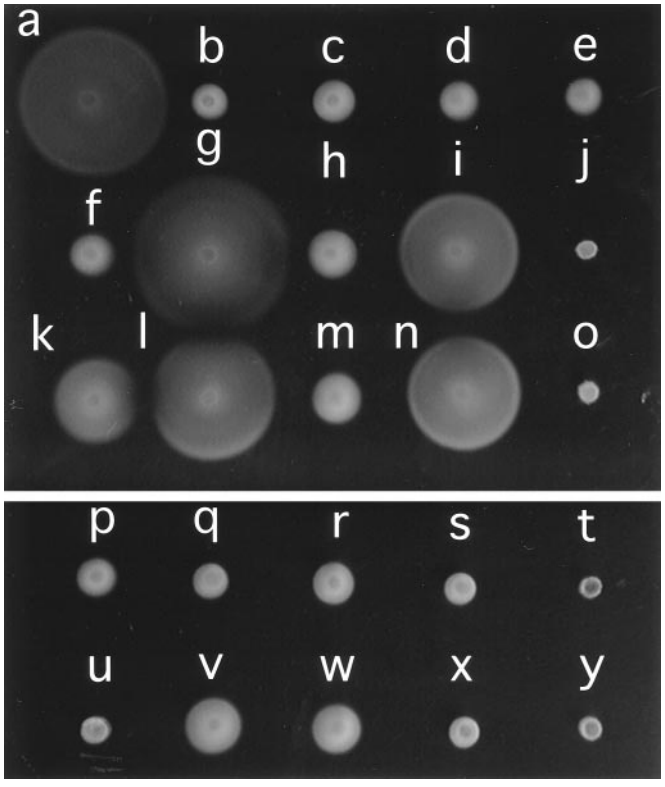

FIG. 8. ***Trans* suppression of A19K by Asp or Glu substitutions in TM2.** RP4372*recA* cells co-expressing Tar-A19K and Tar-T154P with an Asp or Glu substitution from compatible plasmids were examined for their swarming ability as described in the legend to Fig. 2. The Tar proteins present are: *a*, wild-type Tar; *b*, no Tar; *c*, Tar-A19K; *d*, Tar-T154P; *e*, Tar-A19K + Tar-T154P; *f*, Tar-A19K + Tar-T154P·A198D; *g*, Tar-A19K + Tar-T154P·V201D; *h*, Tar-A19K + Tar-T154P·I204D; *i*, Tar-A19K + Tar-T154P·L205D; *j*, Tar-A19K + Tar-T154P·A208D; *k*, Tar-A19K + Tar-T154P·A198E; *l*, Tar-A19K + Tar-T154P·V201E; *m*, Tar-A19K + Tar-T154P·I204E; *n*, Tar-A19K + Tar-T154P·L205E; *o*, Tar-A19K + Tar-T154P·A208E; *p*, Tar-T154P·A198D; *q*, Tar-T154P·V201D; *r*, Tar-T154P·I204D; *s*, Tar-T154P·L205D; *t*, Tar-T154P·A208D; *u*, Tar-T154P·A198E; *v*, Tar-T154P·V201E; *w*, Tar-T154P·I204E; *x*, Tar-T154P·L205E; *y*, Tar-T154P·A208E.

V202L substitutions (class 2 suppressors) fail to suppress some signaling defects, and suppressions did not occur between subunits. This finding supports the validity of the experimental design for intersubunit suppression, because it excludes the possibility that apparent intersubunit suppression results from compensation of smooth swimming biased signaling of the Tar-A19K homodimer by tumbling biased signaling of the Tar-T154P·Sup homodimer.

Disulfide-crosslinking studies predict that residues 198 and 201 of TM2 point toward residue 19 of TM1′ (Lys-19′) (Fig. 4). Therefore, the γ-carboxyl groups of the glutamate residues of the class 1 suppressors are likely to form an intersubunit salt bridge with the ε-amino group of Lys-19′. Consistent with this, all of the same-site pseudorevertants isolated from A19K are to introduce uncharged residues (Ile, Thr, and Gln) (16).

The possibility of salt bridge formation might be tested by changing ionic strength. However, such *in vivo* experiments should be difficult, because *E. coli* cells show abnormal responses to higher salt concentrations without chemoreceptors or any other chemotactic signaling proteins (termed pseudotumbling) (37). The hypothesis might also be examined by introducing a negative charge in TM1 and a positive charge in TM2. Although either of these mutations might be detrimental and suppressed by a positive charge in TM2 or a negative charge in TM1, respectively, there is no obvious candidate for such mutation (it should be noted that W192R is harmless).

Perhaps the most realistic way to test the salt bridge formation is to systematically introduce Asp or Glu substitutions on the face of the TM2 helix on which residues 198 and 201 are located. Indeed, some of these mutations (V201D, L205D, and L205E) reversed the defects caused by A19K, whereas others (A198D, I204D, I204E, A208D, and A208E) produced little or no suppression. The effective suppressors result from substitutions for residues 198, 201, and 205 that are predicted to face Lys-19′ (Fig. 4). On the other hand, substitutions that do not suppress, with the exception of A198D, are predicted to be located further from Lys-19′. The positively charged side chain of Lys-19 probably disturbs normal packing of the four TM helices (TM1, TM1′, TM2, and TM2′), and introduction of a negatively charged residue in TM2′ may restore this packing by creating a salt bridge between TM1 and TM2′.

It is striking that A198D and A198E had such different effects. An Asp residue at position 198 might not extend far enough to form a salt bridge with Lys-19′ or, if a salt bridge does form, it might affect the receptor structure and function. Hydrophobic interaction between TM1 and TM2′ might be important for the receptor architecture, and a charge-neutralizing salt bridge might restore such interactions. A similar salt bridge between TM helices has been implicated in the structural stabilization of wild-type lactose permease (38, 39).

The interhelical salt bridges have another, perhaps more important, implication for signal transduction. They would probably be maintained upon binding and release of a small molecule-like aspartate. The enthalpies (ΔH) of formation of the various salt bridges cannot be calculated precisely, but they should be larger than the enthalpy (−18 kcal/mol) reported for serine binding to Tsr (40). Therefore, the salt bridges presumably restrict the potential ability of TM1 and TM2′ to move relative to one another during the signal-transduction cycle.

Recent studies have provided good evidence that TM1 and TM1′ do not have to move relative to one another for effective TM signaling to take place after ligand binding (10–15). Rather, vertical displacement, tilting, or rotation of TM2 relative to TM1 seems to be the critical element in signaling. These findings predict that ligand binding may cause both intra- and intersubunit displacement of TM2. The multiple interhelical salt bridges that are compatible with receptor function argue that such a displacement cannot be too large. These small movements of TM2, however, may trigger structural changes in the cytoplasmic domains that are essential for signal production. Such changes have been suggested to occur between subunits (41–45) as well as within a subunit (or between dimers) (30, 46, 47).

The mode(s) of suppression by W192R and V202L are still unknown. These mutations might exert their effects within a subunit or they might be effective only when they are placed in

both subunits of a dimer. Moreover, A19K can also be suppressed by substitutions in the cytoplasmic "linker" region, which is contiguous from TM2 (16). Further investigation of these suppressors should help elucidate the mechanism of receptor signaling.

Acknowledgments—We thank Dr. K. Oosawa for strains, plasmids, and helpful discussion and Dr. M. D. Manson (Texas A & M University) for critically reading the manuscript.

REFERENCES

1. Manson, M. D. (1992) *Adv. Microb. Physiol.* **33,** 277–346
2. Parkinson, J. S. (1993) *Cell* **73,** 857–871
3. Stock, J. B., and Surette, M. G. (1996) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (Neidhardt, F. C., Curtiss, R., III, Ingram, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) 2nd Ed. pp. 1103–1129, American Society for Microbiology, Washington, D. C.
4. Falke, J. J., Bass, R. B., Butler, S. L., Chervitz, S. A., and Danielson, M. A. (1997) *Annu. Rev. Cell Dev. Biol.* **13,** 457–512
5. Milligan, D. L., and Koshland, D. E., Jr. (1988) *J. Biol. Chem.* **263,** 6268–6275
6. Falke, J. J., and Koshland, D. E., Jr. (1987) *Science* **237,** 1596–1600
7. Gegner, J. A., Graham, D. R., Roth, A. F., and Dahlquist, F. W. (1992) *Cell* **70,** 975–982
8. Schuster, S. C., Swanson, R. V., Alex, L. A., Bourret, R. B., and Simon, M. I. (1993) *Nature* **365,** 343–347
9. Milburn, M. V., Prive, G. G., Milligan, D. L., Scott, W. G., Yeh, J., Jancarik, J., Koshland, D. E., Jr., and Kim, S.-H. (1991) *Science* **254,** 1342–1347
10. Chervitz, S. A., and Falke, J. J. (1995) *J. Biol. Chem.* **270,** 24043–24053
11. Chervitz, S. A., Lin, C. M., and Falke, J. J. (1995) *Biochemistry* **34,** 9722–9733
12. Chervitz, S. A., and Falke, J. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 2545–2550
13. Lee, G. F., Lebert, R., Lilly, A. A., and Hazelbauer, G. L. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 3391–3395
14. Maruyama, I. N., Mikawa, Y. G., and Maruyama, H. I. (1995) *J. Mol. Biol.* **253,** 530–546
15. Hughson, A. G., and Hazelbauer, G. L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 11546–11551
16. Oosawa, K., and Simon, M. I. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 6930–6934
17. Tatsuno, I., Lee, L., Kawagishi, I., Homma, M., and Imae, Y. (1994) *Mol. Microbiol.* **14,** 755–762
18. Jeffery, C. J., and Koshland, D. E., Jr. (1994) *Biochemistry* **33,** 3457–3463
19. Baumgartner, J. W., and Hazelbauer, G. L. (1996) *J. Bacteriol.* **178,** 4651–4660
20. 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
21. Oosawa, K., Mutoh, N., and Simon, M. I. (1988) *J. Bacteriol.* **170,** 2521–2526
22. Grant, S. N., Jessee, J., Bloom, F. R., and Hanahan, D. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 4645–4649
23. Bartolomé, B., Jubete, Y., Martinez, E., and Cruz, F. D. (1991) *Gene* (*Amst.*) **102,** 75–78
24. Landt, O., Grunert, H. P., and Hahn, U. (1990) *Gene* (*Amst.*) **96,** 125–128
25. Nishiyama, S., Nara, T., Imae, Y., Homma, M., and Kawagishi, I. (1997) *J. Bacteriol.* **179,** 6573–6580
26. Okumura, H., Nishiyama, S., Sasaki, A., Homma, M., and Kawagishi, I. (1998) *J. Bacteriol.* **180,** 1862—1868
27. Iwama, T., Homma, M., and Kawagishi, I. (1997) *J. Biol. Chem.* **272,** 13810–13815
28. Bibikov, S. I., Biran, R., Rudd, K. E., and Parkinson, J. S. (1997) *J. Bacteriol.* **179,** 4075–4079
29. Rebbapragada, A., Johnson, M. A., Zhulin, I. B., Harding, G. P., Succarelli, A. J., Fletcher, H. M., and Taylor, B. L. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 10541–10546
30. Tatsuno, I., Homma, M., Oosawa, K., and Kawagishi, I. (1996) *Science* **274,** 423–425
31. Lynch, B. A., and Koshland, D. E., Jr. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10402–10406
32. Pakula, A. A., and Simon, M. I. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 4144–4148
33. Boyd, A., and Simon, M. I. (1980) *J. Bacteriol.* **143,** 809–815
34. Chelsky, D., and Dahlquist, F. W. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 2434–2438
35. DeFranco, A. L., and Koshland, D. E., Jr. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 2429–2433
36. Engström, P., and Hazelbauer, G. L. (1980) *Cell* **20,** 165–171
37. Li, C., and Adler, J. (1993) *J. Bacteriol.* **175,** 2564–2567
38. King, S. C., Hansen, C. L., and Wilson, T. H. (1991) *Biochim. Biophys. Acta* **1062,** 177–186
39. Dunten, R. L., Sahin-Tóth, M., and Kaback, H. R. (1993) *Biochemistry* **32,** 3139–3145
40. Lin, L.-N., Li, J., Brandts, J. F., and Weis, R. M. (1994) *Biochemistry* **33,** 6564–6570
41. Long, D. G., and Weis, R. M. (1992) *Biochemistry* **31,** 9904–9911
42. Cochran, A. G., and Kim, P. S. (1996) *Science* **271,** 1113–1116
43. Surette, M. G., and Stock, J. B. (1996) *J. Biol. Chem.* **271,** 17966–17973
44. Chen, S., and Koshland, D. E., Jr. (1997) *Biochemistry* **36,** 11858–11864
45. Danielson, M. A., Bass, R. B., and Falke, J. J. (1997) *J. Biol. Chem.* **272,** 32878–32888
46. Milligan, D. L., and Koshland, D. E., Jr. (1991) *Science* **254,** 1651–1653
47. Gardina, P., and Manson, M. (1996) *Science* **274,** 425–426