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

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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) 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, seeRefs. 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 certain substitutions at position 198 (W198A and W198G) suppress the signaling ability of Tar (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-

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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 RP4372recA (F- thi thr leu met eda rpsL Δtar-tap5201 tar-1 recA) (20) and K0607 (Δtar-7021 Δstar-tap5201 Δxrg-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 gryA96 supE44 relA1 thi-1 Δ(aroF-lacZYA169 deoRlacZAM15)) (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 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-A19KSup 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-A19KSup 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-A19KSup 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 on the same subunit (collectively referred to as Tar-A19KSup) (Table I). These proteins were expressed in strain K0607, 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-A19KSup 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.

**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-A19KSup) (Table I). These proteins were expressed in strain K0607, 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-A19KSup 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-A19KSup 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-A19KSup adapted to aspartate indistinguishably from cells expressing wild-type Tar.

**Table I**

| Tar | Swarming ability | Adaptation ability |
|-----|------------------|--------------------|
|     | Asp Mal          | Asp Mal Glyc       |
| Wild type | + + + + + | + + + + + |
| A19K | - - NA* NA | - + + + + |
| A19K - W192R | + + | + + + + + |
| A19K - A198E | + + + + | + + + + + |
| A19K - V201E | + + + + | + + + + + |
| A19K - V202L | + + | + + + + + |

* NA, not applicable due to lack of an initial 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 K0607.

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 mM 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 e-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).

In temporal-stimulation assays, the first group of cells showed almost the same threshold for aspartate as cells expressing wild-type Tar (Fig. 6A), 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. 6B). 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. 6C). 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.
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 swelling 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
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 pseudo-tumbling) (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 Tar (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.

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