Evidence for a Helix-Clutch Mechanism of Transmembrane Signaling in a Bacterial Chemoreceptor

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Abstract

The *Escherichia coli* Tsr protein contains a periplasmic serine-binding domain that transmits ligand occupancy information to a cytoplasmic kinase-control domain to regulate the cell's flagellar motors. The Tsr input and output domains communicate through conformational changes transmitted through a transmembrane helix (TM2), a five-residue control cable helix at the membrane-cytoplasm interface, and a four-helix HAMP bundle. Changes in serine occupancy are known to promote TM2 piston displacements in one subunit of the Tsr homodimer. We explored how such piston motions might be relayed through the control cable to reach the input AS1 helix of HAMP by constructing and characterizing mutant receptors that had one-residue insertions or deletions in the TM2-control cable segment of Tsr. TM2 deletions caused kinase-off output shifts; TM2 insertions caused kinase-on shifts. In contrast, control cable deletions caused kinase-on output, whereas insertions at the TM2-control cable junction caused kinase-off output. These findings rule out direct mechanical transmission of TM2 conformational changes to HAMP. Instead, we suggest that the Tsr control cable transmits input signals to HAMP by modulating the intensity of structural clashes between out-of-register TM2 and AS1 helices. Inward displacement of TM2 might alter the sidechain environment of control cable residues at the membrane core-headgroup interface, causing a break in the control cable helix to attenuate the register mismatch and enhance HAMP packing stability, leading to a kinase-off output response. This helix-clutch model offers a new perspective on the mechanism of transmembrane signaling in chemoreceptors.

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Introduction

All organisms use transmembrane signal transduction to monitor and respond to environmental stimuli. In motile bacteria and archaea, chemoreceptors known as methyl-accepting chemotaxis proteins (MCPs) convert information about external attractant and repellent levels into signals that control locomotor behavior. The extensively studied MCPs of *Escherichia coli* serve as important models for understanding transmembrane signal transduction (for recent reviews, see Refs [1–4]).

E. coli has four MCP species (Tsr, Tar, Tap, Trg) that detect various small-molecule ligands. They have similar functional architectures: mainly α-helical protomers of ~550 residues organized as homodimers with a ligand-sensing periplasmic domain connected via a transmembrane helix in each subunit to a cytoplasmic signal-processing domain (Fig. 1a). The cytoplasmic portion of MCP molecules contains a membrane-proximal HAMP domain, a sensory adaptation domain containing sites for reversible covalent modifications, and a hairpin tip that regulates the activity of a histidine autokinase, CheA. CheA phosphoryl groups are, in turn, donated to the CheY response regulator, whose phosphorylated form binds to the base of flagellar motors to initiate random, directional changes in cell swimming trajectory. Whenever the cell happens to head up an attractant gradient, the increasing ligand concentration causes the receptor to down-regulate CheA activity, extending upgradient swimming runs.

Sensory adaptation plays a critical role in the MCP-mediated chemotactic behavior of *E. coli.* The

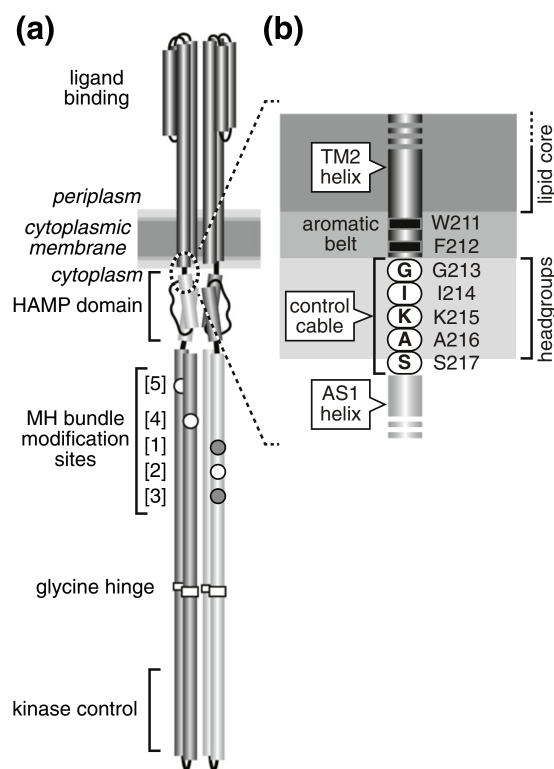

Fig. 1. Tsr structural features and transmembrane signaling elements (a) The Tsr homodimer [1]. Cylindrical segments represent α-helices, drawn approximately to scale. Each Tsr subunit contains five adaptation sites within a methylation-helix (MH) bundle. Sites 2, 4, and 5 (white circles) are translated as glutamic acid residues, the substrate for CheR methylation. Sites 1 and 3 (gray circles) are translated as glutamine residues that can be deamidated to glutamic acid residues by CheB, making them competent for subsequent methylation. This study focuses on the Tsr region at the membrane-cytoplasm interface (dashed circle), enlarged in (b). (b) The Tsr control cable. Detail of the dashed region in (a). The aromatic residues at the C-terminus of the TM2 transmembrane helix and the following control cable residues are shown at their probable positions relative to the hydrophobic (lipid core) and polar (headgroup) membrane regions [14].

sensory adaptation enzymes CheR (MCP methyltransferase) and CheB (MCP methylesterase and deamidase) modify specific residues in the four-helix methylation bundle of the receptor molecule. The overall methylation state of the receptor matches the level of its chemoeffector in the environment. Thus, the MCP modification state serves as a memory store for detecting temporal changes in chemoeffector levels as the cell swims through spatial gradients. Ligand occupancy changes update the modification record by adjusting the CheR/CheB substrate properties of MCP molecules. Attractant ligands shift receptor molecules to a kinase-off state that serves as a substrate for CheR-mediated reactions; reduced ligand occupancy shifts receptors to a kinase-on state that serves as a substrate for CheB-mediated reactions.

The mechanism of transmembrane signaling by *E. coli* MCPs has been explored most extensively with the Tar (aspartate-sensing), Tsr (serine-sensing), and Trg (ribose/galactose-sensing) receptors, and the current mechanistic picture is a montage derived from all three (for reviews, see Refs [5–8]). Hereafter, residue names and coordinates for Tsr, the subject of the studies in this report, will be used to describe the structure–function features important for transmembrane signaling.

In Tsr homodimers, two membrane-spanning segments flank the serine-binding portion of each subunit: an N-terminal TM1 helix and a TM2 helix that connects to the AS1 helix of the HAMP domain (Fig. 1b). TM2 comprises 19 mostly hydrophobic residues (A192-V210) that embed in the membrane lipid core [9,10] and two aromatic residues (W211, F212) at its cytoplasmic end that partition at the core-headgroup interface [11–13]. A five-residue control cable joins the TM2 and HAMP AS1 helices and mediates their signaling transactions [14–16].

Studies of the Tsr control cable led us to a working model of transmembrane signaling in which the control cable mediates structural interactions between the mismatched registers of the TM2 and AS1 helices [14,15]. For example, a proline replacement at any position in the Tsr control cable, except its N-terminal G213, abolishes stimulus responses, suggesting that control cable helicity is important to the signal transmission mechanism [15]. Moreover, charged amino acid replacements at the I214 position interfere with signal transmission, implying that the interaction of less polar sidechains at that position with the membrane interfacial environment might assist stimulus-induced shifts in receptor signal state [14]. We reasoned that a control cable with high helix potential might enable TM2 to destabilize the packing of the HAMP bundle, whereas a control cable with reduced helicity or a distinct helix break might enhance HAMP packing [14]. Various proposed mechanisms of HAMP action, including the gearbox [17], scissors [18,19], and dynamic-bundle [20] models, all predict that altered packing arrangements of the HAMP bundle produce changes in CheA output activity.

Considerable evidence supports the view that piston motions of the TM2 helix normal to the plane of the membrane initiate transmembrane signal transmission in MCPs [8,21–25]. Attractant stimuli, for example, promote inward piston displacements of 1–2 Å in one subunit of the receptor dimer [21,26–28]. The resultant structural asymmetry that impinges on the control cable might be transmitted directly to the AS1 helix of HAMP, as proposed by the gearbox and scissors models, or it could somehow modulate control cable helicity to influence HAMP packing, as specified in the dynamic-bundle

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