Signaling Consequences of Structural Lesions that Alter the Stability of Chemoreceptor Trimers of Dimers

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Abstract

Residues E402 and R404 of the *Escherichia coli* serine chemoreceptor, Tsr, appear to form a salt bridge that spans the interfaces between neighboring dimers in the Tsr trimer of dimers, a key structural component of receptor core signaling complexes. To assess their functional roles, we constructed full sets of single amino acid replacement mutants at E402 and R404 and characterized their signaling behaviors with a suite of *in vivo* assays. Our results indicate that the E402 and R404 residues of Tsr play their most critical signaling roles at their inner locations near the trimer axis where they likely participate in stabilizing the trimer-of-dimer packing and the kinase-ON state of core signaling complexes. Mutant receptors with a variety of side-chain replacements still accessed both the ON and OFF signaling states, suggesting that core signaling complexes produce kinase activity over a range of receptor conformations and dynamic motions. Similarly, the kinase-OFF state may not be a discrete conformation but rather a range of structures outside the range of those suitable for kinase activation. Consistent with this idea, some structural lesions at both E402 and R404 produced signaling behaviors that are not compatible with discrete two-state models of core complex signaling states. Those lesions might stabilize intermediate receptor conformations along the OFF–ON energy landscape. Amino acid replacements produced different constellations of signaling defects at each residue, indicating that they play distinct structure–function roles. R404, but not E402, was critical for high signal cooperativity in the receptor array.

Introduction

Bacteria sense and respond to their environment via transmembrane receptors. Sensitive detection and adaptive response to environmental stimuli are critical to bacterial survival and proliferation. Many motile bacteria use chemoreceptors known as methyl-accepting chemotaxis proteins (MCPs) to modulate their locomotor responses to environmental chemicals (see Refs. [1,2] for reviews). *Escherichia coli's* MCPs have been extensively studied and are good experimental models for elucidating molecular mechanisms of transmembrane and intracellular signaling.

E. coli has four canonical MCPs: Tar, Tap, Trg, and Tsr, which have similar functional architecture and signal transducing mechanisms [1,2]. These receptor proteins detect various small molecules through a periplasmic sensing domain; their cytoplasmic domains modulate the activity of an associated histidine autokinase CheA, which is coupled to receptor control by a third protein, CheW (Fig. 1a). Following autophosphorylation, CheA donates the phosphoryl group to the response regulator CheY. Phosphorylated CheY molecules bind at the bases of flagellar motors, raising their probability of clockwise rotation [3,4]. Counter-clockwise motor rotation produces forward swimming movements, whereas clockwise reversals cause tumbling episodes that randomly reorient the cell's swimming direction. Cellular phosphorylated CheY is short-lived, owing to a specific phosphatase, CheZ [5,6].

Chemoreceptor/CheW/CheA signaling complexes generally conform to two-state behavior, having an "on" state with high kinase activity (ON) and an "off"

state with very low kinase activity (OFF). Chemical stimuli elicit chemotactic responses by shifting MCP signaling complexes toward the ON or OFF state. Subsequent covalent modifications to the receptor signaling domain, catalyzed by a glutamyl methyltransferase (CheR) and a deamidase, and methylesterase (CheB), reverse the shift in ON/OFF equilibrium to terminate stimulus responses. This sensory adaptation system enables the swimming cell to detect and respond to temporal changes in chemoeffector levels over a wide concentration range.

Native MCP molecules are homodimers; protomers of Tsr, the serine chemoreceptor and subject of the current study, are 551 residues in length. Tsr subunits contain five sites for adaptational modifications, initially translated as glutamyl (E304, E392, E502) and glutaminyl (Q297, Q311) residues (Fig. 1a). CheB converts the Q sites to E sites through irreversible deamidation reactions; CheR converts E sites to glutamyl methyl-esters (Em), which can be reversibly hydrolyzed back to E sites by CheB. Q and Em sites shift receptor signaling complexes toward the ON state; E sites shift output toward the OFF state. Receptor modification state is under negative feedback control: signaling complexes in the ON state are substrates for CheB action; complexes in the OFF state are substrates for CheR action [2,7,8]. Stimulus-induced shifts in the ON–OFF equilibrium trigger changes in the relative activities of CheB and CheR that drive the system back to its pre-stimulus set point.

E. coli chemoreceptors operate in networked arrays that have highly cooperative signaling properties. The basic unit of receptor function, the core complex, contains six MCP molecules, organized as two trimers of dimers, two CheW molecules, and one CheA homodimer [9]. The cytoplasmic domains of receptor molecules comprise antiparallel four-helix bundles with contact sites at their tips for trimer and core complex assembly (Fig. 1a). Receptor trimer-of-dimers and CheA/CheW binding interactions are critical for array formation [10] and kinase control [11–13]. Although the architecture of receptor arrays has been elucidated at the molecular level [14–16], the mechanism(s) of CheA control are still unclear, largely due to the many protein–protein interaction surfaces within the signaling arrays, which confound the analysis of any single interaction. For example, residues at the receptor hairpin tip could play multiple roles by promoting interactions with several different partner proteins. Such structure–function complexity presents a difficult challenge to experimental identification of the component interactions and their signaling roles.

Residues E402 and R404 in the hairpin tip of Tsr are highly conserved across the MCP superfamily [17], implying important functional role(s). However, they occupy two different structural environments in the Tsr trimer of dimers (Fig. 1b and c). In each dimer, near the trimer axis, an inner E402 residue packs against an inner R404 residue from a neighboring dimer at the interdimer interface [18] (Fig. 1b and c). At the periphery of the trimer, the outer E402 and R404 residues are solvent exposed, and their side chains lie apart from one another (Fig. 1). The close proximity of two nearly invariant and oppositely charged residues at interdimer interfaces of the trimer suggests that E402 and R404 side chains might participate in an ionic interaction that contributes to trimer structure or stability. But could these residues also play important roles at the trimer periphery, for example, as binding determinants for CheA or CheW?

To investigate whether the E402 and/or R404 side chains are critical for Tsr function, whether they interact structurally and whether that interaction is functionally important, we constructed and characterized a complete set of single amino acid replacement mutants at each residue. Each mutant receptor was subjected to a panel of *in vivo* tests to assess: chemotaxis performance; trimer, core complex, and array formation; kinase activation and stimulus control; and sensory adaptation. These studies revealed that E402 and R404 play functionally critical roles only at the interdimer interface of receptor trimers, not at the trimer periphery. The E402 and R404 side chains may interact, for example, in a salt bridge, to promote trimer stability, but amino acid replacements that disrupt that interaction produce different signaling defects at the two positions, attesting to non-equivalent structural roles. These findings cast new light on the nature of CheA signaling states and their mechanism of control in chemoreceptor core complexes and arrays.

Results

Mutational survey of Tsr residues E402 and R404

We constructed a complete set of amino acid replacement mutants at Tsr residues E402 and R404 in *tsr* expression plasmid pCS53 (see Materials and Methods). This plasmid encodes Tsr molecules with a cysteine replacement at residue S366, a crosslinking reporter site for trimer-of-dimer formation [19]. In receptor-less host strain UU2612, which contains both sensory adaptation enzymes, CheR and CheB (hereafter designated R+B+), pCS53-expressed Tsr-S366C promotes serine chemotaxis (in tryptone soft-agar plates) comparable to that promoted by wild-type Tsr, expressed from plasmid pRR53 [19]. Unless otherwise indicated, the wild-type Tsr phenotypes mentioned below refer to Tsr-S366C. In strain UU2612 (R+B+), some mutant receptors (designated E402* or R404*) supported chemotaxis toward serine in tryptone soft-agar plates, but many of those did so at reduced efficiencies (Fig. 2). Seven E402* mutants (V, L, I, G, P, K, R) and 12 R404* mutants (C, L, M, K, Y, T, N, E, D, W, F, P) could not support any serine