Molecular modeling of flexible arm-mediated interactions between bacterial chemoreceptors and their modification enzyme

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Abstract: Sensory adaptation in bacterial chemotaxis is mediated by methylation and demethylation of specific glutamyl residues in the cytoplasmic domain of chemoreceptors. Methylation is catalyzed by methyltransferase CheR. In *E. coli* and related organisms, methylation sufficiently rapid to be physiologically effective requires a carboxyl terminal pentapeptide sequence on the receptor being modified or, via adaptational assistance, on a neighboring homodimer in a receptor cluster. Pentapeptide-enhanced methylation is thought to be mediated by a ~30 residue, potentially disordered sequence that serves as a flexible arm connecting the receptor body and pentapeptide-bound methyltransferase, thus allowing diffusionally restricted enzyme to reach methyl-accepting sites. However, it was not known how many or which sites on the same or neighboring receptors were accessible to the tethered enzyme. We investigated using molecular modeling and found that, in a hexagonal array of trimers of receptor dimers, CheR tethered to a dimer of chemoreceptor Tar by its native 30-residue flexible-arm sequence could reach all methyl-accepting sites on the dimer to which it was tethered plus 48 methyl-accepting sites distributed among nine neighboring dimers, equivalent to the total sites carried by six receptors. This modeling-determined methylation neighborhood of one enzyme-binding dimer and six neighbors corresponds precisely with the experimentally identified neighborhood of seven. Thus, the experimentally observed adaptational assistance can occur by docking of pentapeptide-bound, diffusionally restricted enzyme to methyl-accepting sites on neighboring receptors. Our analysis introduces the notion that physiologically relevant adaptational assistance could occur even if only a subset of sites on a particular receptor are within reach.

Keywords: bacterial chemotaxis; disordered protein regions; receptor methyltransferase; molecular modeling

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Introduction

Sensory adaptation is central to bacterial chemotaxis. It creates a wide dynamic range of sensitivity to small changes in concentrations of stimulating chemicals and provides the molecular memory that mediates gradient sensing.¹ The molecular basis of this adaptation

Figure 1. Chemoreceptor organization and the carboxyl-terminal tail. (A) Ribbon diagram of a chemoreceptor homodimer² with a tethered CheR. The two receptor subunits are shown in green and red, the methyl-accepting sites as dots numbered 1–4. The carboxyl-terminal pentapeptide is shown in purple. The structure of CheR (yellow) bound to the pentapeptide (PDB ID: 1AF7) is tethered to the receptor body by the flexible arm. An arrow points to the CheR active site. (B) Space-filling representation of trimer of chemoreceptor dimers and CheR. Color scheme and arrow pointing to the CheR active site are as in (A). A double arrow represents the equilibrium binding of CheR to the pentapeptide. (C). Alignment of C-terminal sequences of pentapeptide-bearing receptors from *E. coli* and *S. enterica*. The species of origin is indicated by a subscript “E” or “S.” Residue numbers are for Tar, positions with identical residues in the five sequences are enclosed with black boxes, and the boundary of the structurally defined coiled-coil receptor body³ is marked by a vertical line.

is reversible covalent modification of chemoreceptors: methylation and demethylation of specific glutamyl residues, catalyzed by methyltransferase CheR and methylesterase/deamidase CheB. In the well-studied chemosensory systems of *Escherichia coli* and its close relative, *Salmonella enterica* serovar Typhimurium, physiologically effective action of these modification enzymes requires an activity-enhancing pentapeptide, asparagine-tryptophan-glutamate-threonine or serine-phenylalanine (in the single-letter code NWETF or NWESF), located at the receptor carboxyl terminus (see Fig. 1).^{4–7} For efficient adaptational modification and chemotaxis, this receptor-borne pentapeptide must be on the receptor being modified or, in the process of adaptational assistance, on a neighboring receptor.^{4,6,8–17}

Enhancement of methylation by the receptor-borne pentapeptide appears to result from the restricted diffusion of pentapeptide-bound CheR to the region near its substrate modification sites, thus increasing the effective enzyme concentration several orders of magnitude.¹⁸ For diffusional restriction to result in enhanced methylation, the tethered enzyme must be able to reach the relevant sites, a situation thought possible because a ~30 residue, potentially disordered sequence between the body of the receptor dimer and the pentapeptide can serve as a flexible arm, allowing tethered CheR to reach methyl-accepting sites.^{12,13,17,19} Thus, the extent to which pentapeptide-bound enzyme can reach methyl-accepting sites on its own and neighboring receptors is central to an understanding of the general process of adaptational

modification and the specific process of adaptational assistance. In this study, we investigated this issue using molecular modeling. As background, we review information about the two proteins and their interactions.

The basic unit of chemoreceptor organization is the homodimer,²⁰ an elongated structure [Fig. 1(A)] made up in large part of extended helical coiled coils.^{3,21} Methyl-accepting glutamates are positioned near the middle of the coiled coil cytoplasmic portion of the receptor, facing the solvent. Chemoreceptor Tar has four such residues per subunit, three at seven-residue intervals on the helix extending away from the membrane and one on the antiparallel companion helix of the helical hairpin [Fig. 1(A)].

Methyltransferase CheR^{5,22} consists of an amino-terminal, substrate-interaction domain, a larger carboxyl-terminal catalytic domain and a pentapeptide-binding β -subdomain (see Fig. 1). The β -subdomain is found exclusively and uniformly in chemotaxis methyltransferases.²² CheR binds to the activity-enhancing pentapeptide by adding it as a fourth β -strand to the three-stranded sheet of the β -subdomain²² [Fig. 1(A)].

In the pentapeptide-bearing chemoreceptors of *E. coli* and its close relatives, the five-residue sequence is separated from the conserved, coiled-coil body of the cytoplasmic domain³ by 30–35 residues that are notably variable among otherwise closely related receptor sequences and often include several prolines [Fig. 1(C)]. These features suggest this connecting sequence is relatively unstructured, that is disordered. Thus, it might serve as a flexible arm that could allow a diffusionally restricted modification enzyme, bound to the carboxyl-terminal pentapeptide at the end of the arm, to reach modification sites.^{12,13,17–19} Such dual interaction would not necessarily have to be with the same polypeptide chain. For instance, enzyme bound to the pentapeptide of one subunit of an intertwined homodimer might bind a modification site on the other monomer. Furthermore, receptor dimers join at the membrane distal tips of their cytoplasmic domains to form trimers of dimers^{3,23,24} [Fig. 1(B)]. *In vivo*, thousands of receptors cluster in patches that are likely grouping of dimers and trimers of dimers.^{25–29} In clusters, CheR might bind the pentapeptide of one dimer and dock on a methyl-accepting site of a different dimer in the same or a different trimer. Interdimer binding is thought to be the origin of adaptational assistance, in which a pentapeptide-bearing receptor enables efficient modification of neighboring receptors lacking the pentapeptide^{12,13,17} and thus mediates physiologically effective adaptation and chemotaxis.^{8–11}

To what extent can diffusionally restricted CheR, tethered by binding to a carboxyl-terminal pentapeptide, reach substrate sites on the same or on neighboring receptor polypeptides? We have used molecular modeling methods to address this issue, taking into consideration the constraints of the actual amino acid

sequence of the putative flexible arm, the specifics of the structure of methyltransferase CheR and possible arrangements of neighboring chemoreceptors.

Results

A computational approach to accessibility of methyl-accepting sites by tethered CheR

We used computational modeling to investigate the ability of methyltransferase CheR bound to the carboxyl terminal pentapeptide and thus tethered to a chemoreceptor by the flexible arm to reach target methyl-accepting sites on the same or neighboring receptors. Our approach was to dock the enzyme with a bound pentapeptide at a methyl-accepting site and determine whether a structurally allowed extension of the 30-residue flexible arm of chemoreceptor Tar could span the distance between its origin on a receptor body and its distal end at the amino terminus of the pentapeptide bound to the docked enzyme. For each combination of arm origin and methyl-accepting site, we (1) measured the through-space distance and compared it with the maximal reach of the flexible-arm sequence stretched as a beta sheet or an extended chain and (2) used, for those distances less than the maximal reach, molecular modeling of the 30-residue sequence of Tar to investigate whether that actual amino acid sequence could span the distance in a structurally allowable conformation.

Docking CheR on methyl-accepting sites

We first constructed models of pentapeptide-bound CheR docked at each of the four Tar methyl-accepting sites, residues 295, 302, 309, and 491, hereafter sites 1–4 (see “Materials and Methods” for details). This was done using a homology model of the Tar homodimer and the known structure of CheR bound to the pentapeptide.²² Docking was performed by two independent approaches: (1) manual docking followed by energy minimization plus molecular dynamics and (2) automated docking with a distance restraint to insure the modified residue was positioned in the CheR active site near the cofactor, followed by energy minimization plus molecular dynamics. The respective best structures obtained from the two approaches were very similar (RMSD <1 Å) and had many features in common with a model of CheR bound to the cytoplasmic fragment of receptor Tsr.³⁰ Correspondence of three independent modeling exercises provided confidence that the models were credible representations of an interaction that may not be amenable to high-resolution structural analysis because of the low affinity of the enzyme for the receptor sites of modification.⁴ In the consensus structure from our two modeling approaches (see Fig. 2), the enzyme docked at each methyl-accepting site exhibited highly complementary contact surfaces with the receptor and was stabilized by favorable charge interactions of conserved basic

Figure 2. Consensus structure of CheR docked at a chemoreceptor methyl-accepting site. Stereo view of CheR (yellow) docked to a chemoreceptor methyl-accepting glutamate (green CPK) at residue 309 (site 3) of Tar (red). Note the alignment of the methyl-accepting glutamate for inline S_N2 methyl donation of the methyl group (black CPK) by *S*-adenosylmethionine (cyan CPK).

residues of CheR $\alpha 2$ helix with glutamyl residues on the surface of the receptor structure. The glutamyl residue to be methylated extended neatly into the CheR active site, positioned perfectly for in-line S_N2 methyl donation by *S*-adenosylmethionine.

Modeling flexible arms

Using loop-building algorithms, we investigated whether the relevant 30-residue sequence of the Tar could provide a structurally realistic connection between

the last structurally defined residue of the coiled-coil body of a Tar dimer and the NWETF pentapeptide bound to CheR docked at a methyl-accepting site. For each combination of tether origin and methyl-accepting site, we first tried the relatively simple and computationally efficient random tweak algorithm.³¹ When this approach failed to connect with a structurally plausible flexible arm segment, we used a more computationally demanding restrained molecular dynamics method.³² Physically plausible connections, that is, those with allowed phi and psi angles and devoid of steric clashes, were identified using the PROCHECK algorithm.³³

Access of CheR to methyl-accepting sites on the receptor dimer to which it is tethered

The Tar homodimer has eight methyl-accepting sites, four on each subunit. We found that the through-space distances from the tether origin on the body of either homodimer subunit to the beginning of the pentapeptide bound to CheR docked at each of the eight sites on a receptor dimer were substantially less than the maximal reach of the tether sequence [Fig 3(A), Table I, top two rows]. Note that the 16 combinations (two tether origins and eight sites) constitute only eight unique combinations for the symmetrical homodimer. All 16 combinations are shown in Table I, but only the eight unique combinations are evident in Figure 3(A). Structurally reasonable three-dimensional arrangements of the flexible arm could connect each tether origin with all eight enzyme positions (see Fig. 4 for examples), consistent with the conclusion of

Figure 3. Through-space distances and structurally allowed connections between chemoreceptor flexible arm origins and CheR docked at methyl-accepting glutamates. Data in Tables I and II are displayed diagrammatically. Horizontal lines are axes of through-space distances. Vertical lines mark each measured distance between a flexible arm origin and the pentapeptide bound to CheR at a methyl-accepting site. Vertical lines above a horizontal line represent structurally allowed connections (Arm Reaches = yes). Those below the line represent structurally impossible connections (Arm Reaches = no). Dashed lines indicate maximal extensions of the flexible arm sequence as a β sheet (max β) or an extended chain (max extended).

Table I. Connecting Pentapeptide-Bound CheR Docked at a Methyl-Accepting Site to Flexible-Arm Origins: Analysis of a Dimer and a Trimer of Dimers

Tether origin ^a	Che R docked at ^a							
	Site 1: I-a/I-b		Site 2: I-a/ I-b		Site 3: I-a/ I-b		Site 4: I-a/ I-b	
	Distance (Å)	Arm reaches?	Distance (Å)	Arm reaches?	Distance (Å)	Arm reaches?	Distance (Å)	Arm reaches?
I-a	41.2/40.8	Y/Y	48.6/50.2	Y/Y	57.3/61.6	Y/Y	70.7/65.7	Y/Y
I-b	40.8/41.2	Y/Y	50.2/48.6	Y/Y	61.6/57.3	Y/Y	65.7/70.7	Y/Y
II-a	74.6/80.2	Y/Y	81.8/77.0	Y/Y	93.6/73.0	Y/Y	70.3/103.8	Y/N
II-b	64.6/80.5	Y/Y	72.9/79.0	Y/Y	85.8/76.0	Y/Y	69.1/99.4	Y/N
III-a	57.6/102.1	Y/N	64.4/103.6	Y ^b /N	75.4/102.0	Y ^b /N	84.8/98.2	Y/N
III-b	45.4/87.5	Y/Y ^b	54.2/90.6	Y/Y	66.6/91.2	Y/Y	77.7/87.8	Y/Y ^b

^a Dimers in the trimer of dimers are designated I–III, numbering counterclockwise from dimer I as viewed from the periplasm. Dimer subunits are designated “a” and “b.” Subunit “a” has its methyl-accepting sites and tether origins facing the exterior of the trimer. See Figure 5 for a diagram of the labeling.

^b Random tweak did not generate a structurally plausible connection but molecular dynamics simulation did.

Windisch *et al.*¹⁸ The greatest extension of the tether was required for CheR docked at the two sites 4, even though these sites are nearest the tether origin [Figs. 1(A) and 4]. This reflects the orientation of sites on the receptor helical hairpin structure. Sites 1–3 are on the helix extending away from the membrane in the amino-to-carboxyl direction and site 4 is on the companion, antiparallel helix. CheR docking to sites 1–3 orients the enzyme’s pentapeptide-binding site toward the membrane and thus toward the tether origin. Site 4 is ~20 Å closer to the tether site than the next closest, site 1, but CheR docking at site 4 orients the pentapeptide-binding site away from the membrane and thus more distant from the tether origin. Furthermore, the tether must curl around the enzyme. This greater distance and restricted access could contribute to the low rate of methylation at site 4 relative to the other three sites.³⁴

Access of tethered CheR to methyl-accepting sites in a trimer of dimers

Adaptational assistance^{12,13,17} implies that CheR tethered to a chemoreceptor dimer carrying the pentapeptide can reach and thus efficiently modify methyl-accepting sites of adjacent dimers lacking the pentapeptide. The structurally well-characterized way to create specifically adjacent receptors is formation of trimers of receptor dimers.^{3,23,24} Thus, our first step in investigating adaptational assistance was to analyze the potential for assistance in a trimer of dimers. We constructed a model of a trimer of intact Tar dimers, based on the structure of the trimer of Tsr dimer fragments³ and used that model to create a family of eight trimer models in which the target dimer had CheR docked, respectively, at each of the eight methyl-accepting sites. Those models were used to investigate whether the pentapeptide bound to CheR docked at each methyl-accepting site of one dimer could be connected by the flexible arm to tether origins on the other two dimers of the trimer. The through-space distances for all 32 combinations of eight methyl-accept-

ing sites and four tether origins were less than the distance spanned by the completely extended conformation of the flexible arm sequence and 26 had a through-space distance less than the distance spanned by the flexible arm with β -sheet angles [Fig. 3(B) and Table I, bottom four rows]. Structurally reasonable three-dimensional arrangements of the flexible arm could connect all of these 26 combinations [see Fig. 5(A) for an example]. However, structurally reasonable connections could not be constructed for the six combinations with longer through-space distances even though those distances were less than the distance covered by the fully extended chain [Fig. 5(B) and Table I, bottom four rows]. Among these six combinations, four were between the exterior-facing tether origin on the dimer clockwise from the target dimer (viewed from the membrane) and the four sites that faced the interior of the trimer. Connecting this origin and the four positions of docked CheR required the flexible arm to bend around the dimer to which it was attached and thread between dimers in a way that did not allow it to reach those interior-facing sites. The two other structurally prohibited connections were from the two tether origins of the dimer counterclockwise from the target dimer to the interior-facing site 4, a path too long for the tether to thread through the center of the trimer or stretch far around the periphery of the trimer to contact with an inverted CheR.

Access of tethered CheR to methyl-accepting sites in nearby trimers of dimers

Quantification of adaptational assistance *in vitro* implied that CheR tethered to one dimer can assist in methylation of sites on six neighboring dimers,¹⁷ requiring the tethered enzyme to reach beyond its own trimer of dimers. Thus, we modeled connections between CheR docked on one dimer and tether origins on dimers in nearby trimers. This required creating a model of a neighborhood of receptor trimers of dimers. However, experimental data about neighboring trimers was not sufficiently detailed to define a specific

Figure 4. Examples of flexible arm connections. CheR (yellow) docked at a methyl-accepting site (green) of the Tar homodimer (red) and bound to the pentapeptide (purple) is connected by a flexible arm (cyan) to the body of the same receptor dimer. For clarity, only the cytoplasmic domain of Tar is shown. A. Docking at site 3 (residue 309). B. Docking at site 4 (residue 491).

three-dimensional model. Instead, we constructed a rudimentary model in which each dimer would have the maximal number of neighboring dimers, that is, a hexagonal arrangement, an organization for which evidence has recently been published.^{28,29} Using the atomic resolution structure that provides angles and positioning among the dimers in a receptor trimer,³ we arranged six trimers in a hexagon with one dimer from each trimer pointing to the center of the hexagon [Figs. 6 and 7(A)]. With this symmetry, a hexagonal pattern could be extended outward by adding additional hexagonal groupings of trimers extending from the initial hexagon.

Using this model, we investigated which tether origins on neighboring dimers in other trimers could be connected to CheR on the eight methyl-accepting sites of a target dimer. We measured the through-space distance between CheR docked on the target dimer and tether origins on dimers in neighboring trimers. Distances that were less than the maximal reach of an extended chain occurred for origins on

seven dimers in neighboring trimers. All these were members of a single hexagon of trimers of dimers (see Fig. 6). Of the 112 possible combinations (seven dimers $\times$ two tether origins/dimer $\times$ eight sites for CheR), 80 were less than the distance spanned by the completely extended conformation of the flexible arm sequence and 62 had a through-space distance less than the distance spanned by the flexible arm with exclusively β -sheet angles [Fig. 3(C) and Table II]. Structurally allowed three-dimensional arrangements of the flexible arm could connect 53 combinations that

Figure 5. Adaptational assistance in a trimer of chemoreceptor dimers. CheR (yellow) docked at a methyl-accepting glutamate (green) of the Tar homodimer (red) and bound to the pentapeptide (purple) is connected by a flexible arm (cyan) to the body of an adjacent dimer in a trimer of dimers. The complex is viewed from the periplasmic end of the receptors toward their membrane-distal tip where they interact to create the trimer. A. A structurally allowed connection to site 3. B. A structurally prohibited connection to site 4 (steric clash between the flexible arm and CheR).

Figure 6. Schematic of the modeled hexagonal array of trimers of chemoreceptor dimers. Trimers (A–F) of chemoreceptor dimers (I–III in each trimer) are arranged in a hexagon (see text) with each dimer I toward the center of the hexagon. Subunit “a” faces the outside of the trimer and subunit “b” faces the inside. Structurally allowed connections between a flexible arm origin on dimer A-I and CheR docked at a methyl-accepting site on a neighboring dimer are indicated by connecting lines, with the line thickness proportional to the number of sites to which connections were allowed and the line labeled with that number.

had through-space distances less than the β -sheet extension and three additional combinations that had through-space distances less than the fully extended chain, for a total of 56 structurally allowed connections [Fig. 3(C) and Table II] between CheR docked on the target dimer and tether origins on dimers in neighboring trimers [see Figs. 7(B),(C) for examples of a structurally allowed and disallowed connection].

Modeled adaptational assistance

The concept of adaptational assistance is that CheR tethered to a single pentapeptide-bearing receptor can dock on and thus modify methyl-accepting sites on neighboring dimers. We used the allowed connections we identified between CheR docked on a selected dimer and tether origins on neighboring dimers in the same or neighboring trimers to identify the methyl-accepting sites accessible to CheR tethered to one assisting receptor dimer. Because chemoreceptors are homodimers, assistance would occur if an allowed connection could be made from only one tether origin of the assisting dimer. Thus, we identified the allowed connections between at least one of the two tether origins on an assisting homodimer and candidate methyl-accepting sites on neighboring dimers. The results are shown diagrammatically in Figure 6. CheR tethered to

one or the other site on a pentapeptide-bearing receptor dimer (A-I) would have sterically allowed access to (1) all eight methyl-accepting sites on the assisting dimer, (2) eight plus seven sites on the two other

Figure 7. Adaptational assistance in an array of trimers of chemoreceptor dimers. A. Ribbon diagram of a hexagonal arrangement of six trimers of dimers created as described in the text. For clarity only cytoplasmic domains are shown. Each trimer is in a different color and dimers facing the center of the hexagon are labeled as in Figure 6. The view is at an angle approximately halfway between those of Figs. 4 and 5. Pentapeptide (purple)-bound CheR (yellow) is docked on methyl-accepting site A-I-a (see Fig. 6 for explanation of labeling) and connected by the flexible arm (cyan) to tether origin D-I-a on a dimer in a distant trimer. B and C. Views from the center of the hexagon and parallel to the membrane showing only the dimer on which CheR is docked plus the tether-origin dimer. Color coding as in panel A. B. An allowed connection: CheR at site 3 of A-I-a and arm origin at B-I-b. C. A steric clash connection: CheR at site 3 of A-I-b and arm origin at F-I-b.

Table II. Connecting Pentapeptide-Bound CheR Docked at a Methyl-Accepting Site to Flexible-Arm Origins in an Array of Trimers of Dimers

Tether origin ^a	Che R docked at ^a							
	Site 1: A-I-a/ I-b		Site 2: A-I-a/ I-b		Site 3: A-I-a/ I-b		Site 4: A-I-a/ I-b	
	Distance (Å)	Arm reaches?	Distance (Å)	Arm reaches?	Distance (Å)	Arm reaches?	Distance (Å)	Arm reaches?
B-I-a	73.0/40.7	Y/Y	77.6/53.1	Y/Y	82.9/67.4	Y/Y	85.5/76.8	N ^b /Y
B-I-b	87.0/50.5	Y/Y	91.7/60.2	N ^b /Y	97.4/73.1	N ^b /Y	93.7/90.3	N ^b /N ^b
B-III-a	94.0/77.1	Y/Y	100.0/73.2	N/Y	109.5/72.1	N/Y	83.0/110.6	Y/N
B-III-b	84.6/64.4	Y/Y	91.1/63.8	Y/Y	100.7/66.5	N/Y	79.5/101.4	Y/N
C-I-a	102.4/69.2	N/Y	104.8/81.6	N/Y	106.5/96.8	N/Y	116.3/95.1	N/Y
C-I-b	120.8/85.8	N/Y ^c	123.0/97.5	N/N ^b	124.3/112.4	N/N	133.1/111.7	N/N
D-I-a	109.6/89.1	N/Y	110.9/102.3	N/N	110.5/117.1	N/N	131.5/100.1	N/N
D-I-b	125.3/107.7	N/N	126.4/120.7	N/N	125.3/135.4	N/N	149.1/115.8	N/N
E-I-a	91.5/90.4	Y/Y	93.0/103.2	Y/N	92.8/116.3	Y/N	123.0/89.9	N/Y
E-I-b	98.9/105.0	Y/N	100.3/117.3	Y/N	100.1/129.4	Y/N	133.4/100.0	N/N
F-I-a	56.7/71.0	Y/Y	60.6/82.6	Y/Y	64.7/93.2	Y/Y	94.4/70.2	N ^b /Y
F-I-b	52.6/78.8	Y/Y	57.8/88.8	Y/Y ^c	63.7/97.2	Y/N ^b	93.9/75.4	N ^b /Y
F-II-a	72.1/118.5	Y/N	76.2/123.5	Y/N	83.1/124.5	Y/N	107.1/106.0	N/N
F-II-b	78.0/121.7	Y/N	81.7/127.4	Y/N	87.3/130.5	Y/N	116.0/107.5	N/N

^a In a modeled hexagonal array of six trimers of dimers (see text), the trimer that carried CheR docked on a methyl-accepting site of one of its dimers was designated “A” and the other five trimers “B” through “F,” lettering clockwise from trimer A as viewed from the periplasm. Dimers in each trimer of dimers were numbered I–III, counterclockwise from dimer I as viewed from the periplasm, with dimer I facing the interior of the six-trimer array. Dimer subunits are designated “a” and “b.” Subunit “a” has its methyl-accepting sites and tether origin facing the exterior of the trimer. See Figure 5 for a diagram of the labeling. Analysis of connections within trimer A are in Table I.

^b Even though the through-space distance was less than the limit of the flexible arm, neither loop modeling approach could generate a structurally plausible connection.

^c Random tweak did not generate a structurally plausible connection but molecular dynamics simulation did.

dimers in its trimer (A-II and A-III, respectively), (3) seven plus three sites on two dimers of one of the nearest trimers in the hexagonal array (B-I and B-III) and seven plus six sites on two dimers of the other nearest trimer (F-I and F-II), (4) five and four sites, respectively, on the two inward-facing dimers one trimer displaced around the trimer hexagon (C-I and E-I), and (5) one site on the most distant inward-facing dimer (D-I). Thus, modeled adaptational assistance occurred for 48 methyl-accepting sites on neighboring dimers in the same or different trimers, a number equivalent to the total number of sites on six receptor dimers.

Discussion

We used molecular modeling to investigate the ability of the potentially disordered 30-residue sequence of chemoreceptor Tar to allow pentapeptide-bound, diffusionally restricted methyltransferase CheR to reach methyl-accepting sites on its own or neighboring receptors.

Accessibility of tethered CheR to methyl-accepting sites on the dimer to which it is tethered

The simplest case was the chemoreceptor dimer. For the eight methyl-accepting sites of the Tar dimer, the through-space distance between the two possible origins of the flexible arm sequence and its termination at the pentapeptide bound to a docked CheR ranged from 41 to 71 Å. These values were substantially less than the 98 Å reach of the Tar flexible arm oriented as a β-

strand ($\phi = -120^\circ$, $\psi = 113^\circ$) or the 108 Å reach in an extended chain conformation ($\phi = 180^\circ$, $\psi = 180^\circ$). However, such straight-line extensions would not place the end of the tether near the target. For pentapeptide-tethered CheR to reach the eight methyl-accepting sites on the dimer, the 30-residue flexible arm had to bend almost 180° from the initial direction of the polypeptide chain (see Fig. 4), traverse the distance between its origin and the pentapeptide and avoid steric clashes with receptor and enzyme, yet maintain allowed phi and psi angles. This meant that the putative flexible arm had to trace a path that was longer than the direct, through-space distance. Even with these requirements, each connection could be made by an arrangement with allowed phi and psi angles.

Accessibility of tethered CheR to methyl-accepting sites on neighboring dimers

Adaptational assistance^{12,13,17} implies that CheR tethered to a pentapeptide-bearing receptor dimer can reach methyl-accepting sites on neighboring dimers. Within a single trimer of receptor dimers, we found that all but 6 of 32 combinations of the eight methyl-accepting sites and four tether origins could be connected by structurally allowed arrangements of the flexible arm. For four of the structurally disallowed combinations, the connection could be made from the other tether origin in the same dimer. Since chemoreceptors are exclusively homodimers,²⁰ then one pentapeptide-bearing receptor dimer in a trimer could be

modeled to assist in methylation of all but one site in two neighboring receptor dimers in its own trimer.

Our modeling of a trimer of Tar dimers with CheR docked at each of the eight possible methyl-accepting sites of a target dimer revealed that the spacing among the dimers, a spacing derived from the crystal structure of the trimer of the fragments of the cytoplasmic domain of Tsr,³ did not provide sufficient space for CheR to dock without steric clash at one of the eight methyl-accepting sites, site 3 facing the interior of the trimer. In addition, accessibility to some other inward-facing sites was limited. This implied that the trimer conformation captured in the crystals is not the fully methylation-competent receptor conformation because the dimers are too close to one another, an implication consistent with observations *in vivo* that dimers in trimers move farther apart upon ligand binding³⁵ and ligand binding favors the methylation-competent state. To get a sense of the magnitude of a separation among receptor dimers necessary to make the eighth methyl-accepting site available to CheR, we chose a conservative, albeit artificial, strategy of shifting each dimer outward along a radius emanating from the axis of trimer symmetry. Shifting dimers in concert in 0.5 Å steps, we found a 3 Å radial translation of the three dimers was necessary to allow CheR to dock reasonably at an inward-facing site 3. This separation of the dimers would disrupt the dimer-dimer contacts that create the trimer, so the resulting structure cannot represent an actual trimer organization. However, distances between dimers in a trimer of dimers are variable^{35,36} and thus not limited to those in the crystal structure of the receptor fragments.

For dimers in adjacent trimers in a receptor array, structurally allowed connections could be made to a methyl-accepting site on the target dimer and flexible arm origins on seven dimers in neighboring trimers for a total of 33 sites, equivalent to one more than the sites carried by four receptor dimers.

Correspondence of modeled and experimentally determined adaptational assistance

How does the extent of modeled adaptational assistance compare to the extent deduced from *in vitro* experiments? In the experimental studies,¹⁷ the size of an adaptational neighborhood was calculated from the dependence of initial rates of methylation on the ratio between a pentapeptide-bearing (Tar) and a pentapeptide-lacking (Trg) receptor. Fitting the data to a mathematical model for adaptational assistance indicated that one pentapeptide-bearing receptor dimer could mediate a maximal methylation rate, that is, the rate for a receptor bearing its own pentapeptide, for an adaptational neighborhood of approximately seven, that is, the assisting receptor and six assisted receptors. In our molecular modeling, CheR tethered to one assisting receptor could reach 48 methyl-accepting

sites distributed across nine neighboring dimers, 15 on the two other dimers of the trimer containing the assisting dimer and 33 on dimers in neighboring trimers. Forty-eight sites is the equivalent of six assisted receptor dimers, each with eight sites. Thus, the modeled assistance neighborhood was seven, six assisted plus one assisting receptor. This is the same neighborhood size deduced experimentally.¹⁷ The striking correspondence between experimental and modeling results strongly supports the notion that adaptational assistance is mediated by tethered and thus diffusion-restricted CheR docking at methyl-accepting sites on neighboring receptors.

We previously investigated the effects of truncating the 30-residue flexible arm of receptor Tar and found that a truncation of 15 residues resulted in substantial reduction in chemotaxis and a truncation of 20 residues eliminated response, a pattern mirrored by effects of *in vitro* rates of methylation.³⁷ Qualitatively, these observations are consistent with our current analysis of allowed connections. As seen in Figure 3 and Tables I and II, reducing the length of the flexible arm by half would eliminate possible accessibility to most but not all methyl-accepting sites and the greater reduction would eliminate all accessibility.

Additional insights

Experimental measurements did not distinguish whether assistance might occur for some but not all methyl-accepting sites on neighboring receptors. In contrast, modeling studies were able to assess the possibility for assistance at each individual methyl-accepting site. The results revealed that assistance could be provided to a neighboring receptor dimer for some but not necessarily all methyl-accepting sites. Such partial assistance could be sufficient to provide effective adaptational assistance *in vivo* by two lines of reasoning. At the level of individual dimers, *in vivo* the steady state level of methylation in the absence of the receptor ligand is ~1.5 sites per receptor subunit and complete adaptation to a saturating stimulus results in a 1.5–2-fold increase. Thus, fewer than two sites per subunit need be methylated to provide effective adaptational assistance. At the level of signaling teams, it is thought that the important parameter is not modification of a specific ligand-occupied dimer but the aggregate modification of the group of interacting receptors.^{38,39}

In wild-type *E. coli*, adaptational assistance is crucial for the ability of chemoreceptors Trg and Tap, which lack the pentapeptide, to mediate effective chemotaxis.^{7–11,14} However, each is a “low-abundance” receptor, outnumbered among the ~7500 receptor dimers in a cell⁴⁰ ~30:1 by pentapeptide-bearing receptors. The probability of a dimer of Trg or Tap being in a trimer without at least one pentapeptide-bearing partner is sufficiently low that it would seldom occur in a wild-type cell. Thus, adaptation to stimuli

recognized by these low-abundance receptors could be mediated by adaptational assistance within a trimer of dimers. However, the wider issue is the ratio of CheR to receptors. There is only one enzyme for ~60 receptor dimers, every 20 trimer-of-dimers,⁴⁰ indicating most trimers-of-dimers will not have a resident CheR. This implies that adaptational assistance among neighboring pentapeptide-bearing receptors must be an important contributor to physiologically relevant adaptational modification.

Materials and Methods

Docking CheR at the methyl-accepting sites of the E. coli Tar homodimer

We constructed a three-dimensional homology model for residues 1–518 of the homodimer of chemoreceptor Tar from *E. coli* from a model of the homodimer of *S. enterica* Tar² by manually changing the few residues that are different for the two species as well as changing the two glutamines that are deamidated *in vivo* to methyl-accepting glutamates. In this model, the region of the HAMP domain is represented simply as a helical coiled coil, since no high-resolution structure is available for this segment of a chemoreceptor and the details of the arrangement of this region would not impact our analysis, all of which investigates connections between sites on receptors that are membrane-distal to HAMP. The structure of CheR bound to the chemoreceptor carboxyl-terminal pentapeptide NWETF and its inhibitor *S*-adenosyl homocysteine²² (PDB code 1BC5) was altered by superimposing the cofactor *S*-adenosylmethionine (AdoMet) on the inhibitor to create an enzyme-cofactor-pentapeptide complex. This complex was docked manually at the glutamate of methyl-accepting site three of *E. coli* Tar using the interactive molecular graphics program PSSHOW.⁴¹ The CheR-AdoMet-pentapeptide complex was positioned to place the glutamate side chain in close juxtaposition to the cofactor in the CheR active site. Multiple CheR orientations were explored manually and models with obviously bad steric interactions were discarded. Remaining structures were refined with limited conjugate gradient energy minimization and low-temperature molecular dynamics using AMBER 7.0⁴² and AMBER99 all-atom potential function parameters^{43,44} to relieve residual conformational strain and unfavorable van der Waals interactions introduced during manual model building. Docked receptor-CheR complexes were also generated automatically using the protein docking program RosettaDock.^{45,46} In the automated docking calculations, one distance constraint was applied to insure that the receptor glutamate side chain was positioned in the CheR active site near the AdoMet cofactor. The 10 top scoring complexes from the RosettaDock calculations were then refined with 1000 steps of energy minimization and 10–20 ps of low-temperature molecular

dynamics. The best model complexes from both automated and manual docking protocols, as determined by structure assessment tools QPACK⁴⁷ and PRO-CHECK³³ were structurally quite similar, with RMSD < 1.0 Å. Partial atomic charges for the AdoMet molecule were derived from ab initio molecular electrostatic potential calculations using a 6-31G* basis set followed by fitting the electrostatic potential to an atom-centered point charge model. Quantum mechanical calculations were performed with Gaussian98.⁴⁸ The RESP program⁴⁹ was used for charge fitting. The model of CheR docked at site 3 was used to generate models of the pentapeptide-bound enzyme at the other three methyl-accepting sites. Note that sites 1–3 are on a receptor helix that extends away from the membrane in the amino-to-carboxyl direction, whereas site 4 is on the other helix of the helical hairpin, extending away from the membrane with the opposite polarity. For docking CheR at each methyl-accepting site, we maintained same orientation of enzyme relative to the helix carrying the methyl-accepting site and thus maintained the relative positioning and polarity of interacting helices of enzyme and substrate.³⁰ For site 4, this positioning meant that the orientation of CheR was inverted relative to the membrane and its pentapeptide-binding site pointed away from the origin of the flexible arm (see “Results” for the consequences of this orientation).

Models of the trimer of Tar dimers with CheR docked at a methyl-accepting site

A model of a trimer of fragments of the cytoplasmic domains of *E. coli* Tar homodimers was constructed using the X-ray structure of the trimer of dimeric cytoplasmic domain fragments of the highly homologous *E. coli* chemoreceptor Tsr.³ We replaced the dimers of the Tsr trimer structure with our model of the Tar dimer by superimposing the backbone atoms of residues 298–515 of the Tar dimer on the corresponding residues of Tsr that make up the membrane-distal helical hairpin.⁵⁰ This superposition defined the angles among the three Tar dimers. Two dimers in the Tar trimer were completed by superimposing the backbone atoms of the model of the complete Tar homodimer. The third dimer in the Tar trimer was completed by superimposing the backbone atoms of the model of homodimer onto which was docked the CheR-AdoMet-pentapeptide complex at each of the individual eight methyl-accepting glutamates on the two subunits of the receptor dimer. To investigate steric overlap of CheR docked on inward-facing methyl-accepting site 3 with neighboring receptor homodimers, we modified the trimer model by shifting in concert the three dimers outward in 0.5 Å steps along a radius emanating from the axis of trimer symmetry normal to the plane of the membrane. At each step, we tested whether CheR could be accommodated using the

criterion that the nearest distance between backbone atoms of adjacent molecules was $>6 \text{ \AA}$.⁵¹

Models of a cluster of receptor trimers

Models for clusters of trimer assemblies were generated using a rigid-body transformation function in the NAB package.⁵² A receptor trimer assembly was rotated in 60° increments about residue position 143 in homodimer 1 and an axis perpendicular to the membrane plane. The six rotated trimer complexes were each then translated laterally from the origin by $19.5 \text{ \AA}$ along vectors radiating from the origin at 0° , 60° , 120° , 180° , 240° , and 300° , to yield a hexagonal cluster of six chemoreceptor trimers placed as close as possible without steric clash. This arrangement shares features with the hexagonal arrangement of chemoreceptor trimers from *Caulobacter crescentus* identified by cryoelectron tomography.^{28,29} Center-to-center distances between cytoplasmic, membrane-distal tips of adjacent trimers in the hexagon and between tips of the trimers on opposite sides of the hexagon were 8.5 nm and 17 nm , respectively, for our hexagonal arrangement of *E. coli* chemoreceptor trimers and 7.5^{28} or 6.9^{29} nm and 14.5^{28} or 14.1^{29} nm , respectively for two independent models of the *C. crescentus* hexagonal arrangement. In our model for *E. coli*, the angle between dimers in the trimer was that defined by the X-ray crystallographic structure of the trimer of cytoplasmic domains of an *E. coli* receptor and one dimer of each of the six trimers pointed toward the center of the hexagon. For the models of the *C. crescentus* hexagonal array, the three receptor dimers that were fit into densities at hexagonal vertices were essentially parallel, thus the trimer tip to trimer tip distances were somewhat less than those in our model. To obtain the six-trimer cluster with a CheR molecule bound to a single chemoreceptor, a trimer of dimers-CheR complex described in the previous section was substituted for one of the trimers in the cluster.

Modeling the disordered flexible arm

The flexible arm sequence was added to each combination of receptor and bound CheR so that it connected its origin at the last structurally defined residue of a subunit of the coiled-coil body of a Tar dimer (residue 518) and the amino-terminal end of the NWETF pentapeptide (residue 549) bound to CheR docked at a methyl-accepting site. We generated flexible arm connections using a computationally efficient algorithm³¹ and, as necessary, a more computationally demanding method.³² The random tweak algorithm³¹ as implemented in Sybyl (Tripos, Inc. version 6.8) is computationally efficient; it can construct loops connecting two predefined anchor positions. When this algorithm failed to generate a loop segment with allowable backbone torsional angles and acceptable steric positioning relative to CheR and the receptor body, a low-temperature constrained molecular dynamics technique³² was

implemented using AMBER 8.0.⁴² Steric interactions and backbone phi and psi angles were examined visually and by using PROCHECK.³³ Resulting models were used without further adjustment or refinement.

Distance measurements

Through-space distances between the origin of the flexible arm (the carboxyl terminus of residue 518) and the amino-terminal end of the pentapeptide (the amino terminus of residue 549) bound to CheR docked at methyl-accepting sites were calculated using the coordinates for the respective models and scripts developed for this study.

The maximum length of the Tar flexible arm (residues 519–548) in different secondary structure conformations was determined by constructing the 30-amino acid sequence in MOE (Molecular Computing Group Inc. version 2004.03) and measuring the length of the polypeptide in the extended ($\phi = 180^\circ$, $\psi = 180^\circ$), strand ($\phi = -120^\circ$, $\psi = 113^\circ$), and helix ($\phi = -65^\circ$, $\psi = -39^\circ$) conformation.

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