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Quantitative approaches to utilizing mutational analysis and disulfide crosslinking for modeling a transmembrane domain

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Abstract

The transmembrane domain of chemoreceptor Trg from *Escherichia coli* contains four transmembrane segments in its native homodimer, two from each subunit. We had previously used mutational analysis and sulfhydryl crosslinking between introduced cysteines to obtain data relevant to the three-dimensional organization of this domain. In the current study we used Fourier analysis to assess these data quantitatively for periodicity along the sequences of the segments. The analyses provided a strong indication of α-helical periodicity in the first transmembrane segment and a substantial indication of that periodicity for the second segment. On this basis, we considered both segments as idealized α-helices and proceeded to model the transmembrane domain as a unit of four helices. For this modeling, we calculated helical crosslinking moments, parameters analogous to helical hydrophobic moments, as a quantitative way of condensing and utilizing a large body of crosslinking data. Crosslinking moments were used to define the relative separation and orientation of helical pairs, thus creating a quantitatively derived model for the transmembrane domain of Trg. Utilization of Fourier transforms to provide a quantitative indication of periodicity in data from analyses of transmembrane segments, in combination with helical crosslinking moments to position helical pairs should be useful in modeling other transmembrane domains.

Keywords: bacterial chemoreceptors; Fourier analysis; helical bundle; helical moments; scanning mutagenesis

Determination of three-dimensional structures of transmembrane proteins by direct physical methods has proven to be notably difficult. The difficulties have encouraged the use of mutational analysis as one means of deducing structural features of transmembrane segments and domains. A particularly useful form of mutational analysis of transmembrane segments involves the introduction of cysteine by directed mutagenesis because the side chain that is introduced can be characterized by sulfhydryl chemistry (Altenbach et al., 1990; Lynch & Koshland, 1991; Akabas et al., 1992; Pakula & Simon, 1992; Sahin-Tóth & Kaback, 1993; Sahin-Tóth et al., 1994). We have used this approach (Lee et al., 1994, 1995), as well as random mutagenesis (Baumgartner, 1992), to characterize the transmembrane domain of the chemoreceptor Trg from *Escherichia coli*. Trg mediates the chemotactic response of *E. coli* to galactose and to ribose by recognition of the two respective periplasmic sugar-binding proteins in their ligand-bound conformation. It is one of a family of related proteins found in a wide array of bacteria (Morgan et al., 1993). Detailed information about the best-studied members of this family of receptors and references to the observations outlined here can be found in reviews by Bourret et al. (1991) and Hazelbauer (1992). The chemoreceptors are homodimers of 60-kDa subunits, each of which has two transmembrane segments (Fig. 1). We constructed a set of *trg* genes, each coding for a protein containing a single cysteine at one of the 54 positions in the two transmembrane segments of the receptor monomer (Lee et al., 1994). For each position, we determined the propensity for oxidative crosslinking between the two cysteines present in the Trg homodimer (Lee et al., 1994), the ability of the cysteine-containing receptor to mediate chemotaxis toward galactose and toward ribose, and the effect of the substitution on transmembrane signaling (Lee et al., 1995). These properties varied with the position of the substituted cysteine along the two transmembrane segments, and, qualitatively, the variation appeared related to the periodicity of an α-helix. In the current study, we describe a quantitative approach for analyzing the significance of the periodicity and a quantitative method for utilizing the data to build an initial model of the three-dimensional orientation of the transmembrane segments.

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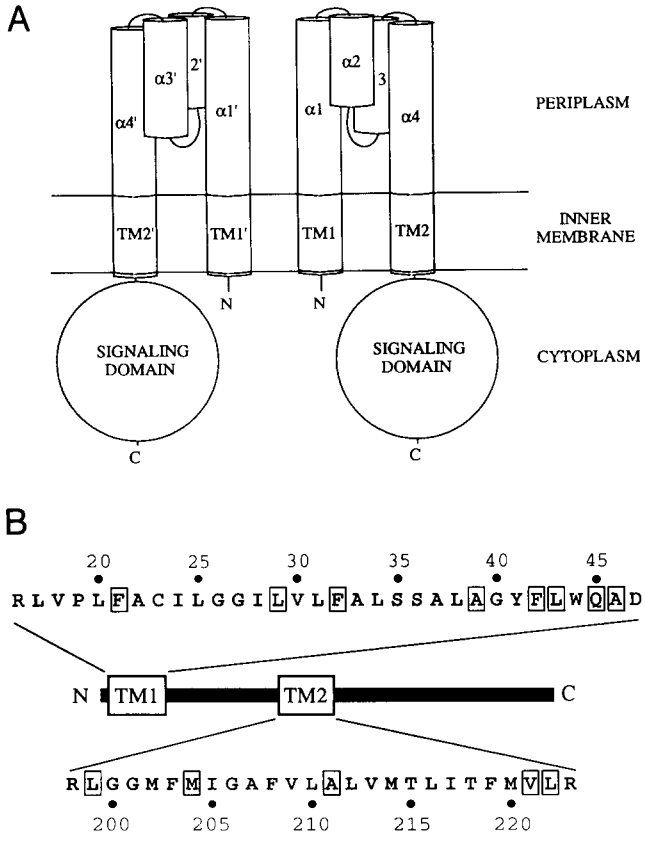

Fig. 1. Diagrams of chemoreceptor organization. **A:** Schematic diagram of the chemoreceptor dimer. One subunit is distinguished from the other subunit of the homodimer by prime symbols. The four-helix bundle of the periplasmic domain, as elucidated by X-ray crystallography (Milburn et al., 1991), is represented as four cylinders ($\alpha 1$-$\alpha 4$) and the two transmembrane segments are labeled TM1 and TM2, respectively. The cytoplasmic, signaling domain, for which no significant structural information is available, is represented as a circle. **B:** Primary structure of Trg. The 537-residue sequence of Trg is represented by the solid line with the positions of TM1 and TM2 indicated. The amino acid sequences of TM1 and TM2 are shown above and below the line, respectively. Positions at which introduced cysteines exhibited substantial (≥20%) propensity for oxidative crosslinking with a cysteine at the homologous position in the other subunit of the dimer (Lee et al., 1994) are indicated by boxes around the residues.

Results

We were interested in quantitative assessments of the apparent periodicities we observed in the data from our genetic and biochemical characterizations of the transmembrane segments of Trg, as well as in a means of comparing and integrating these periodicities. With these purposes in mind, we applied Fourier analysis to our data.

Fourier analysis of periodicity in transmembrane segment 1

The four features of chemoreceptor structure and activity that we had measured were displayed as a function of the position of the cysteine along the 30-residue sequence of transmembrane segment 1 (TM1) and analyzed to produce Fourier power spectra in which the normalized Fourier power, $P(\omega)$ was plotted versus frequency (in degrees) (Fig. 2). The power represented the amount of periodicity inherent in the data at a given frequency, and the frequency was the angle between sequentially adjacent residues. For each feature assayed, a major peak was detected at ≈100°, the characteristic frequency of an α-helix. For the crosslinking data, the 100° peak was slightly lower than the maximum peak at 8° (Fig. 2A). In the data for chemotactic response to ribose and galactose (Fig. 2B,C) and for transmembrane signaling (Fig. 2D), the peak at ≈100° was the maximum. Thus, for the assay that probed structure (crosslinking propensity) as well as for the three assays that assessed function, variation as a function of the position of the introduced cysteine reflected a major contribution by α-helical periodicity. For the transmembrane segments of a bacterial chemoreceptor, appearance of a structural periodicity in the patterns of results from functional assays is plausible because the function of the transmembrane domain of this protein is to undergo a conformational change that conveys an informational signal across the membrane, and this conformational shift is thought to involve relative movements of units of secondary structure (Kim, 1994). On this basis, we reasoned that a composite spectrum (Fig. 2E) would best represent the significant periodicity of this transmembrane segment. The composite has a clear maximum at 101°, reinforcing the significance of the α-helical periodicity.

Fourier analysis of periodicity in transmembrane segment 2

Our analysis of periodicity along TM1 of Trg provided consistent indications of an ≈100° period characteristic of an α-helix. A parallel analysis of our data for TM2 produced a less distinct picture. This was due in part to the unusual effects of cysteine substitutions at two positions in TM2. Among the 54 cysteine-containing receptors, only Trg-L199C exhibited anomalous properties in crosslinking assays (Lee et al., 1994) and only Trg-G200C failed to mediate taxis, specifically toward ribose (Lee et al., 1995). We suspect that, at these positions, unlike the other 52 positions in TM1 and TM2, introduction of a cysteine caused disruption to an extent that the properties of those particular cysteine-containing receptors would not necessarily be relevant to the organization of the wild-type protein. This notion was supported by comparison of power spectra (Fig. 3) for the four assays described above, computed for TM2 using the data for all 24 positions, including the suspect positions 199 and 200 (dotted lines), or computed using the data excluding those two positions (solid lines). The result was striking. With all 24 positions analyzed, only one assay, response to galactose, exhibited a major peak near 100°, but spectra computed without the suspect positions exhibited substantial peaks at this frequency for all four assays. The composite power spectrum of the four assays for the truncated data set had a major peak at 104° (Fig. 3E). These observations were consistent with an α-helical organization of TM2, at least from residues 201 through 222.

However, even with the corrected data set, the composite power spectrum for TM2 (Fig. 3E) did not provide as strong an indication of α-helical periodicity as observed for TM1 (Fig. 2E). This may well reflect a difference for the two transmembrane segments in the sensitivities of the assays we had used. In contrast to cysteines placed in TM1, few cysteines in TM2 exhibited significant crosslinking or caused a significant alteration in

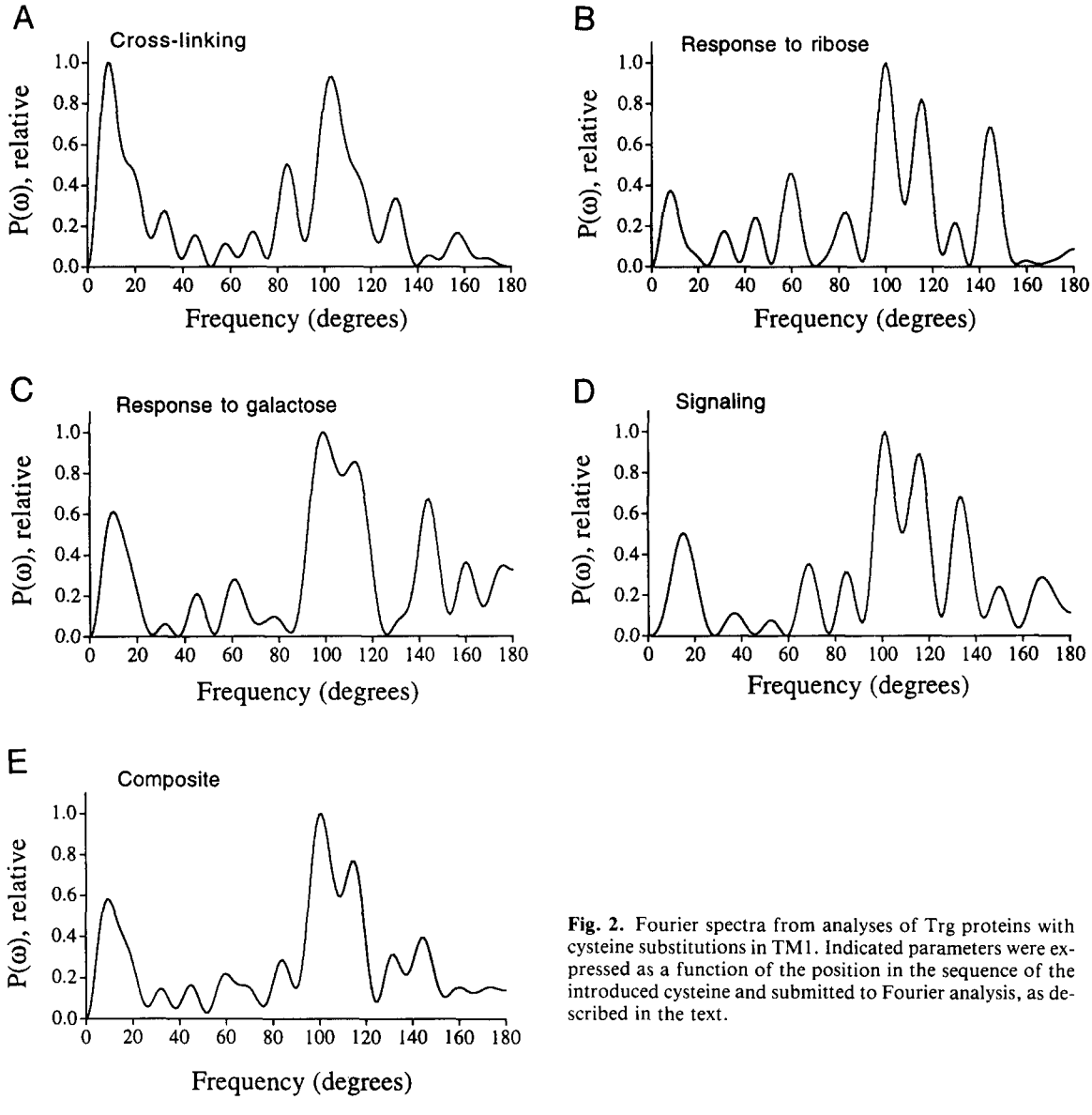

Fig. 2. Fourier spectra from analyses of Trg proteins with cysteine substitutions in TM1. Indicated parameters were expressed as a function of the position in the sequence of the introduced cysteine and submitted to Fourier analysis, as described in the text.

receptor function, and the effects detected were for the most part modest. This is consistent with the notion that the pair of TM1 segments are in close contact in the three-dimensional structure of the transmembrane region whereas the TM2 pair are more distant (Lynch & Koshland, 1991; Pakula & Simon, 1992; Lee et al., 1994). Thus, the less convincing patterns for TM2 are likely to reflect a lower probability for cysteines in TM2 and TM2′ to be close enough, no matter what the orientation, to participate in substantial crosslinking or for a cysteine in TM2 to be in a position to disrupt an interaction in a way that altered function. Because of this, we considered additional data generated from a mutational analysis of random substitutions in TM2 of Trg. Thirty-eight altered receptors, each with a single amino acid substitution at one of 22 positions in the 24-residue sequence, were characterized qualitatively for response to a spatial gradient of ribose and for transmembrane signaling upon stimulation by that sugar (Baumgartner, 1992). The power spectra of the quantitized data from these two assays exhibited maxima at 100° and 107°, respectively (Fig. 4A,B), and a composite spectrum had a predominant peak at 103° (Fig. 4C). A power spectrum created from the normalized sum of all six TM2 spectra (Fig. 5), representing the four assays of proteins with cysteine substitutions at positions 201–222 and the two assays of proteins with random substitutions, had its maximum at 103°, strengthening the evidence for an α-helical organization of TM2.

Orienting helices to construct a model for the transmembrane domain of Trg

The Fourier analyses described in the previous sections provided a quantitative basis for considering the two transmembrane segments of the Trg monomer as α-helices. With defined units of secondary structure, we could consider their relative orientations. The possibilities were limited because each transmembrane helix must span the lipid bilayer and thus would be oriented approximately normal to the plane of the membrane. In addition,

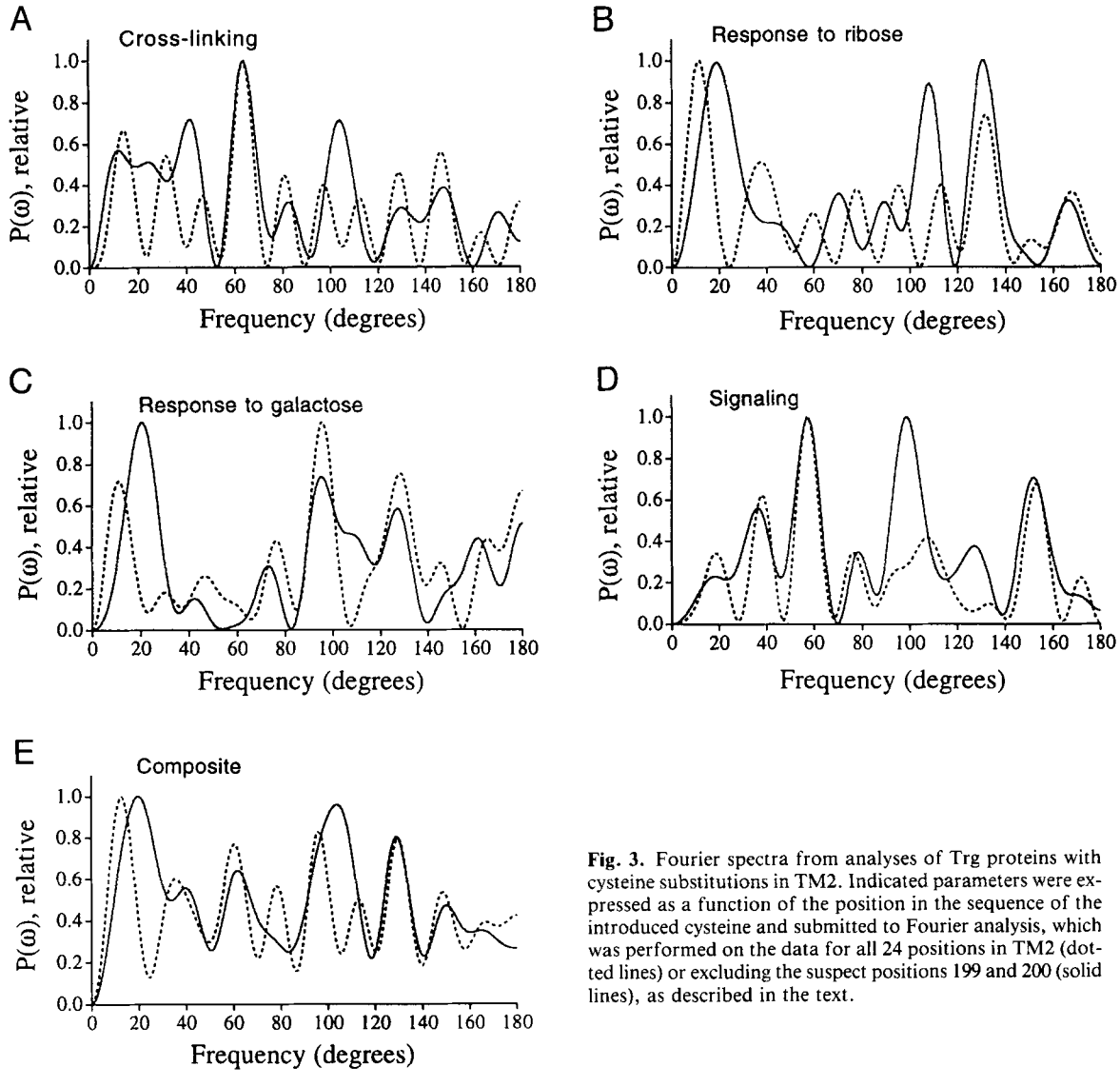

Fig. 3. Fourier spectra from analyses of Trg proteins with cysteine substitutions in TM2. Indicated parameters were expressed as a function of the position in the sequence of the introduced cysteine and submitted to Fourier analysis, which was performed on the data for all 24 positions in TM2 (dotted lines) or excluding the suspect positions 199 and 200 (solid lines), as described in the text.

the two helices within the same subunit would be necessarily antiparallel and the homologous helices in the two different subunits would be necessarily parallel. With these limits, the tertiary structure of the transmembrane domain of Trg could be defined in large part by identifying the proximity and specific orientations of the four helices. Those features would be reflected in measurements of propensity for oxidative crosslinking between pairs of cysteines placed on different units of secondary structure. Our studies of crosslinking had produced substantial data about not only the 1-1′ and 2-2′ pairs discussed in previous sections but also 1-2 pairs (Lee et al., 1994). Thus, we considered quantitative methods for using crosslinking data to identify orientation and proximity of α-helices within a transmembrane domain. We adapted the notion of helical hydrophobic moments, developed by Eisenberg et al. (1982), to calculate helical crosslinking moments, defined as the vector sums of the propensities for crosslinking of a cysteine placed at positions along a helix. The magnitude of the vector at each residue position was proportional to the crosslinking propensity, and its direction was defined by the position of the side chain around the helical axis. The crosslinking moments and the individual vectors from which they were derived for TM1-TM1′, TM2-TM2′, and TM1-TM2 interactions are diagrammed in Figure 6 on idealized helical wheels in which the angle between sequentially adjacent residues is a constant 100°. In the characterization of crosslinking between homologous positions on the two subunits of the native dimer, crosslinking propensity was assessed for cysteines at every position in the two transmembrane segments, and thus the resulting crosslinking moments represent contributions from measurements at all possible positions. Crosslinking moments for heterologous crosslinks between positions on TM1 and TM2 were the result of a selected set of contributions. It was not practical to test all possible pairs of TM1-TM2 cysteines for the extent of crosslinking, so random combinations of cysteines in TM1 and TM2 were screened and those exhibiting significant crosslinking were identified (Lee et al., 1994). The crosslinking moments between TM1 and TM2 were the sum of the crosslinking vectors for these identified

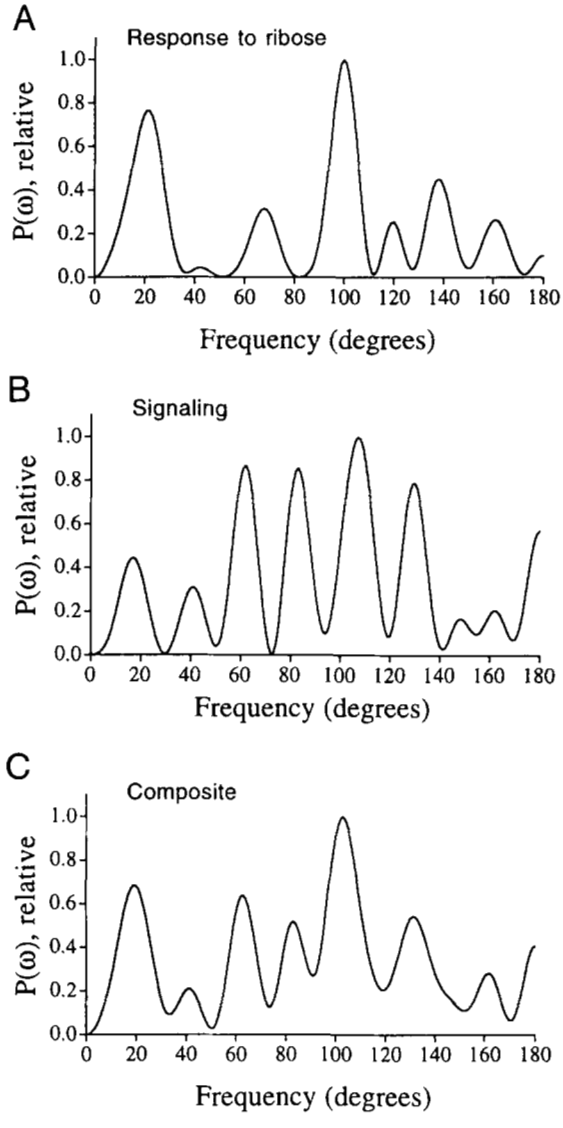

Fig. 4. Fourier spectra from analyses of Trg proteins with random substitutions in TM2. Indicated parameters were expressed as a function of the position in the sequence of the mutational change and submitted to Fourier analysis, as described in the text.

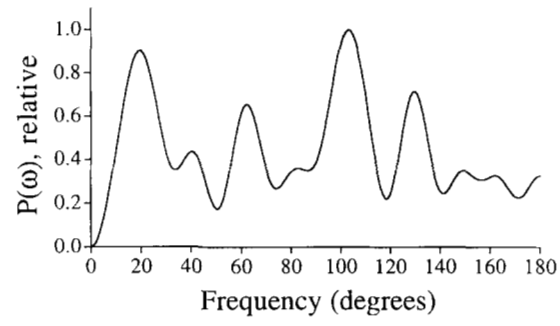

Fig. 5. Composite of Fourier spectra from analyses of TM2. Spectra shown in Figure 4A, B, C, and D and Figure 5A and B were summed and the resultant spectrum normalized by assigning a value of 1 to the maximum value on the $P(\omega)$ axis.

pairs. Because other pairs were not identified by the screening procedure, many of these were likely to exhibit little or no crosslinking, but others may have been missed by chance.

We used the idealized helical wheels and crosslinking moments shown in Figure 6 to create a model of the transmembrane domain of Trg (Fig. 7) in which relative orientation and separation between helices were defined by using, respectively, the direction and magnitude of the moment vectors. In the absence of complicating local effects, crosslinking propensity should be an inverse function of the distance between the sulfhydryls. In a study of disulfide crosslinking in a protein of known structure (Careaga & Falke, 1992), the relationship appeared to be an inverse log function. We assumed that our crosslinking moments would have a similar relationship to the average separation between helices, and thus we made the relative separation between pairs of helices proportional to the inverse of the log of the magnitude of the crosslinking moment. The separations were scaled by placing TM1 and TM1′, the pair with the largest crosslinking moment, at a 10-Å separation between helical centers, the average distance between closely packed helices. The four helices were oriented by aligning first the TM1–TM1′ moments and then the TM1–TM2 moments. These vectors were the largest,

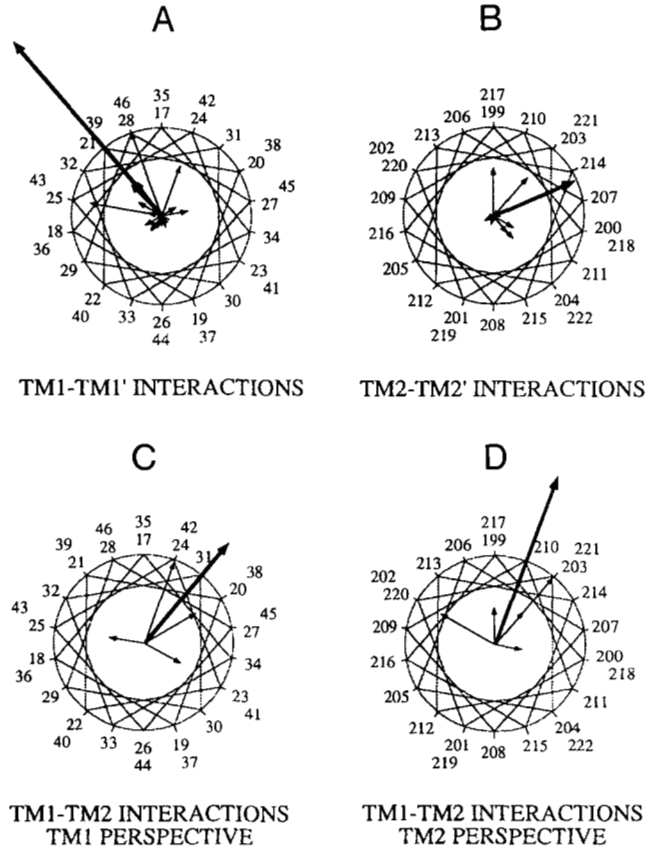

Fig. 6. Crosslinking vectors and resulting crosslinking moments between pairs of helices in the transmembrane domain of Trg. Individual vectors and resulting moments were determined as described in the text.

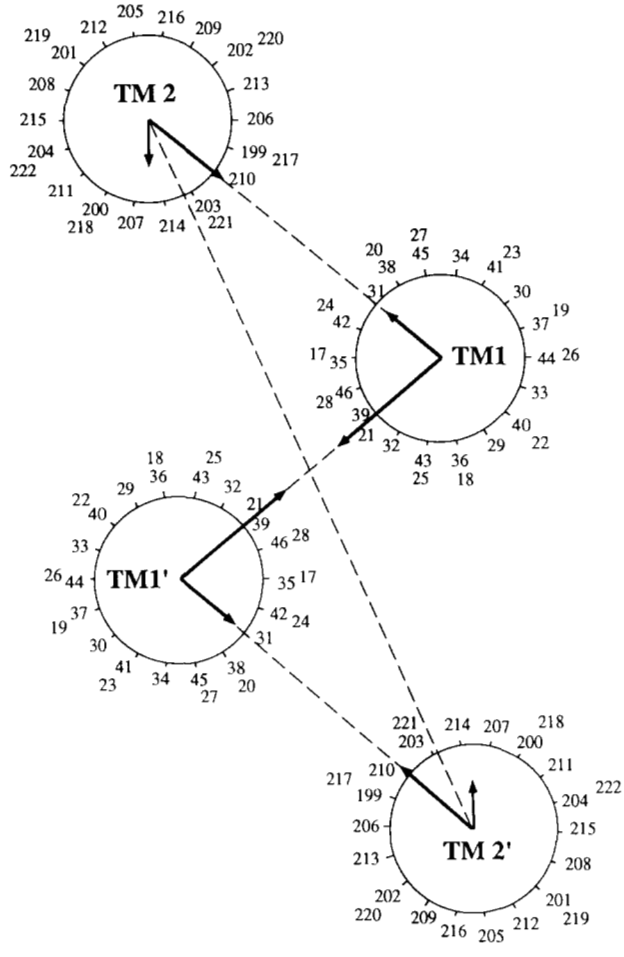

Fig. 7. Model of the transmembrane domain of Trg based on crosslinking moments. Helical wheel representations of the transmembrane helices of Trg were aligned and placed using the crosslinking moments, illustrated in Figure 6, as described in the text. Dashed lines mark axes through helical centers.

reflecting a substantial number of cysteine pairs that exhibited efficient crosslinking between helices. In contrast, only two cysteine pairs on the TM2 helices exhibited even moderate levels of crosslinking, resulting in a crosslinking moment likely to be less reliable than those between other helical pairs. Nonetheless, with the two pairs of larger vectors aligned, and the helical wheels separated by distances inversely proportional to the log of the vector magnitudes between them, the TM2–TM2′ moments deviated by only 20° from the axis between the centers of the TM2 helices. The model also placed the TM2 pair at a substantial separation, consistent with the low level of observed crosslinking.

The angle between adjacent residues of α-helices in proteins of known structure is not always the idealized 100°, particularly for pairs of helices that interact over extended lengths. Thus, we examined the effect of varying the helical periodicities from 95° to 105° for one or both of the TM1–TM1′ and the TM2–TM2′ pairs. Increasing the angular rotation between adjacent positions in TM1 or decreasing the angular rotation in TM2 led to better alignments of moment vectors. Within the range tested, no combination of helical periodicities allowed all three moment vectors to be aligned exactly.

Discussion

We utilized two quantitative approaches for analyzing and applying data obtained from genetic and biochemical studies of transmembrane segments, particularly from cysteine scanning and sulfhydryl crosslinking. These approaches were informative for the specific transmembrane protein we studied and promise to be useful in the characterization of other transmembrane proteins.

Identifying the secondary structure of transmembrane segments

For water-soluble proteins, units of secondary structure are often oriented along a surface between a hydrophobic interior of the protein and the surrounding hydrophilic environment. Thus, analyses of periodicity in the hydrophobic nature of side chains (Eisenberg et al., 1984; Cornette et al., 1987) have revealed periods characteristic of α-helices (≈100°, 3.6 residues) and β-strands (160–180°, 2–2.3 residues). For transmembrane segments, the difference in hydrophobicity between buried and exposed residues is less than for water-soluble protein (Rees et al., 1989), and analysis of periodicity based on such an intrinsic property of the segments might lack sufficient resolution. Alternatively the analysis can address an extrinsic property, the interaction with other transmembrane segments. The segments are likely to interact along specific faces of units of secondary structure, and thus probes of structural periodicity (e.g., sulfhydryl crosslinking) or functional periodicity (e.g., receptor function or signaling properties) could reflect the periodicity of the particular secondary structure. The bacterial chemoreceptors provide an informative test case. Their transmembrane domains, which contain four segments, are sufficiently complex to provide an array of possibilities yet sufficiently limited to pose a tractable problem. We found that Fourier analysis of data from assays of crosslinking between cysteine pairs, or of data about phenotypic features of cysteine-substituted receptors, produced coherent patterns of α-helical periodicity for the TM1 segments of the dimeric chemoreceptor Trg, and an indicative but less convincing helical pattern for the TM2 segments. For neither segment was there a significant periodicity at the position of β-sheet. Qualitative assessment of the body of data for Trg (Lee et al., 1994, 1995) and for the related chemoreceptor Tar (Lynch & Koshland, 1991; Milburn et al., 1991; Pakula & Simon, 1992; Yeh et al., 1993) suggested that TM1 and TM1′ were closely apposed along much of their length but that TM2 and TM2′ were in general more distant. Thus, it is reasonable that assays that depended upon structural or functional interactions would delineate a clear pattern for TM1–TM1′ interactions and only a suggestive pattern for the TM2–TM2′ interactions.

All the spectra we generated, whether from a specific data set or from composites of data, had, in addition to a maximal or predominant peak at ≈100°, several peaks not obviously related to known periodicities in units of secondary structure. The significance of those peaks was not clear, but such peaks were also observed in spectral analyses of hydropathies of helices in proteins of known structure (Cornette et al., 1987). The peaks might

reflect periodic features of the particular helices analyzed or be essentially noise in the analyses. The patterns of data for Trg suggested that there was at least a partial contribution of noise. Secondary peaks were more evident in the spectra for TM2 than for TM1, and, as discussed in the previous paragraph, the data for TM2 were of lower quality than the data for TM1.

Orienting transmembrane helices

Crosslinking studies with our collection of cysteine-containing forms of Trg generated values for crosslinking propensities between 67 different cysteine pairs in the transmembrane domain of that chemoreceptor (Lee et al., 1994). Initial analyses of that large body of data focused on local maxima in the patterns of crosslinking propensities, displayed as a function of sequence position, and provided the qualitative basis for a model of the transmembrane domain as a loose four-helix bundle (Lee et al., 1994). We sought a quantitative and objective method for using the crosslinking data to begin detailed modeling, and chose to use the notion of helical moments. Calculation of helical crosslinking moments had several important features. A large body of data relevant to the interaction between a pair of helices could be condensed into a single parameter, and yet all the available data contributed to that value. The resulting moments could be used to define not only relative orientation but also relative separation between helices. However, the approach has some important limitations. The most fundamental is that the helical moment condenses information about three-dimensional structure into two dimensions, and thus the transmembrane dimension of the helices is not addressed. For instance, it seems likely that TM1 and TM1′ are closely packed along much, if not all, of the transmembrane dimension and thus would be expected to be oriented at an angle, perhaps $\approx 20°$. Thus, the two helices would not interact along precisely the interface identified by the alignment of the moment vectors, but rather as a coiled coil along an axis containing positions 46, 39, 32, etc. Perhaps a feature of coiled coils, an angle between residues slightly greater than 100° (Cohen & Parry, 1990) was reflected in the observation that the vectors in our model were most closely aligned at residue angles for TM1 above 100°. Condensation of crosslinking data into a two-dimensional representation also treats the entire transmembrane dimension as equivalent, yet helices might be bent or kinked, or specific helical pairs might interact along only a part of the transmembrane dimension. The pattern of crosslinking for cysteine-containing Trg and Tar proteins (Pakula & Simon, 1992; Lee et al., 1994) suggests that splaying might occur for some of the helical pairs in the transmembrane domain of those chemoreceptors. We will explore these issues by using the organization shown in Figure 7 as the starting point for detailed, computer-assisted modeling of the transmembrane domain of chemoreceptors.

Materials and methods

Fourier transforms

Discrete Fourier transform power spectra were generated using the program of Cornette et al. (1987) with mesh setting 5, allowing analysis of power spectra at 1° frequency intervals. Spectra were normalized to give the highest peak a maximal value of 1. Composite power spectra were generated by adding individual spectra and normalizing as for the individual spectra. The data for crosslinking propensities, expressed in percent crosslinking after 10 min, were those shown in Figure 5 of Lee et al. (1994), and the data for chemotactic responses to galactose or ribose, expressed as the rate of progress of cells containing the altered receptor relative to the rate exhibited by cells containing the wild-type receptor were those shown in Figure 1 of Lee et al. (1995). In the characterization of Trg proteins containing random substitutions in TM2 (Baumgartner, 1992), chemotactic responses to spatial gradients of ribose were classified as essentially normal, partially defective, or defective. For our quantitative analysis, these classes were assigned values of 1, 0.5, and 0, respectively. Analysis of transmembrane signaling by altered receptors (Yaghmai & Hazelbauer, 1992) had previously identified three distinct phenotypes: (1) essentially wild type, (2) inefficient signaling, and (3) induced signaling. For our quantitative analysis, the phenotypes shown in Figure 3 of Lee et al. (1995) or Figure 19 of Baumgartner (1992) were assigned numerical values of 0, −1, and +1, respectively, and intermediate phenotypes were assigned values of −0.5 and +0.5. For positions at which more than one random substitution occurred, the numerical values of the multiple phenotypes were averaged. The Fourier assessment of non-numerical data by assigning discrete values to phenotypic classes, although unorthodox, has been used previously to determine the periodicity of transmembrane segments (Lemmon et al., 1992).

Crosslinking moments

Vector moments were determined using a program, VECTORS, developed at our request by Susan Jean Johns of the Center for Visualization, Analysis and Design for Molecular Sciences (VADMS) at Washington State University, and based on the software and subroutine libraries contained in the Wisconsin Package from the Genetics Computer Group (1994). The VECTORS program source code is available from the anonymous FTP site ftp.wsu.edu in the /molbio/vectors location. The readme file gives background and installation information on the program. The source code is dependent on version 8.0 GCG procedure library subroutines in order to compile. Parameters and variables in the VECTORS program are indicated in boldface type in the following discussion. In this procedure, sequential **relative position** numbers, beginning with 1, were assigned to the sequence of residues in the segments of interest, starting with position 17 for TM1 and position 199 for TM2. The angle between sequentially adjacent residues in the helix was specified by the variable **angle**. Negative angular values were entered for analyses of TM1, and positive for analyses of TM2. The opposite signs corresponded to the antiparallel organization of the helices. Each position was assigned a crosslinking vector, of magnitude **r**, determined as the percent crosslinking after 10 min of exposure to the oxidation catalyst Cu(II) (1,10-phenanthroline)$_3$ (Lee et al., 1994). Crosslinking was between a cysteine at the particular position and either a cysteine at the homologous position in the other subunit of the dimer (for 1–1′ or 2–2′ crosslinking) or a cysteine on the other transmembrane segment of the subunit (1–2 crosslinking). For crosslinking between TM1 and TM2, cysteines at residues 25, 42, 202, and 221 exhibited crosslinking to more than one position on the opposite segment. In those cases, the largest crosslinking value was used, represent-

ing what we would expect to be the closest positioning of the particular residue to the opposite segment. Each vector was placed with its tail at the center of a helical wheel and its head pointing to the residue position on the circumference of the wheel, an orientation specified mathematically by the relative angle ϕ (in radians):

$$\phi = (\textbf{relative position} - 1)(\textbf{angle})(\pi/180).$$

Thus, relative position 1 was at 0 radians. Individual vectors, each specified by an **r** and ϕ value, were converted to Cartesian coordinates using the relationships:

$$\mathbf{x} = \mathbf{r}\cos(\phi) \text{ and } \mathbf{y} = \mathbf{r}\sin(\phi).$$

Cartesian coordinates for the crosslinking moment vector (values **X** and **Y**) were calculated by summing **x** and **y** values separately, and the magnitude of the crosslinking moment vector, **R**, was calculated as the square root of the sum of $\mathbf{X}^2$ and $\mathbf{Y}^2$. The relative angle, ϑ, of the moment vector could not be calculated using any single inverse trigonometric relationship because inverse relationships are valid only over ranges where the corresponding trigonometric relationships are nonperiodic. To circumvent this, the VECTORS program plotted the relative position of the moment vector based on its **X** and **Y** coordinates, calculated above. The value of ϑ was empirically obtained by measuring the angle relative to the 0 radian position on a wheel diagram. The output of the program was a helical wheel diagram drawn with the appropriate **angle** between adjacent residues and with individual and moment vectors superimposed. The helical wheels were drawn to represent the helices of the transmembrane domain viewed from the periplasm. Thus, the α-carbon backbones of the right-handed helices rise in the CCW direction for TM1, and in the CW direction for TM2. The different signs for specified **angles** for TM1 and TM2 reflect this antiparallel orientation of the helices. However, the orientation of the TM1 and TM2 helices is such that a positive value for ϑ means the angle is oriented CW with respect to the 0 degree position (residue 17 for TM1 or residue 199 for TM2), whereas a negative ϑ is oriented in the CCW direction.

Acknowledgments

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