Characterization of Proline-Containing α -Helix (Helix F Model of Bacteriorhodopsin) by Molecular Dynamics Studies

R. Sankararamakrishnan and Saraswathi Vishveshwara

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

ABSTRACT Many of the bilayer spanning segments of membrane transport proteins contain proline residues, and most of them are believed to occur in α -helical form. A proline residue in the middle of an α -helix is known to produce a bend in the helix, and recent studies have focused on characterizing such a bend at atomic level. In the present case, molecular dynamics (MD) studies are carried out on helix F model of bacteriorhodopsin (BR) Ace-(Ala)₇-Trp-(Ala)₂-Tyr-Pro-(Ala)₂-Trp-(Ala)₈-NHMe and compared with Ace-(Ala)₇-Trp-(Ala)₂-Tyr-(Ala)₃-Trp-(Ala)₈-NHMe in which the proline is replaced by alanine. The bend in the helix is characterized by structural parameters such as kink angle (α), wobble angle (θ), virtual torsion angle (ρ), and the hydrogen bond distance d ($O_{p-3} \dots N_{p+1}$). The average values and the flexibility involved in these parameters are evaluated. The correlation among the bend related parameters are estimated. The equilibrium side chain orientations of tryptophan and tyrosine residues are discussed and compared with those found in the recently proposed model of bacteriorhodopsin. Finally, a detailed characterization of the bend in terms of secondary structures such as α_I , α_{II} and goniometric helices are discussed, which can be useful in the interpretation of the experimental results on the secondary structures of membrane proteins involving the proline residue.

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Key words: proline-containing α -helix, backbone-bend parameters, correlation between parameters, side chain conformation, helix F of bacteriorhodopsin

INTRODUCTION

The proline residue in proteins has always been associated with important structural features.¹ Its presence in the middle of a right-handed α -helix, however, has only recently been recognized and characterized, made possible from the studies of the crystal structure analysis,^{2,3} energy minimization,⁴ molecular dynamics,⁵ and free energy⁶ simulations.

Apart from its presence in globular proteins, the proline residue in the middle of the membrane spanning segments of a number of transport proteins has been identified.⁷ The secondary structure of such membrane spanning segments has been characterized as α -helical in bacteriorhodopsin (BR)⁸ and other membrane proteins are also known to have proline-containing α -helices.^{9–12} The possible structural and/or functional role of prolines in transmembrane helices has been recently discussed.¹³

Three of the membrane spanning helices (B, C, F) in BR contain proline residues in the middle. These helices contain the side chains that have been recognized to be functionally important.^{14–16} During the photocycle, large-scale motion of these helices has been proposed to take place.^{17–21} The characterization of the dynamical properties of the individual helices can help in understanding some of the aspects of the photocycle at the molecular level. In this work, helix F model of bacteriorhodopsin is investigated and the features are compared with the helix in which the proline residue is replaced by an alanine residue. The geometrical features of proline-containing α -helices are distinctly different from those of a standard α -helix.³ Since the occurrence of such helices are common in membrane transport proteins,⁷ it was felt that an analysis of the geometrical parameters would be useful in modeling and understanding the three-dimensional architecture of membrane proteins. Such an analysis has aided in recognizing specific parameters involved in the formation of kink and in quantitatively evaluating the flexibility introduced in the α -helix by a proline residue. Also, the secondary structural characteristics of proteins having proline-containing helices are discussed in the light of the results obtained from the molecular dynamics studies.

METHODS

Molecular dynamics (MD) simulations for the helix F model of bacteriorhodopsin (BR) were per-

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Address reprint requests to Dr. Saraswathi Vishveshwara, Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India.

Fig. 1. The conformational parameters of a polypeptide chain containing a proline residue in the middle. R represents the side chain of the residue. The notations ϕ , ψ , and ω refer to the standard backbone torsion angles and χ_1 and χ_2 define the proline ring

conformation. The C^α atoms defining the virtual torsional angle are joined by broken lines. The symbol ρ in the text represents the virtual torsional angle $C_{P-3}^\alpha-C_{P-2}^\alpha-C_{P-1}^\alpha-C_P^\alpha$.

Fig. 2. MD trajectories of proline pucker parameters (χ_1 and χ_2 of proline residue).

formed using the AMBER program,^{22,23} which was run on a VAX 8810 machine. The system chosen for the molecular dynamics studies is Ace-(Ala)₇-Trp-(Ala)₂-Tyr-Pro-(Ala)₂-Trp-(Ala)₈-NHMe (system I) in which the proline, tyrosine, and tryptophan resi-

dues were retained in the same relative positions with respect to the helix F of BR, and the other residues were replaced by alanine residues. This model provides an α -helix model long enough to span the membrane bilayer. For comparison, MD simulations

TABLE I. Average and rms Fluctuations of Backbone Internal Parameters (in Degrees) in the Proline Region

Internal parameters	System I	System II	Crystal structure average*
ϕ_{p-4}	-61.3 (± 9.2)	-58.2 (± 8.5)	-60.9
ψ_{p-4}	-38.7 (± 12.2)	-47.9 (± 8.3)	-30.9
ϕ_{p-3}	-67.4 (± 12.1)	-58.2 (± 9.1)	-75.7
ψ_{p-3}	-44.2 (± 10.0)	-48.9 (± 8.5)	-33.9
ϕ_{p-2}	-69.2 (± 11.4)	-57.7 (± 9.0)	-73.0
ψ_{p-2}	-44.4 (± 12.1)	-50.0 (± 8.5)	-36.4
ϕ_{p-1}	-44.9 (± 11.5)	-55.6 (± 8.6)	-51.8
ψ_{p-1}	-58.5 (± 8.2)	-52.5 (± 8.6)	-51.1
ϕ_p	-62.3 (± 10.2)	-56.8 (± 9.1)	-60.8
ψ_p	-44.7 (± 10.0)	-48.6 (± 8.5)	-34.0
ω_{p-2}	-171.3 (± 8.0)	176.7 (± 6.6)	-175.4
$N_{p-1}-C_{p-1}^{\alpha}-C_{p-1}$	114.3 (± 3.1)	110.5 (± 3.0)	115.3
$C_{p-1}^{\alpha}-C_{p-1}-N_p$	121.2 (± 2.6)	117.5 (± 2.8)	118.6

*The average was obtained as a result of crystal structure analysis of proline-containing α -helical segments from 14 different high-resolution protein structures. Details in ref. 3.

TABLE II. Average and rms Fluctuations of Conformational Parameters (in Degrees) Depending Upon the Proline Puckering

Parameters	Pro-Down	Pro-Up	Minimized structures	
			Pro-Down	Pro-Up
ϕ_{p-1}	-43.3 (± 11.3)	-48.2 (± 11.2)	-39.7	-46.7
ψ_{p-1}	-60.5 (± 7.4)	-54.1 (± 8.3)	-60.3	-54.1
ω_{p-1}	-178.1 (± 6.4)	176.5 (± 7.6)	-177.3	175.2
ϕ_p	-59.4 (± 8.6)	-51.5 (± 10.2)	-59.7	-49.9
ψ_p	-43.1 (± 9.7)	-48.1 (± 9.7)	-41.6	-47.6
χ_1 (Pro)	21.2 (± 7.3)	-25.5 (± 7.6)	23.4	-28.6
χ_2 (Pro)	-30.6 (± 7.3)	33.2 (± 8.3)	-33.6	35.7

TABLE III. Average and rms Fluctuations of Intrahelical Hydrogen Bond Lengths (in Angstroms) and Hydrogen Bond Angles (in Degrees)

Description of hydrogen bond	System I	System II
	O . . . H Distance O . . . H-N Angle	O . . . H Distance O . . . N-H Angle
$O_{p-8} \dots HN_{p-4}-N_{p-4}$	1.92 (± 0.13) 163.4 (± 9.3)	1.91 (± 0.13) 162.4 (± 9.3)
$O_{p-7} \dots HN_{p-3}-N_{p-3}$	1.99 (± 0.23) 154.4 (± 14.8)	1.90 (± 0.13) 161.1 (± 9.5)
$O_{p-6} \dots HN_{p-2}-N_{p-2}$	1.94 (± 0.17) 156.4 (± 12.5)	1.93 (± 0.16) 161.8 (± 9.5)
$O_{p-5} \dots HN_{p-1}-N_{p-1}$	1.94 (± 0.17) 156.9 (± 13.3)	1.92 (± 0.13) 162.1 (± 9.3)
$O_{p-4} \dots HN_p-N_p$	Absent	1.88 (± 0.12) 162.1 (± 9.3)
$O_{p-3} \dots HN_{p+1}-N_{p+1}$	3.48 (± 0.63) 159.7 (± 10.3)	1.92 (± 0.14) 161.9 (± 9.7)
$O_{p-2} \dots HN_{p+2}-N_{p+2}$	1.95 (± 0.18) 163.0 (± 8.7)	1.95 (± 0.16) 161.9 (± 9.6)
$O_{p-1} \dots HN_{p+3}-N_{p+3}$	1.89 (± 0.14) 161.2 (± 9.4)	1.88 (± 0.13) 161.8 (± 9.2)

Fig. 3. MD trajectories of bend related parameters (kink angle (α), wobble angle (θ), virtual torsion angle (ρ), hydrogen bond distance $d(O_{p-3} \dots N_{p+1})$ and (ϕ_{p-1}) . Figure continues on page 30.

were also performed on Ace-(Ala)₇-Trp-(Ala)₂-Tyr-(Ala)₃-Trp-(Ala)₈-NHMe (system II) to investigate the role of the proline residue in the middle of an α -helix in general and the importance of side chains in the proline-containing helix F of BR in particular.

The internal parameters for the starting structure of system I were taken from ref. 4. The energy was minimized using the AMBER minimization module until the rms gradient of the energy was less than 0.1 Kcal/mol Å. The structure thus obtained was taken as the starting point for the simulation. For system II, the minimized ideal α -helical structure was considered as the starting point.

Initially, random velocities were assigned to each atom using the Maxwellian distribution corresponding to a low temperature. The systems were equilibrated to 300 K over a period of 25 ps and continued for a further 100 ps. The AMBER MD program uses a Verlet leapfrog algorithm.²⁴ A time step of 0.001 ps was chosen in this study. All hydrogen atoms were explicitly included and hence the force field corresponding to an all atom model²⁵ was used. All

bonds involving hydrogen atoms were constrained using the algorithm SHAKE, and both the simulations were performed in vacuum. Although the model peptide we have chosen is a transmembrane segment of a membrane protein bacteriorhodopsin, only about one-third of the helical face is in contact with the lipid bilayer. Since the remaining part is exposed to the inner core of the protein, which contains polar and ionizable amino acid residues, a distance-dependent dielectric constant $\epsilon = R_{ij}$ was used in our calculations. The output from the MD run contained a total of 1,000 "snapshots" of the atomic coordinates during 100 ps evolution time with each snapshot taken at 0.1 ps interval for both the systems I and II.

The notation of the conformational parameters is shown in Figure 1. The dihedral angles χ_1 and χ_2 of proline residue is related to the *up* or *down* pucker of proline with (χ_1, χ_2) assuming $(-ve, +ve)$ and $(+ve, -ve)$ in the *up* and *down* conformations, respectively. Apart from the standard parameters, virtual torsional angles of C $^\alpha$ atoms are also depicted. The

Fig. 3. Legend appears on page 29.

averages and rms fluctuations of internal parameters and hydrogen bond parameters were obtained from the AMBER MD analysis program, whereas virtual torsion angles of C^α atoms were calculated in a separate program for both system I and system II.

The backbone internal parameters in the proline region, the side chain dihedral angles, the hydrogen bond parameters, and the virtual torsional angles of C^α atoms were analyzed. Our recent crystal structure analysis³ and MD studies on proline-containing

Fig. 4. A plot of kink angle (α) vs. wobble angle (θ) obtained from MD simulated structures. The points are plotted in different symbols depending on the hydrogen bond distance $d(O_{p-3} \dots N_{p+1})$. The symbols 'o', '+', 'x' and '.' correspond to $d(O_{p-3} \dots N_{p+1}) < 3.2$ Å, 3.2 to 3.8 Å, 3.8 to 4.4 Å and > 4.4 Å, respectively.

helical segments⁵ have shown that the virtual torsion angle $C_{p-3}^\alpha - C_{p-2}^\alpha - C_{p-1}^\alpha - C_p^\alpha$ (designated as ρ) deviates from the average value of a standard α -helix. Hence this parameter has been studied in detail. The dependence of backbone dihedral angles on proline puckering was also investigated in system I by considering all the 1,000 MD simulated structures.

Eight structures from the 100 ps simulation of system I were submitted to energy minimization procedures. Five structures lie in the range 45 to 55 ps in which proline is in the *down* conformation and the other three, in the range 85 to 95 ps, where proline is in *up* conformation. Using the AMBER minimization module, the steepest descent method was used for the first 50 cycles and followed by the conjugate gradient method until convergence was obtained. The resulting structures were further minimized by the Newton-Raphson method using the AMBER NMODE module. All the conformational parameters for these minimized structures were analyzed.

The bend introduced by proline in the right-handed α -helix has been characterized mainly by two parameters viz. kink angle and wobble angle (designated as α and θ , respectively).^{3,26} The kink angle is the angle between two helical axes: one from the N-terminal to proline (helix I) and the other from proline to C-terminal (helix II). An algorithm suggested by Chou et al.²⁷ was used to find the helix axis. The wobble angle is the angle that defines the orientation of helix II in three-dimen-

sional space with respect to helix I. The detailed method for evaluating the wobble angle is described in ref. 3. The main steps involved in the calculation of wobble angle (θ) are as follows: (1) helix I was made parallel to Z-axis with C_p^α atom as the origin; (2) helix I was rotated about z-axis to make the radius vector, joining the C_p^α atom and the helix axis of helix I, collinear with positive X-axis; (3) a point was projected on the XY-plane from helix axis of helix II; and (4) the angle made by the line, joining the origin and the projected point, with the X-axis is the wobble angle (θ).

The parameters virtual torsion angle (ρ), $d(O_{p-3} \dots N_{p+1})$ and ϕ_{p-1} , which are related to kink angle and wobble angle were studied in detail. The correlation coefficient C between any two parameters A and B is given by the formula

$$C = \frac{\sum_{i=1}^N (A_i - \bar{A})(B_i - \bar{B})}{\sum_{i=1}^N |(A_i - \bar{A})(B_i - \bar{B})|}$$

where $\bar{A}$ and $\bar{B}$ are averages and A_i and B_i are the values of the i^{th} simulated point for the parameters A and B , respectively, in a total of N points. The correlation coefficient matrix was obtained for all the bend related parameters. Using this matrix and the trajectories, the interrelationship of these parameters has been discussed.

Separate programs were written to evaluate kink

Fig. 5. A stereo diagram of three MD simulated structures for system I (only backbone atoms and proline side chain are plotted) with the values of $[\alpha, \theta]$ being $[5.5^\circ, -2.0^\circ]$, $[30.5^\circ, 22.0^\circ]$ and $[52.2^\circ, 63.0^\circ]$.

angle, wobble angle, to get the trajectories of different parameters and to calculate correlation coefficient between any two parameters.

RESULTS AND DISCUSSION

Backbone Internal Parameters

The proline residue in the middle of a right-handed α -helix is known to introduce perturbations in some of the bond angles and dihedral angles of the backbone. From the previous studies³⁻⁵ the (ϕ, ψ) values corresponding to ϕ_{p-4} to ψ_p region have shown distortions from the standard values (idealized theoretical value²⁸ $\phi = -57.5^\circ$, $\psi = -47.5^\circ$, crystal structure average of all helical residues in globular proteins²⁹ $\phi = -63.8^\circ (\pm 6.6)$, $\psi = -41.0^\circ (\pm 7.2)$). The MD average and the rms deviation of parameters ϕ_{p-4} to ψ_p , $N_{p-1}-C_{p-1}^\alpha-C_{p-1}$ and $C_{p-1}^\alpha-C_{p-1}-N_p$ for the molecule Ace-(Ala)₇-Trp-(Ala)₂-Tyr-Pro-(Ala)₂-Trp-(Ala)₈-NHMe (system I) are given in Table I. A comparison has been made with the molecular dynamics average for Ace-(Ala)₇-Trp-(Ala)₂-Tyr-(Ala)₃-Trp-(Ala)₈-NHMe (system II) and the recent crystal structure average on proline-containing α -helices.³ The values confirm the trend reported previously that, ϕ_{p-1} is less negative and ψ_{p-1} is more negative than the standard values. In general ϕ_{p-3} , ϕ_{p-2} are more negative and ψ_{p-4} , ψ_{p-3} , and ψ_p are less negative than the standard values. The peptide dihedral angle ω_{p-2} has taken an average value of -171.3° with rms fluctuation of 8.0° . The rms fluctuations in parameters ϕ_{p-4} to ψ_p are larger in the proline-containing helix (system I) compared to the non-proline-containing helix (system II). The bond angles $N_{p-1}-C_{p-1}^\alpha-C_{p-1}$ and $C_{p-1}^\alpha-C_{p-1}-N_p$ are generally wider than the standard values³⁰ ($N-C^\alpha-C = 108.0$ to 111.0° ; $C^\alpha-C-N = 115.0^\circ$) with the crystal structure average of proline-

containing helices given in Table I slightly differing from the MD average.

Proline Puckering Parameters

The proline ring fluctuates between the *up* and *down* conformation during the simulation period. This is represented by the trajectories of the dihedral angles χ_1 and χ_2 of the proline residue (Fig. 2). Some of the parameters (ϕ_{p-1} , ψ_{p-1} , ω_{p-1} , ϕ_p and ψ_p) in the proline region indicate preferential values depending on the proline pucker and the MD averages of these values are given in Table II. A few structures from the MD simulation were selected for energy minimization as described in Methods. The structures minimize either to the *up* or to the *down* conformation with almost same energy (~ -327.5 Kcal/mole). However the parameters that are found to be different in MD average values have clearly different values even after minimization (Table II). The ω_{p-1} deviates from planarity to a slightly greater extent in the *up* conformation than in the *down* conformation.

Bend Characterizing Parameters

Although well-defined changes are noticed in the above mentioned internal parameters, it is difficult to get an idea of the bend in the helix due to proline by examining a single internal parameter. The general characteristics can be measured in terms of other parameters such as virtual torsional angle $C_{p-3}^\alpha-C_{p-2}^\alpha-C_{p-1}^\alpha-C_p^\alpha$ (ρ), a specific hydrogen bond $N_{p-1} \cdots O_{p-3}$, which is weakened due to proline, kink angle (α), and wobble angle (θ). The analysis of and the correlation among these parameters are discussed in this section.

The MD average of the virtual torsion angle ρ is 74° with maximum rms fluctuation of 12° in system

Fig. 6. (a) MD trajectories of χ_1 and χ_2 of W-182 for system I. (b) MD trajectories of χ_1 and χ_2 of W-182 for system II. Figure continues on page 34.

I. The averages of other virtual torsional angles of system I and all the virtual torsion angles of system II are close to the ideal α -helical value³¹ of 50° and have less rms fluctuations. Higher value and maximum deviation in ρ indicates that the kink starts from the helical turn that ends with proline residue and that region is more flexible. Further, the proline-containing helices are known to lack two hydrogen bonds,²⁶ one due to the absence of the amide proton on proline nitrogen and another between N_{p+1} and O_{p-3} . The hydrogen bond lengths ($O_{p-8} \dots HN_{p-4}$ to $O_{p-1} \dots HN_{p+3}$) and the corresponding N-H $\dots$ O bond angles are given in Table III for system I and system II. The lack of the $O_{p-4} \dots N_p$ hydrogen bond for system I is obvious and the average $O_{p-3} \dots HN_{p+1}$ distance is $3.48 (\pm 0.63)$ Å. Apart from the longer $O_{p-3} \dots HN_{p+1}$ distance, some of the hydrogen bond angles in the proline region assume smaller values ($\approx 155.0^\circ$) than the

standard one (164.2° for an ideal α -helix³²; 161° – 162° for system II). Also, the rms deviations of these parameters are greater indicating fluctuations in this region. This is in agreement with the recent ^1H NMR studies³³ on melittin in which the proline has been identified as the residue imparting flexibility to the helix.

The kink angle (α) is a direct measure of the bend in the helix and the wobble angle (ϕ) gives an idea about the mutual orientation of the helical segments below proline and above proline. The trajectories of these parameters along with those of ϕ_{p-1} , ρ and $d(O_{p-3} \dots HN_{p+1})$ are given in Figure 3 for system I. The MD average kink angle is $28.5^\circ (\pm 11.7)$ and wobble angle is $22.9^\circ (\pm 28.8)$. All these parameters vary frequently in the range of a straight helix to that of bent one. Such fluctuations were also observed by ^1H NMR studies on melittin.³⁴ The occurrence of proline in the signal and energy transduc-

Fig. 6b. Legend appears on page 33.

ing proteins⁷ and channel forming peptides like alamethicin³⁵ is significant from its structural and functional point of view. The presence of proline in α -helices allows for changes in pore sizes. The present observations suggest that conformational changes can occur by adjusting the kink and the wobble angles and hence can play an important functional role.

Structures with varying kink angle ($\alpha = 0$ to 50°) and wobble angles ($\theta = -20$ to $+70^\circ$) obtained during MD simulations were minimized as described in Methods. All the structures minimized with $\alpha = 30.9^\circ$ and $\theta = 27.6^\circ$. This indicates a broad shallow minimum around the minimized structure. This is in agreement with the results of the free energy simulation studies in which the free energy difference was found to be small⁶ for a varying kink angle 15 – 45° in a 14-residue α -helix containing proline in the ninth position. (However, the kink in this study was introduced by varying the hydrogen bond distance $d(N_{p-1} \dots O_{p-5})$ instead of $d(N_{p+1} \dots O_{p-3})$.)

There is a significant correlation between many of the parameters that have been discussed here. The correlation coefficients between α , θ , ϕ_{p-1} , ρ , $d(O_{p-3} \dots HN_{p+1})$ are given in Table IV. All these parameters are highly correlated. The correlation of ϕ_{p-1} with the other parameters is, however, less in comparison. This indicates that the bend introduced in the helix due to proline is a cumulative effect of variations in the number of backbone internal parameters in the proline region. The values of α , θ , and $d(O_{p-3} \dots HN_{p+1})$ for the MD simulated structures in which the correlation among these parameters is more explicit have been plotted (Fig. 4). The negative θ (-50° – 0°) and smaller $d(O_{p-3} \dots HN_{p+1})$ ($< 3.2 \text{ \AA}$) values are found for smaller α values ($< 20^\circ$). The θ values assume more positive values and the $d(O_{p-3} \dots HN_{p+1})$ becomes longer ($> 4.4 \text{ \AA}$) for larger α values. The plot exhibits the range and the extreme values that can be taken up by a proline-containing helix during the MD simulation. The MD average, the crystal structure

TABLE IV. Correlation Coefficients Between Various Bend Related Parameters

	α	θ	ϕ_{p-1}	ρ	$d(O_{p-3} \dots N_{p+1})$
α	1.000	0.861	0.589	0.967	0.973
θ		1.000	0.333	0.751	0.892
ϕ_{p-1}			1.000	0.639	0.559
ρ				1.000	0.960
$d(O_{p-3} \dots N_{p+1})$					1.000

average of proline-containing helices,³ and the minimized values of α and θ fall in the range of 20–30° and –30–+30°, respectively. A stereo diagram of system I with varying α and θ is given in Figure 5.

Analysis of Side Chain Conformations

The above studies on the characterization of internal parameters and the bend are applicable to any proline-containing α -helix including those that span membranes. Helix F of BR has Tyr-185, Trp-182, and Trp-189 in the Pro-186 neighborhood, and these residues have been identified as functionally important to the retinal binding pocket.¹⁶ The spatial arrangement of these aromatic residues play an important role in interacting with retinal. In the present study, the inherent dynamical behavior of these residues are characterized.

The MD simulation for system I was started with all side chains (Trp-182, -189, and Tyr-195) in their extended conformation. A similar starting point was also considered for system II. The trajectories of the two dihedral angles χ_1 and χ_2 for Trp-182, -189, and χ_1 for Tyr-185 are shown in Figures 6–8 for both systems I and II.

In Figure 6a, although the extended conformation with $\chi_1 = 180.0^\circ$ for Trp-182 was the starting point, soon it assumed that value $\chi_1 \approx -60^\circ$. A comparison of MD average structure with those from BR model (Henderson, personal communication) is given in Table V. The preference for χ_1 of Trp-182 to be in the –60° region seems to be inherent for helix F since even in the absence of an environmental effect as found from MD simulation, this value is assumed, which is close to *trans* orientation in nonproline helix (system II). The χ_1 of Tyr-185, in contrast, has remained as *trans* in MD simulation of both systems I and II and is in the –60° region in the BR structure. The MD average of χ_1 of Trp-189 is in the *trans* orientation in system I, system II, and in the BR model proposed by Henderson et al.⁸ However, the trajectories of χ_1 of Trp-182 (Fig. 6b) and Trp-189 (Fig. 8b) for system II show that other conformations are also accessed for a short time during the simulation. Such type of transitions were not observed in system I, perhaps due to restrictions brought out by the proline residue.

A stereo diagram of system I with different values of χ_1 for Trp-182 is given in Figure 9a. A similar

diagram for system II in which the Trp-182 and -189 are plotted in different conformations as obtained by MD simulation (Fig. 9b). It is evident that the equilibrium orientation of these side chains is different in system I and system II. Thus the bend introduced by proline influences the equilibrium side chain conformations. A superposition of MD equilibrated system I with the structure of helix F in Henderson's BR model⁸ (with only Trp-182, Tyr-185, and Trp-189 side chains) is shown in Figure 9c. Tyr-185 conformation is different in the two structures because it interacts with Asp-212 of helix G in BR, whereas the helix F model (system I) is considered in isolation in the present MD studies. It should, however, be noted that the comparison of the present results with the BR model proposed by Henderson et al.⁸ has limitations because of the low accuracy of the structure and also the simulations were carried out on the molecule (system I) in vacuum in which only aromatic side chains of helix F were considered.

Analysis of the Nature of Proline-Containing Helix

Although proline-containing helices in globular proteins and membrane proteins have been identified as α -helical in nature, there are several ways in which they differ from an ideal α -helix. From the present MD studies and from crystal structure analysis,^{2,3} it has become possible to characterize the structural properties of proline-containing helices. In order to carry out a detailed characterization, the properties of α -helices in general are outlined below.

Conformational energy calculations show³⁶ that a trough of low energy extends from $\phi = -47^\circ$, $\psi = -60^\circ$ to $\phi = -88^\circ$, $\psi = -25^\circ$ for an α -helix. Though the dihedral angles in the proline region fall within this trough, they indeed belong to "different types of α -helical structures."

The standard α -helix proposed by Arnott and Wonacott²⁸ is usually referred as α_I -helix. The other type of helix in the right-handed α -helical region corresponds to the second of two possible solution for the dihedral angles of a helix with constant n (number of residues per turn) and h (the height per residue). This helix is referred to as α_{II} -helix and was first proposed by Nemethy et al.³⁷ The helical parameters of standard α_I and α_{II} helices are given in Table VI. A comparison of these parameters show

Fig. 7. MD trajectories of χ_1 of Y-185 for system I and II.

differences in the ϕ , ψ angles and hydrogen bond parameters. In α_{II} -helix the hydrogen bonds are slightly weaker since the plane of the peptide unit is tilted with respect to helix axis and the NH group of this helix points towards the axis. The peptide unit is nearly parallel in the α_I -helix. The third type of helix in the α -helical region is designated as a goniometric helix³⁸ in which the values of ϕ and ψ of α_I -helix interchange almost exactly (i.e., $\phi = -47.5^\circ$, $\psi = 57.5^\circ$) with small changes resulting in the helical parameters.

The nature of the proline-containing helix can be characterized in terms of the above different α -helical structures. The analysis of backbone internal parameters are discussed in the first section of Results and Discussion. In fact, the average values of the dihedral angles (ϕ_{p-3}, ψ_{p-3}) and (ϕ_{p-2}, ψ_{p-2}) are close to those for the α_{II} helix (Table I). As a result, the hydrogen bond parameters for residues p-2 and p-3 are also close to those for the α_{II} -helix (Table

III). The average of the hydrogen bond angle NHO is between that of α_I and α_{II} helices and the rms fluctuations of all the parameters (described in Table III) show that these residues can access both α_I and α_{II} structures. The values of (ϕ_{p-1}, ψ_{p-1}) are close to the goniometric helix. The other parts of the helix show that the (ϕ, ψ) values are close to those for the α_I -helix. This can be compared with those parameters obtained from the MD studies of system II, in which all the (ϕ, ψ) angles remain close to α_I -helix throughout the simulation.

The deviation in the value of the virtual torsion angle ρ from the standard value can be explained if the proline-containing helix is considered as a mixture of different α -helical structures. The virtual torsion angles of C'' atoms for various standard α -helical structures and their mixtures are given in Table VII. It is clear that both α_I and α_{II} have the same value 50° though the ϕ, ψ angles are different. Similarly this value for the goniometric helix is al-

Fig. 8. (a) MD trajectories of χ_1 and χ_2 of W-189 for system I. (b) MD trajectories of χ_1 and χ_2 of W-189 for system II. Figure continues on page 38.

most the same as that for the α_I and α_{II} helices. However, the virtual torsion angle $C_i^\alpha-C_{i+1}^\alpha-C_{i+2}^\alpha-C_{i+3}^\alpha$ has the value 68° when residues $i, i+1$, are in the α_{II} -helical, $i+3$ in the α_I -helical conformation and residue $i+2$ is in the goniometric conformation. This is analogous to ρ in the proline-containing helix. Thus the proline-containing helix is mainly α_I in character with pronounced α_{II} and goniometric helical character in the helical turn ending with the proline residue. The MD studies have shown that fluctuations in this region are higher, with the values approaching near α_I character at times.

High-resolution crystal structures and analysis of proteins containing proline in the middle of α -helix are slowly emerging. A number of experimental studies characterizing the secondary structures of membrane proteins with proline-containing helices have been carried out.³⁹ However, the secondary structural information derived from these studies

has not been precise. The shift and the intensity differences in amide I and amide II IR bands in BR, almethicin, and other membrane proteins³⁹ suggest a deviation from the ideal α_I -helical structure. However, no unique structure has been arrived at using this technique. In the case of BR, a mixture of $\alpha_I + \alpha_{II}$,⁴⁰ $\alpha_I + \beta$,⁴¹ $\alpha_I + 3_{10}$,⁴² and predominantly α_{II} ⁴³ structures have been suggested. Far-UV CD spectra of BR also suggest that the secondary structure of BR is not pure α_I -helix, but has substantial α_{II} character.⁴⁰ Nevertheless, the exact position and nature of the deviation become available only from high-resolution crystal structures. The crystal structure analysis of globular proteins³ and the energy minimization studies⁴ have given more details on the precise structural details of proline-containing α -helices. Further, the present MD simulation has given information on the flexibility in different parts of these helices. These studies also give the

Fig. 8b. Legend appears on page 37.

extreme limits of values that can be taken up by such structures and hence can be useful in interpretation of experimental results.

CONCLUSIONS

The proline residue in the middle of an α -helix has an important role to play in the structure of proteins. The presence of such secondary structures is particularly significant in membrane proteins. The following features have emerged from the present MD studies on helix F model of bacteriorhodopsin (BR) as well as from our recent crystal structure analysis.³

1. Some of the backbone internal parameters in the helical turn ending with proline deviate from ideal α -helical values.

2. The deviations in the internal parameters from an ideal α -helix give rise to a bend in the helix. This bend can be characterized directly by several parameters such as kink angle (α), wobble angle (θ), vir-

tual torsion angle $C_{p-3}^{\alpha}-C_{p-2}^{\alpha}-C_{p-1}^{\alpha}-C_p^{\alpha}$ (ρ), and a specific hydrogen bond ($O_{p-3} \cdots N_{p+1}$). These parameters are evaluated for the MD simulated structures. The MD average and the rms fluctuations are given for all parameters. The MD trajectories of the bend related parameters show that the helix frequently fluctuates between an ideal α -helix and a bent structure. A high degree of correlation is exhibited between the bend related parameters. Among the backbone internal parameters, only the dihedral angle ϕ_{p-1} is correlated to a reasonable extent with the bend.

3. The nature of distortions in the proline region is characterized in detail in terms of related secondary structures such as α_I , α_{II} and goniometric helices. The interpretation of the experimental results regarding the secondary structures of membrane proteins are discussed.

4. The MD average side chain conformations of helix F model of BR (Trp-182, Tyr-185, Trp-189) is

Fig. 9. (a) Stereo diagram of two structures of system I (without alanine residues) are superposed. One of them corresponds to the MD average structure given in Table IV and the other structure is close to the starting conformation obtained at the beginning of the equilibration. (b) Stereo diagram of three structures of system II (without alanine residue) in which the side chain orientations are different. Two of the plotted structures were obtained at 10 ps (χ_1 (W-182) = -167.1° ; χ_2 (W-182) = 69.5° ; χ_1 (Y-185) =

168.9° ; χ_1 (W-189) = -78.8° ; χ_2 (W-189) = 45.8°) and at 90 ps (χ_1 (W-182) = -72.9° ; χ_2 (W-182) = 174.7° ; χ_1 (Y-185) = 167.8° ; χ_1 (W-189) = -172.8° ; χ_2 (W-189) = 13.4°). The third structure is close to the starting conformation. (c) Stereo diagram of the average structure obtained for system I superposed on the crystal structure of the helix F of BR (only trptophan, tryosine, and proline residues are represented and the values corresponding to the conformation of these residues are given in Table VI).

TABLE V. Average and rms Fluctuations of Tryptophan and Tyrosine Side Chain Dihedral Angles (in Degrees)*

Side chain dihedral angle [†]	System I	System II	In bacteriorhodopsin [‡]
χ_1 (W-182)	-56.0 (± 10.9)	-156.2 (± 47.7)	-79.2
χ_2 (W-182)	154.0 (± 18.7)	91.9 (± 31.7)	177.7
χ_1 (Y-185)	178.0 (± 10.0)	172.7 (± 13.8)	-65.2
χ_1 (W-189)	-179.5 (± 11.5)	-167.2 (± 36.3)	-145.1
χ_2 (W-189)	71.2 (± 22.8)	62.1 (± 29.6)	101.5

*The notations for tryptophan and tyrosine residues are W-182, Y-185, and W-189 for systems I and II. This corresponds to the positioning of the residues Trp-182, Tyr-185, and Trp-189 in the helix F of BR. The same nomenclature is followed in the text and also in Figures 6–8.

[†] $\chi_1 = \text{N-C}^\alpha\text{-C}^\beta\text{-C}^\gamma$

$\chi_2 = \text{C}^\alpha\text{-C}^\beta\text{-C}^\gamma\text{-C}^{\delta 1}$

[‡]From the coordinates obtained by Henderson (personal communication).

TABLE VI. Standard Helical Parameters for Idealized α_I - and α_{II} -Helical Structures

Helical parameters*	α_I	α_{II}
n	3.62	3.62
h	1.50 Å	1.50 Å
ϕ	-57.4°	-70.5°
ψ	-47.5°	-30.0°
d (N . . . O)	2.86 Å	3.00 Å
d (H . . . O)	1.88 Å	2.12 Å
N H O	164.2°	145.7°
H N O	10.3°	23.5°
Distance between H's of adjacent NH groups	2.79 Å	2.53 Å

*n = the number of residues per turn; h = the height per residue; d (A . . . B) = the distance between atoms A and B. See also ref. 32.

TABLE VII. Virtual Torsion Angle of C $^\alpha$ Atoms (in Degrees) for Various Standard α -Helical Structures

Description*	Values
$\alpha_I\text{-}\alpha_I\text{-}\alpha_I\text{-}\alpha_I$	50.0
$\alpha_{II}\text{-}\alpha_{II}\text{-}\alpha_{II}\text{-}\alpha_{II}$	50.0
gm-gm-gm-gm	49.5
$\alpha_I\text{-}\alpha_I\text{-gm-}\alpha_I$	57.6
$\alpha_{II}\text{-}\alpha_{II}\text{-gm-}\alpha_I$	68.0

*The conformation assumed by each residue is indicated; gm—goniometric helix.

compared with that given for BR structure⁸ and with corresponding position in an ideal α -helix. The bend in the helix due to proline gives rise to a specific Trp-182 orientation, which is different if it were to be present in an ideal α -helix. Also, the flexibility in the side chain conformations is less in the proline-containing helix. The conformation of Tyr-185 in the BR structure is decided by the residues of neighboring helices such as Asp-212.

In summary, the conformation and the flexibility involved in proline-containing α -helices are characterized in detail by a set of geometrical parameters. This has aided in quantitative evaluation of the conformational properties of such helices, which acts as a tool for model building and refinement of low-resolution structures. The results on secondary structures of membrane proteins by spectroscopy and the conformational properties of channel forming peptides passing through the membranes can be better understood in light of present studies on proline-containing α -helices.

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