A model for transmembrane helix with a *cis*-Proline in the middle

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Abstract The presence of a higher percentage of Proline in the transmembrane helices of transport proteins indicates that they are involved in the function of these integral membrane proteins (IMPs). In many cases, the possible involvement of *cis-trans* isomerization in function/folding of IMPs has been suggested. The introduction of *cis*-Pro in an ideal α-helix results in a helix-turn-helix motif. A molecular dynamics (MD) simulation is carried out on the sequence ACE-$(ALA)_{10}$-*cis*-Pro-$(ALA)_{10}$-NME with ideal α-helical structure to investigate if and how a straight helix can accommodate a *cis*-Pro. The analysis of the conformations accessed during MD simulation showed that the residues near *cis*-Pro can adopt alternate conformations other than the right-handed helical conformation such that an almost straight helix is obtained. This may have implications in the involvement of *cis-trans* isomerization in folding and/or function of IMPs.

Key words: Helix; Molecular dynamics; *Cis*-Proline; Transmembrane helix; *cis-trans* Isomerization

1. Introduction

The structural and functional role of the imino acid proline in proteins has indeed been a fascinating one. It has been recognized long back that proline is a chosen residue for turns in globular proteins [1] and it exists as *cis*-peptide in many cases [2,3] as shown in Fig. 1. Since 10% of peptidyl-proline imide bonds occur as *cis* isomer in native globular proteins, *cis-trans* isomerization can be the rate limiting step in the folding process [4–6]. The wide distribution of rotamases like FK506 binding protein (FKBPs) [7,8] and cyclophilins [9], catalyzing the *cis-trans* prolyl isomerization in cells, suggests that isomerization of the imide torsion angle may be important in vivo [10,11]. It has also been suggested that the prolyl isomerization may be involved in regulatory switches [12]. Computational studies on the *cis-trans* isomerization of the proline dipeptide has also been conducted and a strong dependance of the activation barrier on ψ has been reported [13]. Inspite of the severe restrictions that it places on the backbone geometry, proline also occurs in the middle of helices in globular proteins [14–16] and membrane proteins [17–19] producing a kink [14,16,20,21]. The conformations and dynamics of such helices are well characterized [22–25]. It is also seen more often in the transmembrane helices (TMHs) than in globular proteins' helices [26]. Indirect evidence such as its higher percentage of occurrance clearly indicates a functional role in transport proteins [17–18]. Experimental studies on the conformational change in Bacteriorhodopsin (bR) and Rhodopsin (Rh) associated with their function indicate the involvement of the peptide bond at the Pro residues as 'hinges' [27–29]. The findings of Strader et al., on Pro to Ser mutation in β-adrenergic receptor had suggested the involvement of P323 in a receptor processing event such as folding [30]. Also, the ninaA visual transduction mutant of *Drosophila melanogaster* was found to be a PPIase, and it was suggested that it may be involved in the processing of rhodopsin or it may function as a Pro isomerase involved in the conformational changes during visual transduction [31]. The rate of XXX-Pro isomerization observed in peptides is in the order of several seconds to fractions of a second, and the opening and closing of channels is in millisecond time scales [18]. The ease with which Pro can be mutated in several membrane proteins and the protein activity retained may suggest that peptidyl-proline isomerization has no role to play in the functioning of these. However, these mutations studies assay for the activity and do not involve the detection of conformational changes [18]. Thus, as yet, these experimental observations do not constitute conclusive and direct evidence to reject or accept the involvement of *cis-trans* isomerization of Pro in membrane protein folding and/or function and more studies are needed to be carried out to understand this problem.

It is interesting to investigate the above problem from the structural point of view. If *cis-trans* isomerization were to take place during the folding and/or functioning of IMPs, it is natural to ask whether a *cis*-Pro can be accomodated in an α-helix and if so, how stable is the structure. The *cis*-Pro residues observed in globular proteins generally occur in turns [3] and reverse the chain direction as shown in Fig. 1. Introduction of a *cis*-Pro in an ideal helix will result in a helix-turn-helix motif. Such a change makes it difficult for the helix to traverse across the membrane, resulting in a drastic change in the protein structure. Hence, in order to maintain a near-native structure of the IMP, it is necessary that a *trans* to *cis* isomerization at XXX-Pro in TMH should be accompanied by changes in other local parameters. Deber and coworkers in their pursuit along this direction have shown by ^{1}H and ^{13}C-NMR that the Pro leads to a good amount of flexibility of the main chain [36]. However, this study does not indicate the presence of *cis*-Pro. Further, they did a random generation of *cis*- and *trans*-Pro containing helices, and energy minimized and clustered them on the basis of their minimum energies [37]. The best *cis*-Pro structure generated was found to be bent on the same side as the *trans*-Pro helix. The bend, however, was too large (about 90°) and such a conformation can significantly alter the protein structure. In our previous simulation studies [38–39], we have characterized the alternative conformation, flexibility and rigidity of TMHs of bR and Rh by molecular dynamics (MD) simulation. We have adopted a similar procedure in this study to investigate the possible conformation of *cis*-Pro containing

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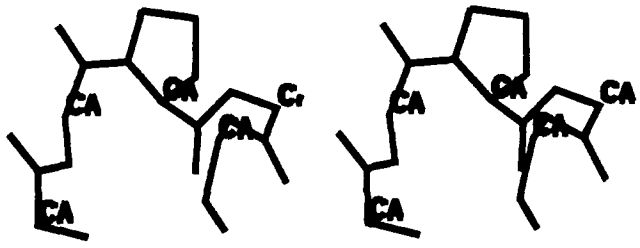

Fig. 1. A stereo plot of a peptide with a *cis*-Pro turn.

α-helix (ACE-$(ALA)_{10}$-*cis*-Pro-$(ALA)_{10}$-NME). A variety of helical structures with *cis*-Pro, including an almost straight helix have been accessed during the simulation and the details of these studies are given below.

2. Methods

An ideal right-handed α-helix with the amino acid sequence ACE-$(ALA)_{10}$-*cis*-Pro-$(ALA)_{10}$-NME was generated using the Biopolymer module of InsightII (Biosym Inc.). The protocol followed for simulation and analysis was the same as that given in our previous studies [38,39]. We give a short account of it below. The helix was minimized to a root means squared deviation of 0.01 kCal/Mol/Å in energy and it was heated to 300 K in steps of 50 K/5 pico second (ps). During the heating, using the coupled to heat-bath algorithm [40], the backone atoms were restrained. The equilibriation was continued up to 40 ps as mentioned earlier [38,39] and a free simulation conducted for 500 ps. Four consecutive residues which were in the right handed helical (RH) region was considered as a turn. The kink angle of the helix was defined as follows. Two helices consisting of residues 1 to $(p-1)$, where p is the sequence number of Pro, and $(p+1)$ to 21 were considered. The axis for each helix was defined as the line passing through the centroid of the C_α atoms and at a least squared distance from them [41]. The angle between these two lines defines the kink angle. A donor-acceptor of <3.5Å was considered as a hydrogen bond (H-Bond). All simulation were done using the AMBER 4.0 suite of programmes [42] and the analysis was conducted using the in-house developed programmes.

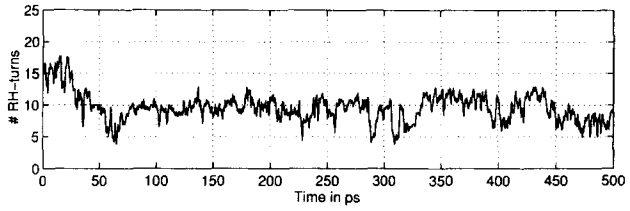

Fig. 2. Number of RH-turns trajectory.

3. Results and discussion

The number of RH-turns shows an initial value of 15 which settles down to 10 during the course of the simulation (Fig. 2). This indicates the presence of a considerable amount of helical character throughout the simulation period. The reduction in the number of RH-turns is primarily due to the deviations in (ϕ,ψ) of residues $p-2$ to $p+2$, where p is the sequence number of proline. These structural transitions seen in residues A9 to A12 are shown in Fig. 3. The residue $(p-1)$ i.e., A10 shows a tendency to switch over to the extended region. By ≈190 ps both (ϕ_{A10}, ψ_{A10}) go to ±180° region. Φ_{A10} comes back to g− region but ψ_{A10} stays in the extended region for the rest of the simulation period. Thus, roughly in the first half of the

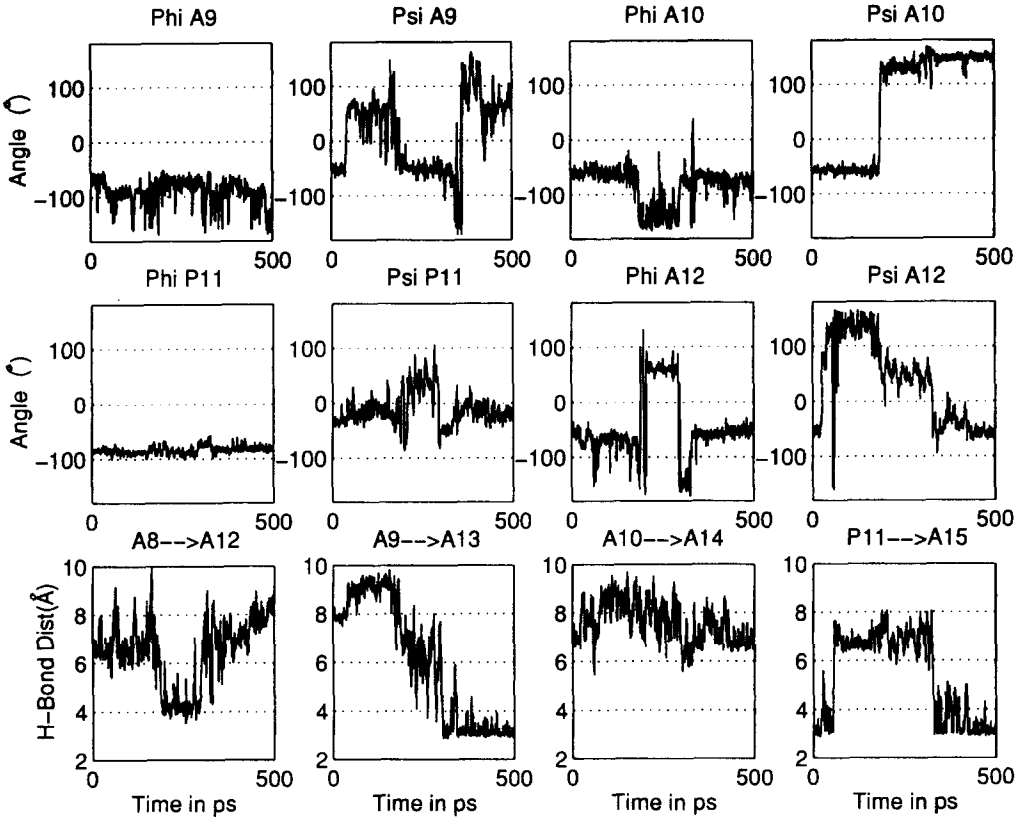

Fig. 3. Trajectories of selected (ϕ, ψ) and main chain $i-i+4$ H-Bonds. The titles on H-Bond trajectories indicate the residues involved, for e.g. A8–>A12 indicates the distance $O'_{A8}-N_{A12}$.

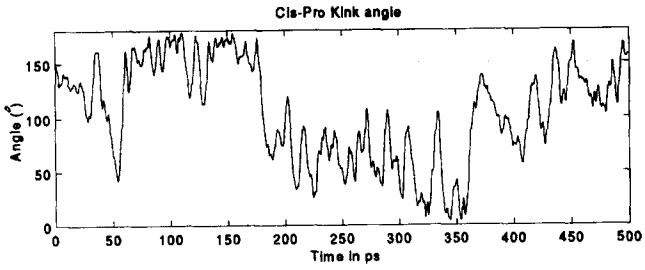

Fig. 4. The kink angle trajectory of the helix.

simulation period, the residue $(p-1)$ is in the α-helical conformation and in the second half it is in the extended β-conformation. This is in agreement with the crystal structure analysis [3] in which the β-region was found to be predominant for the residue preceeding *cis*-Proline. Associated changes in (ϕ,ψ) of A9–A12 are also shown in Fig. 3. As a result of this, the backbone hydrogen bond chain in the vicinity of the Pro residue is broken. The backbone $i-(i+4)$ H-Bonds involving the above residues are also shown in the lowest panel of Fig. 3. The H-Bond A10–>A14 is clearly broken through out the simulation period with the O'_{A10}--N_{A14} distance being >6Å for most of the simulation period. (The absence of HN at position p means that the O'_{p-4}-->N_p H-Bond is absent in the helix). Similar behaviour is exhibited by the $(p-3)-(p+1)$, i.e. A8–>A12 H-Bond except that corresponding to (ϕ_{A12}, ψ_{A12})'s transition to $g+$ region, this H-Bond comes down to ≈4.5Å. A correlation is also seen in the reduction of the distance O'_{A9}--N_{A13} and the behaviour of (ϕ, ψ) of residues $p-2$ to $p+1$. The H-Bond distance involving the carbonyl oxygen of P11 with N_{A15}, shows a transition to >6Å after ≈50 ps of simulation but it comes back to within ≈3.5Å after 320 ps. This is the time by which the dihedral angles ψ_p, ϕ_{p+1} and ψ_{p+1} come back to $g-$ region. The gross structural transformation as measured by the kink angle between the two helices (Helix I: residues 1 to $(p-1)$; Helix II: residues $((p+1)$ to 21) is shown in Fig. 4. The starting structure of an ideal α-helix with *cis*-Pro in the middle is highly bent with kink angle of ≈150°. Structures fluctuating about this value are seen upto ≈175 ps. Drastic structural transformations have taken place in the next 200 ps. In this interval, the kink angle is around 20–30° which corresponds to the value in the case of a *trans*-Pro containing helix

Table 1
Summary of the conformational parameters of the minimized snapshots

Snapshot	Kink Angle (degrees)	(ϕ, ψ) out of RH-region	H-Bonds below-above Pro
min	154.8		3–4
50 ps	75.5	$\phi_{p-2}, \psi_{p+1}, \phi_{p+2}$	6–5
100 ps	170.7	$\phi_{p-2}, \psi_{p+1}, \phi_{p+2}$	5–6
234.6 ps	85.7	$\phi_{p-3}, \phi_{p-2}, \phi_{p-1}$ $\psi_{p-1}, \psi_p, \phi_{p+1}$ $\phi_{p+1}, \phi_{p+8}, \psi_{p+8}$ ϕ_{p+9}, ψ_{p+9}	3–5
300 ps	92.5	$\phi_{p-3}, \psi_{p-3}, \phi_{p-1}$ $\psi_{p-1}, \phi_{p+1}, \psi_{p+1}$	5–6
354.2 ps	17.2	$\phi_{p-2}, \psi_{p-2}, \psi_{p-1}$	6–6
400 ps	75.5	$\phi_{p-3}, \psi_{p-2}, \psi_{p-1}$	6–5
450 ps	149.9	ψ_{p-2}, ψ_{p-1}	5–5
500 ps	156.6	$\phi_{p-2}, \psi_{p-2}, \psi_{p-12}$	6–5

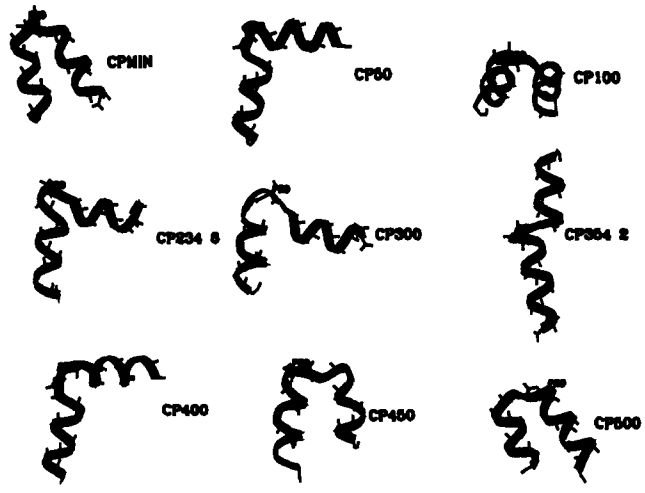

Fig. 5. Ribbon diagram of the minimized snapshots.

[22–25,43,44]. It is interesting to note that at times the kink angle has reached values close to 0°, indicating a straight helix. The behaviour after 400 ps is similar to that in the initial period and leads to a highly bent structure. Throughout the simulation period the A10–P11 peptide bond remained in the *cis*-conformation.

Thus, the simulation gave rise to a variety of *cis*-Pro helix conformations, some of which are minimized and the ribbon diagram are presented in Fig. 5. It can be seen that a multiplicity of conformations ranging from completely bent to an almost straight helix (CP354_2) are obtained during the simulation. The striking result of this simulation is that *cis*-Pro can indeed be accomodated in a helix and we have obtained the parameters for such an almost straight helix. Summary of the structural parameters of these minimized snapshots given in Table 1 shows that the number of backbone $i-(i+4)$ hydrogen bonds is highest in this structure. Further, only the parameters ϕ_{p-2}, ψ_{p-2} and ψ_{p-1} are out of the RH-region and their values are −110°, −172° and 147°, respectively. Thus, we see that a local distortion near the Pro residue effectively cancels out the chain reversal property of the *cis*-Pro peptide bond as shown in Fig. 1. Such an ability of helices to undergo local distortions while maintaining the overall helical character and direction of propogation has been observed in our previous simulation studies of bR and Rh helices [39,40]. During the simulation of the 7 helices of bR and 7 helices of Rh we had frequently encountered straight helices with a pair of residues (i and $i+1$) such that, $\phi_i = -90°$, $\phi_{i+1} = -150°$ and $\psi_{i+1} = -47°$, and this pair is out of the RH-region. Hence, we believe that appropriately chosen non-Rh values of (ϕ, ψ) can be accommodated in a straight helix, which is energetically feasible and gives rise to local distortions only. It is significant that such a feature is common to a general sequence, since it lends support to biological activities mediated through TMHs. A similar feature has also been observed by Deber et al. in their minimization studies on randomly generated *cis* and *trans* Pro containing helices and they have suggested this as being important in the structure and function of IMPs which contain Pro helices [37]. Further, it is to be noted that it is not only important to retain the length of the helix, but also important that the helices on either side of the distortion are not significantly rotated with respect to each other, since the changes in the interactions with the rest of the

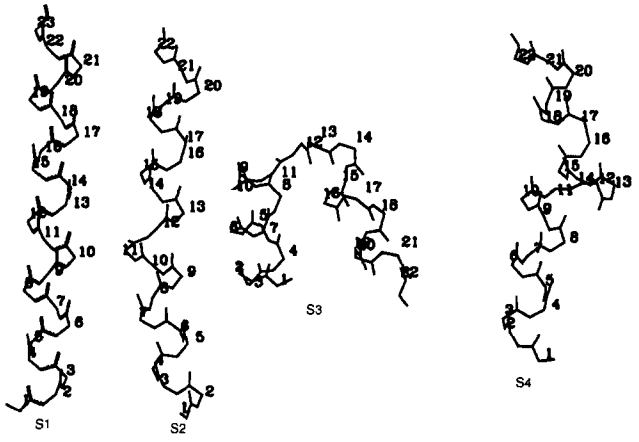

Fig. 6. Helices with variations in (ϕ, ψ) values. S1: ideal α-helix; S2: ideal α-helix with $\phi_{11} = -90°$, $\psi_{11} = -40°$, $\phi_{12} = -150°$ and $\psi_{12} = -47°$; S3: ideal α-helix with $\phi_{11} = -110°$, $\psi_{11} = -172°$ and $\phi_{12} = -60°$; S4: all parameters same as in S3 except for a *cis*-peptide bond at peptide 12–13.

protein should be minimal. In order to verify this fact, helices with local distortions have been generated and plotted in Fig. 6. S1 is an ideal α-helix and S2 is also an α-helix with $\phi_{11} = -90°$, $\psi_{11} = -40°$, $\phi_{12} = -150°$ and $\psi_{12} = -47°$. A major change in the position of only the residue 10, 11 and 12 is seen by this distortion. The change in the helix orientation after these residues is not significant. The helix S4 is a near straight helix with a *cis*-peptide bond at 12–13 and $\phi_{11} = -110°$, $\psi_{11} = -172°$ and $\phi_{12} = -60°$, (the values observed for the minimized snapshot at 354.2 ps during the present *cis*-Pro simulation). In this helix, the position of the residues 11–15 are changed with respect to that of an ideal α-helix. Thus the residues in one helical turn are displaced and the helix direction proceeds normally with the i–$(i+4)$ residues, 2–6–10–18–22, facing the same side. The incorporation of these (ϕ, ψ) values in an ideal α-helix with all the peptide bonds in *trans* conformation results in a helix-turn-helix structure as shown in S3.

In summary, we have shown that *cis*-Pro residue can be accommodated in a straight helix and the parameters are obtained to model such a helix. The MD simulation results show that a helix with *cis*-Pro can access multiple conformations, ranging from a helix-turn-helix like structure to a straight helix and it is possible to introduce local distortions without affecting the general direction and propogation of the helix. These findings are significant in the light of the plausible involvement of *cis-trans* isomerization in the folding and function of IMPs.

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