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Effect of the valine–threonine constraint on the dynamics of the proline helix – A molecular dynamics study

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Abstract. Proline residues in helices play an important role in the structure of proteins. The proline residue introduces a kink in the helix which varies from about 5° to 50°. The presence of other residues such as threonine or valine near the proline region can influence the flexibility exhibited by the kinked helix, which can have an important biological role. In the present paper, the constraint introduced by threonine and valine on a proline helix is investigated by molecular dynamics studies. The systems considered are (1) a poly-alanine helix with threonine-proline residues (TP) and (2) a poly-alanine helix with valine–threonine–proline residues (VTP), in the middle. Molecular dynamics simulations are carried out on these two systems for 500 ps. The results are analyzed in terms of structural transitions, bend-related parameters and sidechain orientations.

Keywords. Proline helix; valine-threonine constraint; molecular dynamics.

1. Introduction

It has been well-recognized that proline residues in proteins have important structural and functional roles to play. Their structural roles in turns, bends and helices are well established (Chou and Fasman 1974, 1977; Anfinsen and Scheraga 1975; Robson and Suzuki 1976; Rose *et al* 1985; Brandl and Deber 1986; Barlow and Thornton 1988; Richardson and Richardson 1988; Woolfson and Williams 1990; MacArthur and Thornton 1991). We have been particularly interested in characterizing the bend introduced by proline in helices and examining the effect of constraints on such helices (Sankararamakrishnan and Vishveshwara 1990–1993). Recently, we had focused on the constraints introduced by threonine in different positions on a proline helix which gave rise to a variety of bent structures during molecular dynamics simulation (Shobana and Vishveshwara 1993). It appears that the nature of the bend introduced by proline in helices and the extent of flexibility exhibited by the secondary structure can be modulated by certain types of constraints.

Recent studies have shown that repetitive sequences containing alanine–threonine–proline (ATP) and valine–threonine–proline (VTP) appear in nucleolin, a ubiquitous protein in eukaryotic cells, which is thought to play a direct role in pre-RNA transcription and ribosome assembly (Lapeyre *et al* 1987; Suzuki 1991). Hence we believe it is interesting to study the structure and dynamics of the helices which contain ATP and VTP sequences. The present work is intended to gain an

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understanding of the constraint introduced by a bulky residue (valine) on the proline helix.

The dynamics of a helix with ATP sequence was previously investigated for 200 ps (Shobana and Vishveshwara 1993), in which a highly bent structure (DP2) was found until 140 ps and a normal helix (DP1) with proline was observed between 140 ps and 200 ps. In the present study, the simulation on ATP is extended up to 500 ps in order to see if further structural transitions occur. The VTP system is simulated for 500 ps under identical conditions. The results of the two simulations are compared and the extent of rigidity introduced by valine on the structural transitions, fluctuations in the bend in the helical structure, and the sidechain orientation of the threonine residue, are discussed.

2. Methods

To explore the conformational properties of proline containing α-helices with constraints, two systems were chosen. They are (1) ATP: Ace–$(\text{Ala})_{12}$–Thr–Pro–$(\text{Ala})_{10}$–NHMe, and (2) VTP: Ace–$(\text{Ala})_{11}$–Val–Thr–Pro–$(\text{Ala})_{10}$–NHMe. The system 2 has a valine in addition to threonine as the constraint. The valine orientation was chosen such that the N–C^{α}–C^{β}–H^{γ} is + 60° (Bhat *et al* 1979; Benedetti 1977). In the two systems, the initial conformation includes the hydrogen bond interaction between the O^{γ} of threonine sidechain and the carbonyl oxygen of the *p*-5th residue. The 200 ps simulation of ATP has been already reported (Shobana and Vishveshwara 1993). The nomenclature used for the ATP system and the two structures obtained during the simulation are DP, DP1 and DP2 in the earlier study (Shobana and Vishveshwara 1993) and TP, TP1 and TP2 in the present study respectively. The structure for VTP is generated from the starting unoptimized structure of TP by replacing the alanine below threonine by valine and is designated as VTP.

The initial structures were energy minimized using the AMBER program (Weiner and Kollman 1981; Weiner *et al* 1984, 1986) adapted to run on an INTEL 860 machine in which the potential function describing the interaction of the system has the following form

$$\begin{aligned}E_{\text{total}} = & \sum_{\text{bonds}} K_R(R-R_0)^2 + \sum_{\text{angles}} K_\theta(\theta-\theta_0)^2 \\ & + \sum_{\text{dihedrals}} V_n/2[1+\cos(n\phi-\gamma)] \\ & + \sum_{i<j\ \text{non-bonded}} [B_{ij}/(R_{ij})^{12} - A_{ij}/(R_{ij})^6 + q_i q_j/\varepsilon R_{ij}] \\ & + \sum_{\text{H-bonds}} [C_{ij}/(R_{ij})^{12} - D_{ij}/(R_{ij})^{10}].\end{aligned}$$

The partial atomic charges and the force field parameters used were those provided in AMBER. All atoms including the hydrogens were considered in the calculations. A distance-dependent dielectric constant $\varepsilon r_{ij} = r_{ij}$ was employed for the evaluation of electrostatic interaction energies. The structures were refined until the r.m.s. gradient of energy was less than 0·001 kcal/mol Å. For the minimization of the two systems, a steepest descent method was used for the first 1000 cycles and then a conjugate gradient method was used until convergence.

MD studies were carried out on TP and VTP using the AMBER-MD module. All bonds involving hydrogens were constrained using the SHAKE algorithm. Simulations were carried out in vacuum at 300 K. During the heating process the temperature was raised drastically and the system reached the temperature of 300 K in about 0·3 ps. A time step of 0·001 ps was chosen and the MD run was performed for 500 ps including an equilibration period of 25 ps. The atomic coordinates were stored at 0·1 ps interval. A few points from the MD trajectories of TP and VTP were minimized to a convergence of 0·005 kcal/mol Å.

The two simulations were then analyzed in terms of their conformational parameters; backbone dihedral angles ϕ, ψ and ω, and the virtual torsion angles as described in figure 1. The bend due to proline was characterized by means of kink angle (θ) (Sankararamakrishnan and Vishveshwara 1991), and the virtual torsion angle $C^{\alpha}_{p-3} - C^{\alpha}_{p-2} - C^{\alpha}_{p-1} - C^{\alpha}_{p}(V_{\rho})$. In addition, to monitor the alpha helical nature, hydrogen bond interactions were analyzed as described in the earlier report (Shobana and Vishveshwara 1993). The backbone hydrogen bond $N_{p+1} \cdots O_{p-3}$ is designated as h_{ρ}. The sidechain-backbone hydrogen bonds of threonine $O^{\gamma} \cdots O_{p-4}$ and $O^{\gamma} \cdots O_{p-5}$ are designated as d_{i-i-3} and d_{i-i-4}, respectively. The proline pucker was monitored by means of the ring dihedrals χ_1 $(N - C^{\alpha} - C^{\beta} - C^{\gamma})$ and $\chi_2(C^{\alpha} - C^{\beta} - C^{\gamma} - C^{\delta})$. When χ_1 has positive values and χ_2 has negative values, the proline is said to have DOWN pucker and when χ_1 is negative and χ_2 is positive it is said to have UP pucker (Sankararamakrishnan and Vishveshwara 1991). The valine sidechain was monitored by means of dihedral angle χ_1 $(N - C^{\alpha} - C^{\beta} - H^{\beta})$.

3. Results and discussion

MD studies have been carried out on proline containing α-helices with constraints in the form of threonine and threonine with valine. The results and analysis of the MD simulation of TP and VTP systems are presented here.

The trajectories of helix bend parameters (θ and V_{ρ}) and threonine-related structural parameters (χ_t, d_{i-i-3} and d_{i-i-4}) of the TP system are given in figure 2a. Similar trajectories for VTP system are given in figure 3. The TP system assumes a highly bent conformation between 25 and 140 ps. The potential energy of the system given in figure 2b (including the equilibration period) do not exhibit any conspicuous changes which indicates that the system is stable at 300 K and sufficiently equilibrated. The characteristics of the structure (TP2) between 25 and 145 ps was discussed earlier (Shobana and Vishveshwara 1993) and is a bent structure in which an $i \rightarrow i-2$ hydrogen bond $(N_{p-1} \cdots O_{p-3})$ has gained stability and ψ_{p-2} has assumed a value around +45°. The features observed are characteristic of bends and are clear indications of a non-helical nature. The kink angle (θ), the virtual torsion angle (V_{ρ}) and the threonine sidechain (χ_t) assume values around 76°, 156° and −17°, respectively, which can be seen from figure 2a. The threonine O^{γ} has a good d_{i-i-3} hydrogen bond (d_{i-i-3}) with the carbonyl backbone O of the $i-3$ residue in this conformation. After 140 ps, the system returns to a normal proline helix structure ($V_{\rho} \sim 71°$, $\theta \sim 39°$) and remains in this conformation until 500 ps. During this period the threonine χ value is maintained around −60° with a brief transition to +60°

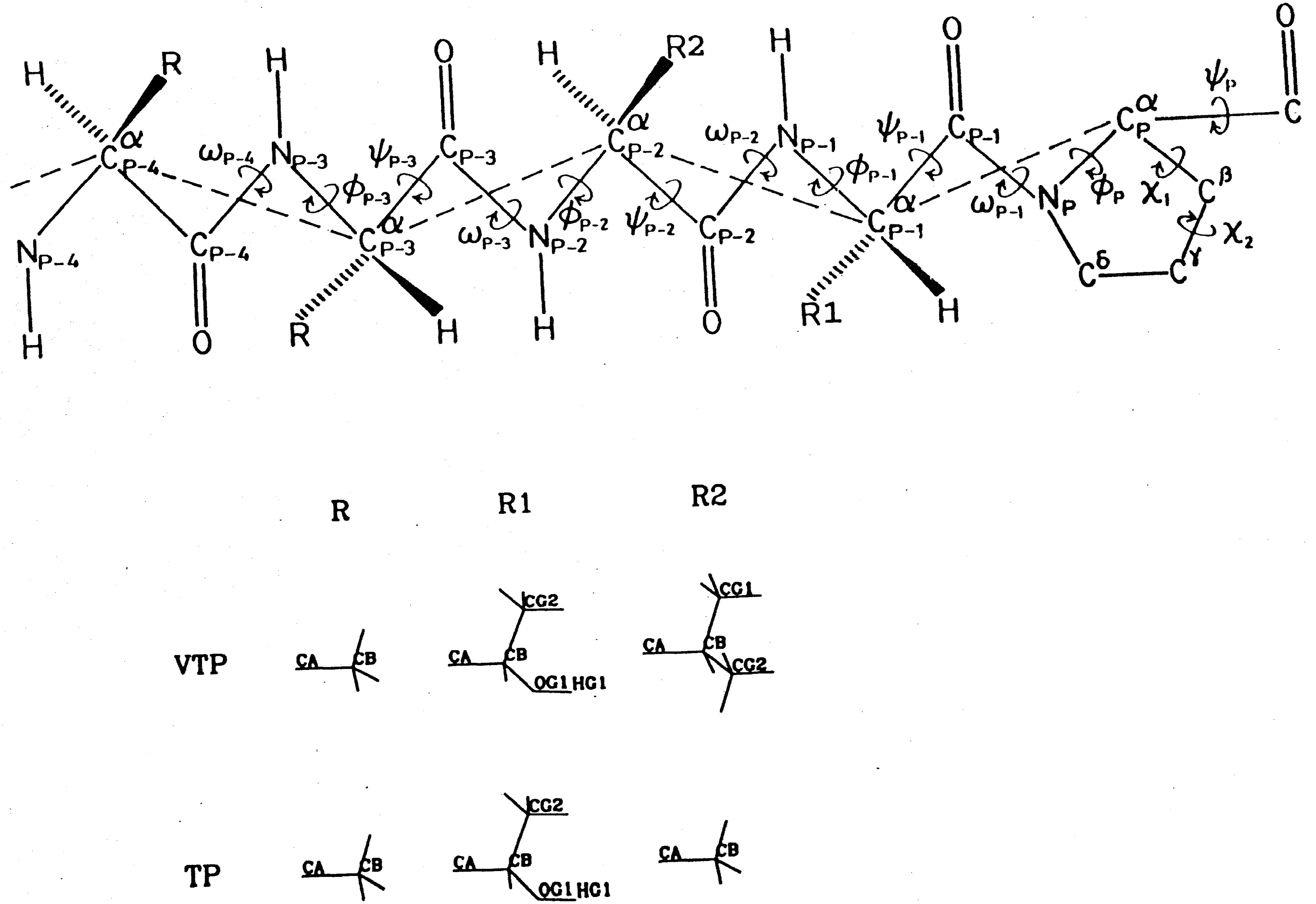

Figure 1. The conformational parameters of the polypeptide chain containing proline, threonine, valine and alanine residues. The relative positions of these residues and the backbone torsion angles are indicated in the figure. The virtual torsion angle is represented by broken lines.

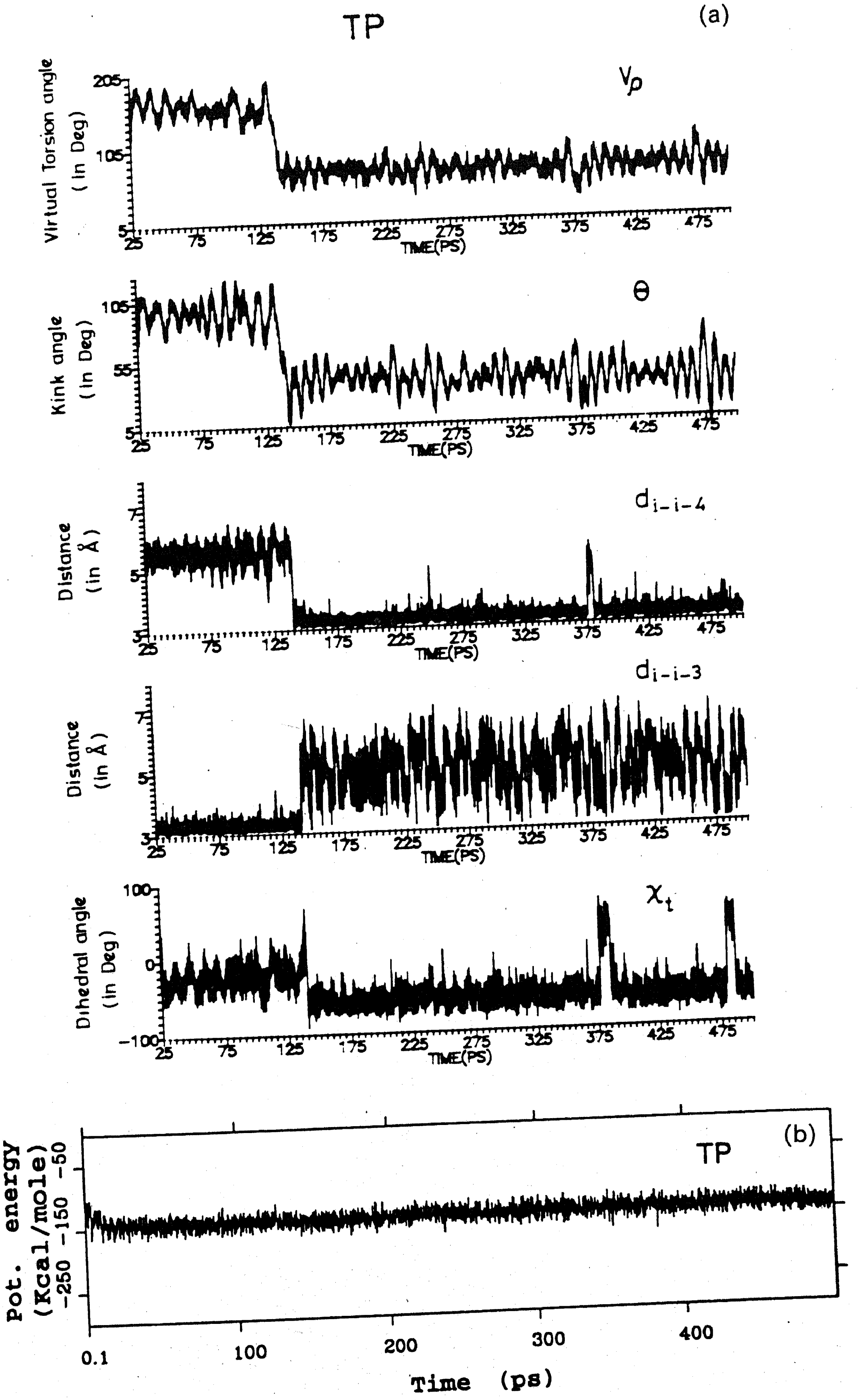

Figure 2. The MD trajectories of (a) dihedral angles: V_ρ, θ and χ_t, and hydrogen bond distances: d_{i-i-4} and d_{i-i-3}, (b) potential energy (including the equilibration period) of the simulation with TP as the initial conformation.

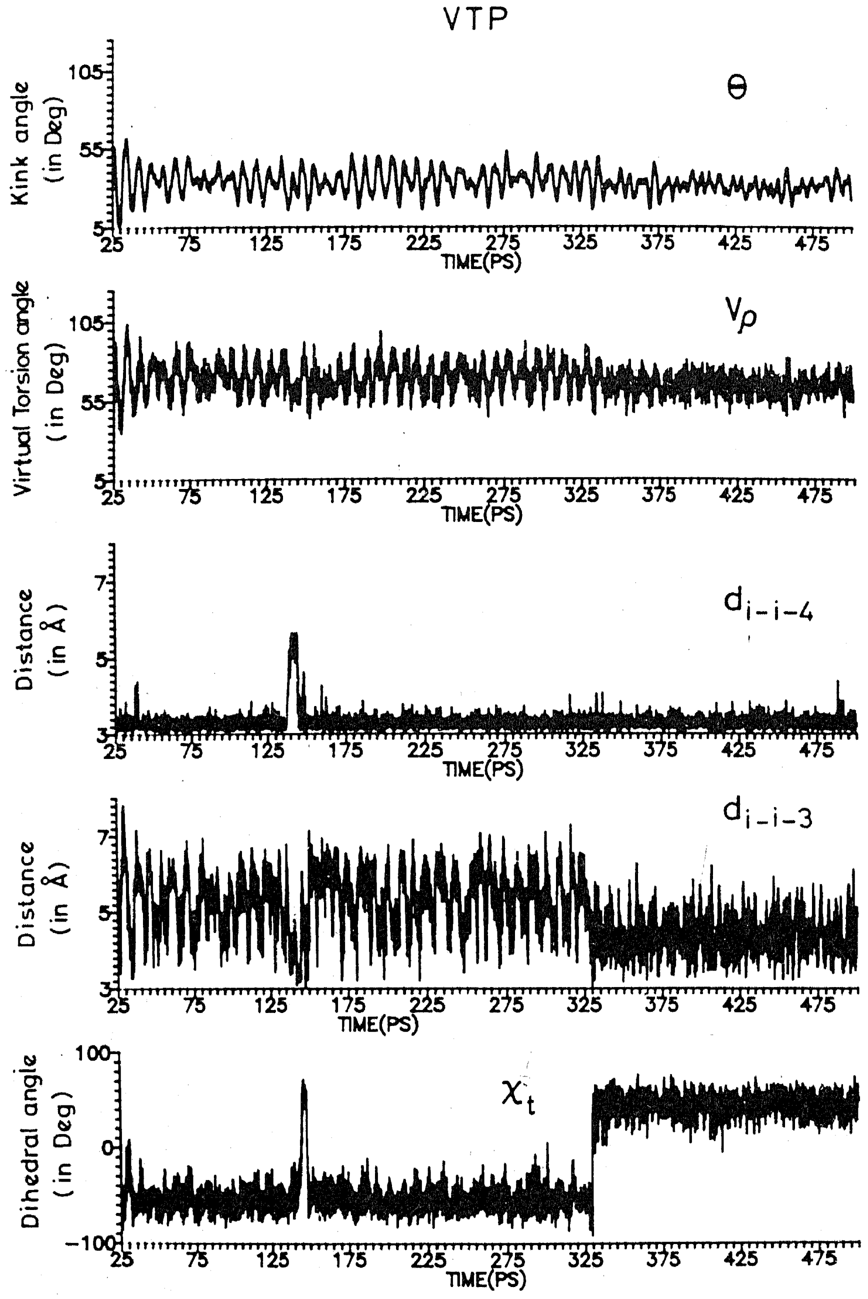

Figure 3. The MD trajectories of dihedral angles: V_p, θ and χ_t and hydrogen bond distances: d_{i-i-4} and d_{i-i-3} of the simulation with VTP as the initial conformation.

around 140, 380 and 480 ps. In this conformation d_{i-i-4} is stable whereas the distance between O^{γ} of threonine and the $i-3$rd carbonyl oxygen varies between 3·28 Å and 5·31 Å. However, these transitions have no major significance from the overall structure point of view since the analysis of the backbone parameters did not exhibit any marked fluctuations. The kink and virtual torsion angles exhibit an interesting periodicity of large fluctuations about the mean value dampening to small fluctuations. The fluctuations lead to structures ranging from an almost straight helix ($\theta \sim 7°$) to a kink of about 75°. A stereo plot of the two minimized structures with extreme kink angles are given in figure 4.

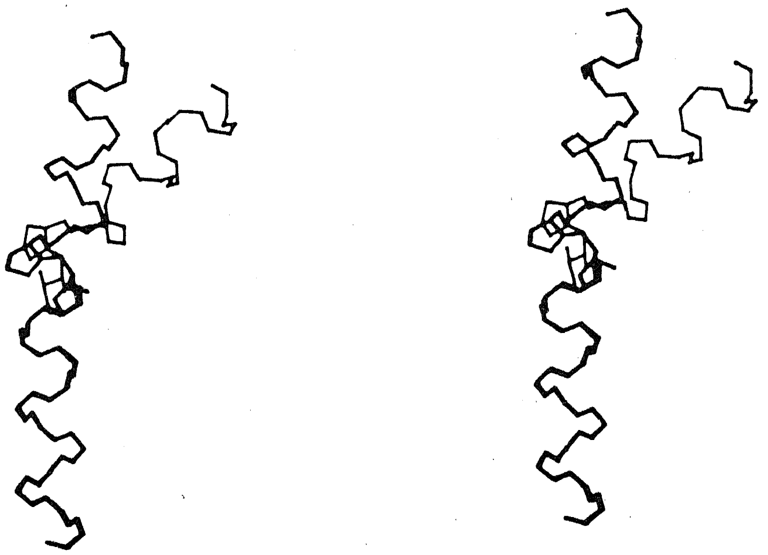

Figure 4. A stereo plot of the backbone atoms along with proline and threonine sidechain atoms of the superposed minimized structures taken from TP simulation at 475·2 ps (high kink angle) and 480·1 ps (low kink angle).

A comparison of TP and VTP systems shows that the highly bent TP2 structure (25–140 ps) is not accessed by the VTP system (figure 3). The VTP system remains in the normal proline helix form throughout the 500 ps simulation in which the d_{i-i-4} is stable. The virtual torsion and kink angles fluctuate around 69° and 34° respectively. The valine sidechain has remained at the initial conformation of $\chi_1 \sim +60°$ throughout. The threonine sidechain (χ_t) has however changed from $-60°$ to $+60°$ around 320 ps and has remained at $+60°$ until 500 ps.

A correlation is exhibited between the bending behavior of the helix (fluctuations in V_p and θ) and χ_t. When χ_t is 60°, the fluctuations in the bend-related parameters are lesser than when χ_t is $-60°$. Hence the system has assumed two structures VTP1 (25–320 ps) and VTP2 (320–500 ps) of very similar bend with subtle differences in their flexibility.

TP1 structure of TP, VTP1 and VTP2 structures of VTP have a bend similar to that of a normal proline helix while few differences are seen in their flexibility. The ϕ, ψ, ω parameters of significance, χ_t and bend-related parameters of the three structures are listed in tables 1 and 2, respectively. The backbone parameters listed in table 1 show that all the three structures exhibit the characteristics of a normal proline helix. For instance, the average values of dihedral angles (ϕ_{p-3}, ψ_{p-3}), (ϕ_{p-2}, ψ_{p-2}) correspond to the α_{11} helix and that of (ϕ_{p-1}, ψ_{p-1}) correspond to the goniometric helix values (Sankararamakrishnan and Vishveshwara 1993). There is a small difference in the flexibility of the backbone dihedral angles as can be seen in the r.m.s. deviations (table 1). However, significant differences can be seen in the threonine χ_t values which correlate with those of the bend-related parameters (θ, V_p and h_p) as shown in figures 2a and 3 and table 2. Thus, the orientation of the threonine sidechain which is influenced by preceding valine residue has considerable influence on the flexibility of the bend due to proline in the helix.

The proline pucker is another interesting parameter for analysis (Sankararamakrishnan and Vishveshwara 1992). The trajectories of χ_1 and χ_2 of proline for TP and VTP

Table 1. The backbone dihedral angles (in degrees) for the two simulations–TP and VTP are given. The three entries for every parameter listed are the MD average, the r.m.s. deviation (in parentheses) and the MD minimized structure (in square brackets).

Dihedral angles	TP1	VTP1	VTP2
ϕ_{p-4}	−57·580	−57·978	−58·770
	(±9·572)	(±9·874)	(±9·400)
	[−54·887 −61·737]*	[−57·943]	[−55·646]
ψ_{p-4}	−44·046	−40·852	−36·411
	(±11·649)	(±12·941)	(±11·591)
	[−51·361 −30·255]	[−41·443]	[−42·835]
ϕ_{p-3}	−66·889	−69·788	−73·241
	(±12·433)	(±13·638)	(±12·109)
	[−57·964 −77·103]	[−67·985]	[−67·736]
ψ_{p-3}	−43·763	−45·680	−47·397
	(±10·040)	(±10·792)	(±9·512)
	[−45·033 −52·992]	[−51·236]	[−49·872]
ϕ_{p-2}	−66·696	−66·394	−67·865
	(±11·370)	(±11·603)	(±11·223)
	[−64·159 −54·980]	[−70·760]	[−66·330]
ψ_{p-2}	−44·451	−46·292	−50·173
	(±12·324)	(±11·141)	(±9·634)
	[−43·648 −46·633]	[−51·710]	[−52·747]
ω_{p-2}	−171·325	−171·486	−170·269
	(±8·133)	(±7·991)	(±7·871)
	[−172·239 −175·258]	[165·157]	[−171·271]
ϕ_{p-1}	−47·534	−47·460	−51·196
	(±12·786)	(±11·508)	(±10·292)
	[−27·206 −60·896]	[−55·252]	[−42·642]
ψ_{p-1}	−57·642	[−57·748	−54·560
	(±9·098)	(±8·749)	(±9·133)
	[−63·892 −63·892]	[−48·846]	[−56·919]
ϕ_{p}	−56·055	−57·589	−57·986
	(±9·966)	(±9·594)	(±9·918)
	[−57·641 −42·377]	[−56·111]	[−61·037]
ψ_{p}	−45·900	−44·347	−44·783
	(±9·955)	(±9·767)	(±9·883)
	[−46·811 −52·126]	[−48·103]	[−43·962]
ϕ_{p+1}	−61·084	−60·906	−60·322
	(±9·823)	(±9·765)	(±9·824)
	[−65·050 −56·265]	[−60·960]	[−61·112]
ψ_{p+1}	−47·786	−47·996	−48·569
	(±8·455)	(±8·577)	(±8·452)
	[−45·259 −51·229]	[−48·929]	[−47·897]

*The two entries correspond to the two minimized structures: (a) a high kink structure at 475·2 ps and (b) a low kink structure at 480·1 ps.

Table 2. The constraint parameters and the bend related parameters in the two simulations – TP and VTP are given. The three entries for every parameter listed are the MD average, the r.m.s. deviation (in parentheses) and the MD minimized structure (in square brackets). The distances given are in Å and the angles are in degrees.

	TP1	VTP1	VTP2
Constraint parameters			
$O^{\gamma}\cdots O_{p-5}$ (d_{i-i-4})	2·827 (±0·245) [2·752 2·736]*	2·829 (±0·312) [2·748]	2·786 (±0·127) [2·761]
$O^{\gamma}\cdots O_{p-4}$ (d_{i-i-3})	4·495 (±0·815) [3·465 4·096]	4·767 (±0·828) [5·501]	3·896 (±0·537) [3·433]
$N–C^{\alpha}–C^{\beta}–O^{\gamma}$ (χ_t)	−51·034 (±22·243) [−63·519 37·046]	−52·488 (±17·300) [−48·312]	44·140 (±11·721) [+44·873]
$N–C^{\alpha}–C^{\beta}–H^{\beta}$ (χ_1)		+57·415 [±7·216] [+52·493]	+57·013 [±7·011] [+53·465]
Bend-related parameters			
$N_{p+1}\cdots O_{p-3}$ (hp)	4·188 (±0·603) [4·821 2·905]	4·094 (±0·546) [4·046]	3·939 (±0·436) [4·002]
V_p	71·557 (±11·749) [89·926 52·810]	69·521 (±9·713) [62·643]	64·263 (±6·416) [68·384]
Kink angle (θ)	39·452 (±11·184) [55·823 20·617]	34·060 (±8·559) [29·716]	30·691 (±5·293) [35·668]

*The two entries correspond to the two minimized structures: (a) a high kink structure at 475·2 ps and (b) a low kink structure at 480·1 ps.

simulations are given in figure 5. Both UP and DOWN conformations are accessed throughout the simulation, a result which is consistent with the crystal structure analysis (Milner-White *et al* 1992). Further in the TP system the UP conformation is predominantly seen for the TP2 structure. In the VTP system, valine seems to have played a role in keeping the proline in the DOWN conformation for most of the time.

4. Conclusions

A comparison of the characteristics derived from the two simulations

$$\text{Ace–(Ala)}_{12}\text{–Thr–Pro–(Ala)}_{10}\text{–NHMe (TP) and}$$
$$\text{Ace–(Ala)}_{11}\text{–Val–Thr–Pro–(Ala)}_{10}\text{–NHMe (VTP)}$$

have shown that the bulky hydrophobic group valine restricts major structural transitions in the proline helix.

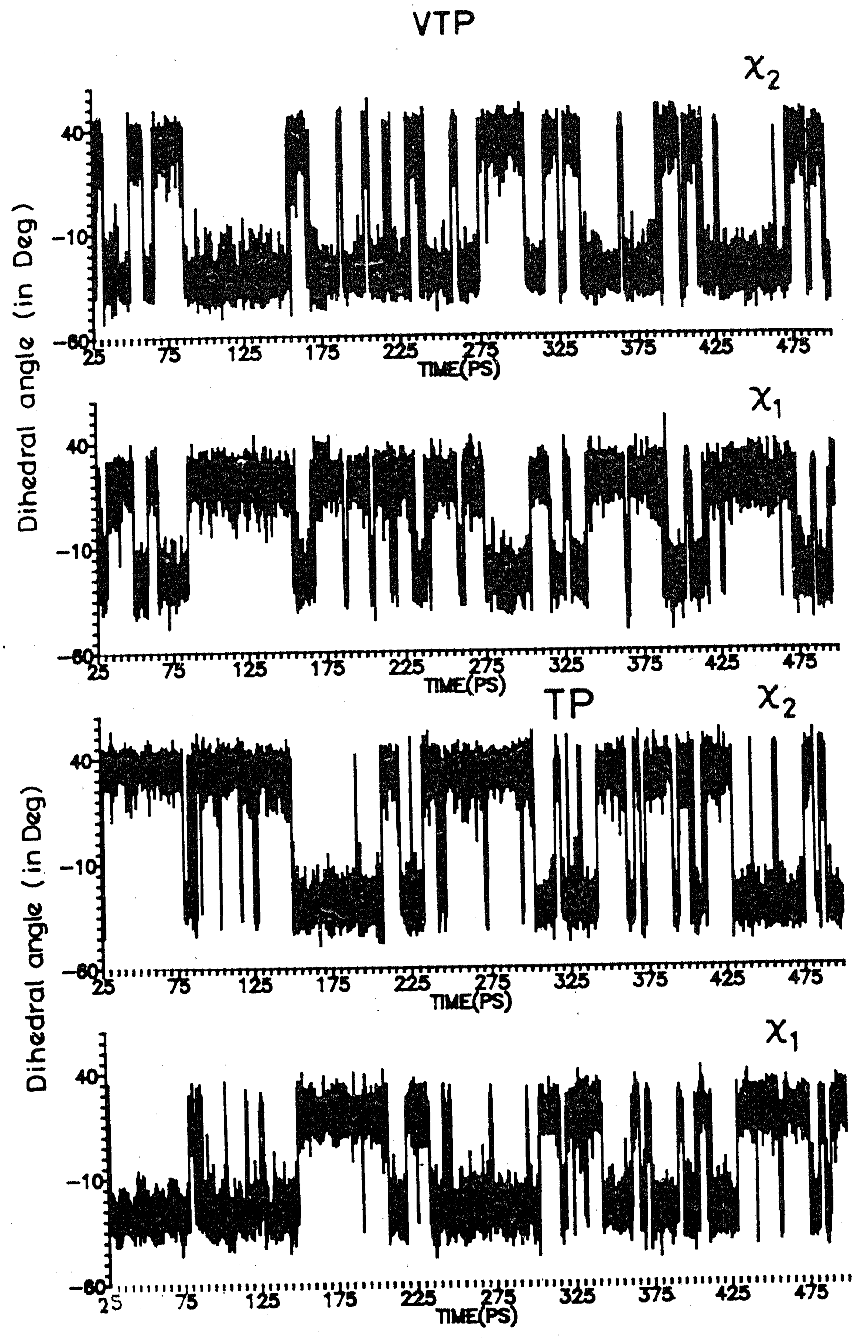

Figure 5. The MD trajectories of the proline sidechain dihedral of the simulation with TP and VTP as the initial conformation.

The normal proline helix conformation has a flexible bend wherein the structure fluctuates with kink angle varying from 5° to about 55°, such periodic behaviour is seen throughout the VTP simulation and from 150–500 ps of the TP simulation.

Both g^+ and g^- orientations are taken up by the threonine residue. In VTP system, when N–C$^\alpha$–C$^\beta$–O$^\gamma$ (χ_t) is g^+ ($\sim 60°$), the average bend and the fluctuations in bend related parameters (kink angle (θ), virtual torsion angle (V_p) and the hydrogen bond $N_{p+1} \cdots O_{p-3}(h_p)$ are relatively less.

The proline ring accesses both the UP and DOWN pucker consistent with crystal structure analysis. In TP2 structure, the proline ring spends most of the time in UP pucker. In VTP system the valine residue has played a role in keeping the proline ring in the DOWN conformation for most of the simulation period.

Thus, the introduction of residues like threonine and valine in a proline containing α-helix modulates the dynamics and the bend characteristics of the proline helix and the conformation adopted by proline.

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