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Cis Peptide Bonds in Proteins: Residues Involved, their Conformations, Interactions and Locations

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An analysis of a non-redundant set of protein structures from the Brookhaven Protein Data Bank has been carried out to find out the residue preference, local conformation, hydrogen bonding and other stabilizing interactions involving *cis* peptide bonds. This has led to a reclassification of turns mediated by *cis* peptides, and their average geometrical parameters have been evaluated. The interdependence of the side and main-chain torsion angles of proline rings provided an explanation why such rings in *cis* peptides are found to have the DOWN puckering. A comparison of *cis* peptides containing proline and non-proline residues show differences in conformation, location in the secondary structure and in relation to the centre of the molecule, and relative accessibilities of residues. Relevance of the results in mutation studies and the *cis-trans* isomerization during protein folding is discussed.

Keywords: *cis* peptide; pyrrolidine ring puckering; turn conformation; residue preference in *cis* peptides; weak interactions

Introduction

In proteins, the partial double bond character of the peptide bond results in two conformations depending on the value of the dihedral angle, ω [$C_\alpha(1)$-C(1)-N(1′)-$C_\alpha(1')$]: *cis* and *trans* (with $\omega = 0$ and 180°, respectively) (Pauling, 1960; Ramachandran & Sasisekharan, 1968) (Figure 1(a)). The isomer with the two C^α atoms *trans* to each other is favoured overwhelmingly due to the lesser steric conflict involving the substituents at these positions, and only when a Pro residue is in position (1′) is there a substantial steric clash involving the C^α atom at position (1) and C^δ atom of Pro at position (1′), even in the *trans* conformation, to give the *cis* imide bond, X-Pro, a higher frequency of occurrence than what is observed for the amide bond, X-Xnp.

A difference in energy of approximately 2.5 kcal/mol between the *trans* and the *cis* isomers (corresponding to only 1.5% occurrence of the *cis* form), regardless of the solvent, and a rotational barrier of about 20 kcal/mol have been found for the peptide bond analog *N*-methylacetamide (LaPlanche & Rogers, 1964; Christensen *et al.*, 1970; Drakenberg & Forsén, 1971; Perricaudet & Pullman, 1973; Radzicka *et al.*, 1988; Jorgensen & Gao, 1988; Schnur *et al.*, 1989; Scherer *et al.*, 1998). For an imide bond in Pro-containing peptides, however, the *trans* isomer is favoured over the *cis* by only 0.5 kcal/mol (Maigret *et al.*, 1970), so that a higher abundance (10-30%) of the *cis* form is observed (Brandts *et al.*, 1975; Grathwohl & Wüthrich, 1976; Juy *et al.*, 1983); the activation energy barrier for *cis-trans* isomerization is also less, 13 kcal/mol (Schulz & Schirmer, 1984). Using conformational energy calculations, Ramachandran & Mitra (1976) found expected frequencies for the *cis* isomer to be 0.1% and 30% (corresponding to an enthalpy difference of 4.0 and 0.5 kcal/mol, respectively) for an Ala-Ala and Ala-Pro peptide bond, respectively. A survey of protein structures by Stewart *et al.* (1990) found only 0.05% of all X-Xnp, but 6.5% of all X-Pro peptide bonds to occur in the *cis* conformation. The analysis of MacArthur & Thornton (1991) provided a value of 5.7% for the latter group, whereas a recent work (Weiss *et al.*, 1998; Jabs *et al.*, 1999) gave values of 0.03% and 5.2%, respectively, for the two types of peptide bonds.

Due to the energy barrier, *cis-trans* isomerization of peptide bond is a rather slow process at room temperature and has been shown to play an important role in protein folding (Brandts *et al.*, 1975; Creighton, 1978; Schmid & Baldwin, 1978; Cook *et al.*, 1979; Lin & Brandts, 1984; Brandts &

Abbreviations used: X, any amino acid residue; Xnp, any non-Pro amino acid residue; Ar, any aromatic residue.

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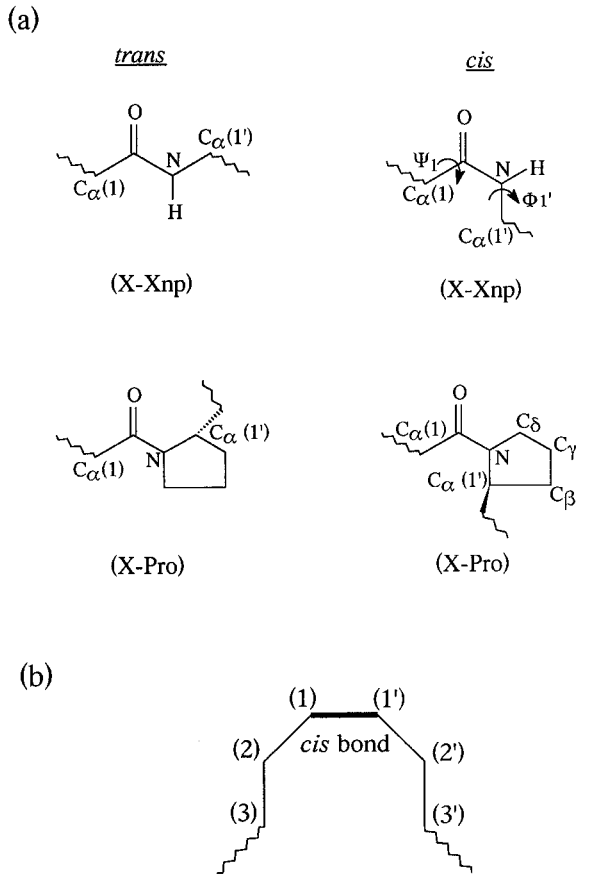

Figure 1. Schematic representation of *cis* and *trans* conformations around X-Xnp and X-Pro peptide bonds (where X = any residue, Xnp = any non-Pro residue). (b) Convention for numbering residues flanking a *cis* peptide bond.

Lin, 1986; Kim & Baldwin, 1990). An enzyme, prolyl isomerase is known to catalyze the *cis-trans* isomerization of X-Pro bonds (Schmid *et al.*, 1993). Experimental data have been derived on the thermodynamics and kinetics of *cis-trans* isomerization by substituting a Pro at (1′) by a non-Pro residue (Schultz & Baldwin, 1992; Mayr *et al.*, 1993; Tweedy *et al.*, 1993; Odefey *et al.*, 1995; Vanhove *et al.*, 1996). However, to understand the structural effect of such mutations it is important to know the conformational features of residues in and around (Figure 1(b)) the X-Xnp *cis* peptide linkages *vis-à-vis* the X-Pro bond. Moreover, although a *cis* peptide bond can cause reversal of chain direction (Lewis *et al.*, 1973) leading to two types of turns with the two central residues having canonical ϕ,ψ (degree) values of (−60,120; −90,0) and (−120,120; −60,0) (Richardson, 1981; Rose *et al.*, 1985). These values, though widely quoted in literature (Wilmot & Thornton, 1988), need a reassessment as ψ of the second residue in the second set is usually significantly off the idealized value. Consequently, we thought it important to make a reclassification of *cis* peptide mediated turns and an evaluation of the torsion angles of the involved residues.

We have recently investigated the interrelationship between the side-chain and the main-chain conformational angles in residues involved in the *trans* peptide units (Chakrabarti & Pal, 1998), and from this perspective it is worthwhile to study the relationship in *cis* peptide bonds. The pyrrolidine ring of Pro can be associated with two types of puckering, designated UP and DOWN, depending on the ring torsion angles (Ramachandran *et al.*, 1970; Ashida & Kakudo, 1974). It has been noted by Milner-White *et al.* (1992) that the puckering of the ring when it is involved in the *cis* linkage is DOWN, and an explanation may be sought in terms of the interaction between the main-chain and side-chain atoms.

The *cis* peptide bonds, especially the ones with non-Pro residues, are located near the active sites or are implicated to have roles in the function of the protein molecule (Herzberg & Moult, 1991; Stoddard & Pietrokovski, 1998; Jabs *et al.*, 1999). Though important, some of the *cis* peptide bonds might have gone unreported in the structures determined at lower resolution (Weiss *et al.*, 1998). To facilitate the identification of such overlooked *cis* peptide bonds it is important to characterize the location of known *cis* peptide units (both X-Pro and X-Xnp) in the three-dimensional structures and their solvent accessibility. A comprehensive analysis of these issues is made here, so as to understand the interactions that stabilize a *cis* peptide bond and possibly identify regions/sequences in protein structures that are likely to adopt a *cis* peptide linkage.

Results and Discussion

Residues forming the *cis* peptide linkage

A total of 50 % (147 out of 294) of well-defined protein structures contain one or more *cis* peptide bonds; 0.3 % of all the bonds in the database exist in the *cis* form (231 in total). Most of them (87 %) are preceding Pro residues (5.7 % of X-Pro bonds have the *cis* conformation). The intrinsic probability of a residue (X) to cause a *cis* conformation of the X-Pro linkage, given by the fraction of occurrence of the bond in the *cis* form, is provided in Figure 2. Stewart *et al.* (1990) found Tyr-Pro sequence to be *cis* 25 % of time, while *cis* Trp-Pro was absent. A high occurrence (19 %) of Tyr in *cis* bonds was also reported by MacArthur & Thornton (1991). From a larger database we find that the percentage of Tyr occurring in *cis* bonds has been reduced considerably (9.7 %), and Trp has become equally conspicuous (10.4 %). A Pro-Pro bond has the highest frequency (11.2 %) to be in the *cis* form. The residue X in X-Pro that causes the bond to be *cis* at least 6 % of the time belongs to one of the following four groups: (i) aromatic residues, (ii) small residues, Gly and Ala; (iii) polar residues Ser, Gln and Arg; and (iv) Pro provide

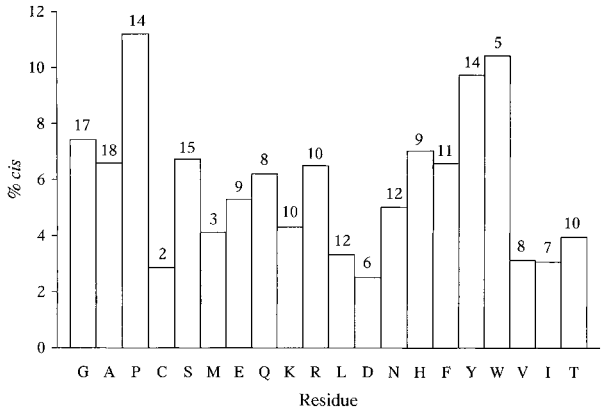

Figure 2. Histogram showing the percentage of occurrence of various residues in the *cis* conformation of the X-Pro peptide bond; the numbers of *cis* cases are given on top of each bar.

61 % of the data points. Branched aliphatic residues Val, Ile, Thr and Leu are less frequent. Recently, Reimer *et al*. (1998) have also calculated the amino acid frequency of *cis* prolyl bonds for every single amino acid preceding Pro. Some of their values are smaller than ours, possibly because of their inclusion of lower resolution (3.5 Å) data, where *cis* bonds are underestimated (Weiss *et al*., 1998).

The number of observations of the X-Xnp bond in the *cis* form is rather small to make any definite statement. Out of 29 cases (Table 1) Gly, Ser, Trp, Ala and Asp have higher occurrences at position (1) and Asn, Ala, Thr, Asp and Phe at (1′) (Table 2B).

Residue preferences in the neighbourhood of *cis* peptide bonds

When considering the neighbours (±six residues) of prolyl residues and their physicochemical properties, Frömmel & Preissner (1990) found six different patterns which contained 75 % of known X-Pro cases. To see if the local sequence has any influence on the occurrence of a X-Pro, Pro-Pro or Xnp-Xnp bond in the *cis* conformation the percentage composition of residues at each position, from (3) to (3′) (Figure 1(b)), was calculated, and the preferred residues are given in Table 2. Being most abundant, the X-Pro cases were analyzed after grouping them into two main turn types (VIa and VIb), as well as the four subgroups (VIa-1, VIa-2, VIb-1 and VIb-2) of the above types. In addition to considering individual residues we also analyzed the occurrence of groups of residues, like small (Gly and Ala), aromatic (Phe, Tyr, Trp and His), β-branched (Val, Ile and Thr) and short polar (Ser, Asp and Asn).

There are interesting trends considering groups of residues (Table 2C). Taking X-Pro (VIa) as an example, aromatic residues have high occurrences at positions (1) and (2′), which decrease sharply on moving outward. On the contrary, the β-branched residues are less at positions (1) and (2′) (especially in the former position, which is also indicated in Figure 2), and increase along the outward locations (especially upstream). X-Pro (VIb) and Xnp-Xnp cases have very similar position-specific variations of these two groups of residues. In Pro-Pro cases, the aromatic residues are abundant at position (2′) and the branched residues at position (2). Short, polar residues (Ser, Asp and Asn) are likely to be a constituent of the *cis* Xnp-Xnp bond, and also be a part of X-Pro (type VIb) bond.

Small residues have relatively higher occurrences in all the positions of Xnp-Xnp, and also in position (2′) of X-Pro (Table 2C). Although taken together as small residues, Gly and Ala are not always found in similar numbers. For example, Gly is more abundant in the location (1) of Xnp-Xnp, whereas Ala predominates in locations (1′) and (2′) (Table 2B). Likewise in X-Pro (turn type VIa), Gly is prominent at position (2′) and is exclusively found in position (2), but does not occur at all in position (1). As to be discussed later, because of the conformations being distinct from other X-Pro cases, Gly-Pro *cis* peptides belong to different turn categories, VIb-3, VIc and VId (Table 3). However, if taken together, they have equal preferences of aromatic and β-branched residues at positions (3) and (2′).

Among the different turn types involving the X-Pro cases (Table 2A), VIa-2 type, in comparison to VIa-1, has a high proportion of small residues, notably Gly in position (2′). Relative to the above two types, VIb-1 has a greater presence of Pro

Table 1. Percentage occurrence in the *cis* conformation of X-Xnp sequences

Range (%)	Sequence[a]
0.0-0.2	AA,GA
0.2-0.4	DA,GT,AD,LT,AT,SV,VN,EI,GF,GG(2),DD
0.4-0.6	QL,FS,RD,SY,DN,SF,SR,PN,NY
0.6-0.8	PY
0.8-1.0	WA,HT
1.0-1.2	HF,CA
4.3	WN(3)

[a] Within a range, the sequences are in an ascending order of occurrence. The number of cases, if more than one, is given in parenthesis.

Table 2. Preference of amino acid residues around various categories of *cis* peptide units

Categories	(3)	(2)	(1)	(1′)	(2′)	(3′)
A. *Xnp-Pro: different turn types*[a]						
VIa-1 (39)	G(15), A,V(10) *[F,E,P]*	D(15),G(13) *[A,N,R]*	**A**(23),Y(13), E(10) *[G,T,N]*	P	G,F(15), T,D,N(10) *[I,K,P]*	S,I(13), D,N,A(10) *[L]*
VIa-2 (13)	T(23),V,I(15)	V(31),G(15)	L,S,W(15)	P	**G**(46),A(15)	S(23),Q(15)
VIb-1 (100)	I(10),V(9)	T,P(11),G(9) *[E]*	S(10)	P	**A**(18)	**P**(14)
VIb-2 (12)	I(25),A,D,S(17)	**G**(42)	N(25),T,H(17)	P	**G**(25),A,I(17)	Q,R(17)
B. *Different possible sequences forming* cis *peptide*[b]						
X-Pro (VIa) (52)	G(13),V,T(12) *[P]*	G,D(13),V(12) *[A,N,R]*	A(19),L,Y(10) *[G,N]*	P	**G**(23),F(12),T, D(10) *[K,P]*	S(15),I(12), P(10) *[L]*
X-Pro (VIb) (116)	I(11),A,V(9)	G(12),T,P(9) *[E]*	N,S,T,Y(9)	P	A(17),V,Y(9)	P(13),T(9)
Xnp-Xnp (29)	**I**(21),A(14), V,R(10) *[D,K,P]*	V,I,L,N(14) *[S,E,K,P]*	G(17),W,S(14), A,D(10) *[I,T,K]*	A,N(17),T(14), D,F(10) *[E,K]*	**A**(24),G,L(14) *[V,F,P]*	**G**(21),Q(14), L,S(10) *[T,E]*
Pro-Pro (14)	K(21)	I,T,K,D(14)	P	P	**F**(29),T,Q(14)	L,T(21),P(14)
Gly-Pro (16)	L(25),V,Y(19)	Q(19),G,S(12)	G	P	V,F(19)	G(19)
C. *Preference of groups of residues*[c]						
X-Pro (VIa) (52)	Sm(21),Ar(8), **Bb**(32),Sp(18)	Sm(13),Ar(16), **Bb**(24),Sp(17)	Sm(19),**Ar**(26), Bb(10),Sp(10)	P	**Sm**(29),Ar(24), Bb(16),Sp(20)	Sm(12),Ar(10), Bb(20),**Sp**(31)
X-Pro (VIb) (116)	Sm(15),Ar(11), **Bb**(27),Sp(17)	Sm(18),Ar(15), Bb(18),Sp(13)	Sm(9),**Ar**(23), Bb(16),Sp(21)	P	**Sm**(23),Ar(13), Bb(20),Sp(11)	Sm(9),Ar(12), Bb(20),Sp(20)
Xnp-Xnp (29)	Sm(21),Ar(7), **Bb**(38),Sp(10)	Sm(20),Ar(13), **Bb**(31),Sp(17)	**Sm**(27),Ar(24), Bb(3),**Sp**(27)	Sm(24),Ar(17), Bb(20),**Sp**(30)	**Sm**(38),Ar(7), Bb(10),Sp(17)	**Sm**(28),Ar(6), Bb(10),Sp(20)
Pro-Pro (14)	Sm(14),Ar(7), Bb(14),Sp(14)	Sm(7),Ar(7), **Bb**(35),Sp(14)	P	P	Sm(0),**Ar**(43), Bb(21),Sp(0)	Sm(7),Ar(7), Bb(21),Sp(14)
Gly-Pro (16)	Sm(0),**Ar**(25), **Bb**(25),Sp(6)	Sm(18),Ar(12), Bb(18),Sp(18)	G	P	Sm(12),**Ar**(31), **Bb**(31),Sp(12)	Sm(19),Ar(12), Bb(18),Sp(12)
D. *Most (and least) likely residues*[d]						
X-Pro (VIa)	Bb,Sm *[P]*	Bb,G *[A,N,R]*	Ar,A *[G,N]*	P	Sm,Ar *[K,P]*	Sp,Bb *[L]*
X-Pro (VIb)	Bb	Bb,Sm *[E]*	Ar,Sp	P	Sm,Bb	Sp,Bb
Xnp-Xnp[e]	Bb,Sm	Bb,Sm	Sm,Sp,W	Sp,Sm	Sm	Sm
Pro-Pro	-	Bb	P	P	Ar	-
Gly-Pro	Ar,Bb	-	G	P	Ar,Bb	-

At each position (Figure 1(b)), the percentage residue composition is calculated and the residues having high values are entered with the percentage composition given in parentheses (when multiple residues have the same value the number is given after the last entry). If the first entry has a distinctly higher value than the next, it is given in bold and underlined. Residues whose average occurrence in protein structures is greater than 4% (Pal & Chakrabarti, 1999a), but are not found at all in a given position, are given in italics within square parentheses. The number of cases in each category is given in column 1.

[a] Given in Table 3 (sparsely populated types are excluded).

[b] X-Pro sequences are broken into two classical VIa and VIb turns.

[c] Residues are grouped as: Sm, small (G, A); Ar, aromatic (F, Y, W, H); Bb, β-branched (V, I, T); and Sp, short polar (S, D, N).

[d] Indicated by either one-letter amino acid code, or a two-letter group designation.[c]

[e] Less likely to have Pro and Lys all throughout.

around the *cis* peptide. Because of steric factors, VIb-2 type needs to have a small residue (Gly in particular) at either position (2) or (2′). Based on the above, the notable presence (or absence) of various residues around the *cis* peptide moieties are summarized in Table 2D. Interestingly, there are only two examples of Pro-X *cis* peptides: 2CTC (PDB file) with sequence, Leu-Tyr-Pro-Tyr-Gly-Tyr and 1MKA, Pro-Ala-Pro-Asn-Met-Leu.

Possible role of neighbouring residues in *cis-trans* isomerization

Data in Table 2C show a contrast in the relative presence of aromatic and β-branched residues around the *cis* peptide units. For X-Pro cases, while aromatic residues have a higher presence at position (1), their numbers decline as one moves out along the sequence from the *cis* bond. On the other hand, the branched residues, show the opposite trend and have the maximum presence at position (3) (even for Xnp-Xnp cases). This observation is suggestive of the steric requirement for the isomerization of a *trans* peptide bond into *cis*. The residues with two bulky alkyl groups at C^{β} (close to main-chain) if located at position (1) hinders the isomerization process. Support for the steric clash having an inhibitory role on the isomerization process also comes from nature of the residue preceding Pro-Pro *cis* peptides. In the sequence X-Pro-Pro, one may ask what determines the second bond to be in the *cis* peptide conformation rather than the first. It appears that a large percentage of these cases have a β-branched residues for X (and in addition, aromatics at position (2′)). Even Xnp-Xnp *cis* peptides have a few such residues at either position (the relatively higher number at position (1′) is due to a large contribution from Thr which, due to its polar features, acts in a different way, as discussed below). The β-branched residues, however, may have a beneficial role when located at position (2) or (3). Because of the larger steric clash

Table 3. Types of turns mediated by *cis* peptide bonds and their geometries

Turn type[a]	Conf.[b]	No.	ϕ_1	ψ_1	$\phi_{1'}$	$\psi_{1'}$	Dist (Å) (2)-(2′)	Secondary structure[c]
A. *Xnp-P*								
VIa-1	BA	39	−74(24)	141(9)	−93(9)	12(16)	5.9(6)	TT(39)
VIa-2	BA	13	−131(24)	145(16)	−79(9)	−16(24)	6(1)	SS(5),CS(3),ET(2),BS,ES,II
VIb-1	BB	100	−117(26)	138(16)	−77(10)	158(17)	6.3(8)	SS(50),CS(16),SC(10),ES(8), CC(8),BS(4),EC(2),BC,EE
VIb-2	BB	12	−134(12)	98(23)	−78(12)	165(9)	4.5(7)	TT(12)
VIb-3	BB	4	−100(20)	183(8)	−72(10)	154(2)	7.7(2)	SC(2),SS,EC
VIc	RA	5	104(38)	188(8)	−83(9)	−16(7)	8.4(4)	CS(4),SS
VId	RB	7	102(20)	186(25)	−69(8)	171(23)	8.3(3)	CC(3),EE(3),BC
B. *P-P*								
VIa-1	BA	7	−54(5)	147(5)	−81(5)	9(10)	5.6(3)	TT(7)
VIb-1	BB	6	−69(6)	160(8)	−77(11)	149(14)	7.4(7)	SS(3),CC,SS,EE
VIb-2	BB	1	−84	149	−96	115	6.3	TT
C. *Xnp-Xnp*								
VIa-1	BA	5	−89(21)	134(30)	−111(17)	14(36)	6.4(9)	TT(5)
VIa-2	BA	3	−113(41)	149(9)	−106(7)	−15(17)	7(1)	CC,ET,EE
VIb-1	BB	15	−108(29)	121(23)	−134(21)	168(15)	8(1)	EC(5),EE(4),SC(3),ES(2),CC
VIb-2	BB	2	−123(6)	121(57)	−102(23)	152(26)	6(1)	TT(2)
VIb-3	BB	1	−155	176	−102	129	8.6	EE
VId	RB	3	131(30)	174(11)	−91(2)	202(13)	9.1(6)	CC(2),EC

Data for eight cases are not included in the Table: two Pro-Xnp cases (with conformations BA and BB); one C-terminal *cis* peptide; one Gly-Pro sequence (LB); and four sterically strained non-Gly-Pro sequence (LB(2), AB(1), AA(1)). Representative diagrams are given in Figure 7.

[a] VIb-3, VIc and VId turns have Gly at position (1). The hydrogen bond (Figure 6) is usually between residues (2) and (2′) (providing CO and NH groups, respectively) in VIa-1, (3) and (3′) in VIb-2, and (1) and (1′) (providing CH and CO, respectively) in VIb-3 and VId.

[b] Conformation based on the location of the two residues in the Ramachandran plot (see Materials and Methods and Figure 5).

[c] Of positions (1) and (1′) as specified by the program DSSP (Kabsch & Sander, 1983): H, α-helix; I, π-helix; E, strand; T, hydrogen bonded turn; S, non-hydrogen bonded turn; C, non-regular structure. The number of observations, if more than one, is given in parentheses.

between the main and side-chain atoms, the ϕ,ψ angles of these residues lie in a limited range (Chakrabarti & Pal, 1998), and thus they can act as a tether or a wrench to hold the chain in position while an adjacent bond is being isomerized.

A corollary of the above hypothesis is that the small residues offering the minimum steric resistance should facilitate the *cis* form. Indeed, a large number of Gly and Ala residues are found in positions (1) of X-Pro, (1) and (1′) of Xnp-Xnp, and (2′) of both. In the case of Xnp-Xnp there may be another factor operating during the *trans* to *cis* isomerization. Most of these have polar residues, Ser, Thr, Asp and Asn at position (1′) and their side-chains are usually within the hydrogen bonding distance of the main-chain NH group at the same position (although the angles, in the range 60-120°, do not fulfill the usual hydrogen bond criterion). Even though the geometry may not be optimum, it is quite plausible that during isomerization such interaction may satisfy the hydrogen bonding potential of the NH group, and thus lower the activation energy of the process. Participation of a nearby residue facilitating the *cis-trans* isomerization is known (Reimer *et al.*, 1997). Once formed, the *cis* peptides may be stabilized by interactions (discussed later) involving aromatic residues which are found in large numbers at positions (1) of X-Pro and (2′) of Pro-Pro and X-Pro (turn type VIa).

Correlation between main-chain and side-chain conformations

Recently, we have shown how the side-chain torsion angle χ_1 is correlated with the backbone angles ϕ and ψ of residues held by *trans* peptide linkage, and how the result can be used to classify the amino acid residues (Chakrabarti & Pal, 1998). The paucity of data for *cis* peptides does not allow one to study the interrelationships of angles for individual residues. However, some general trends can be deciphered (Figure 3). For example, in Xnp-Pro cases, the means of the distributions of the ψ values of Xnp get changed ($130° \rightarrow 135° \rightarrow 148°$) as χ_1 goes from $-180°$ to $-60°$ to $60°$ (conformational states *t*, g^+ and g^-, respectively, which occur in the ratio ≈3:5:1; Figure 3(a)). For a Pro residue in this position, though any value of χ_1 from -30 to $+30°$ is possible, negative values predominate (in the ratio ≈2:1). As noted earlier (MacArthur & Thornton, 1991), ψ is above $60°$ for a residue in this position. Considering ϕ (Figure 3(b)), the points are rare below $-140°$ in the g^+ state, whereas in the other two states, although the spread is from ca -60 to $-170°$, most of the points are closer to the latter value.

Pro in *cis* X-Pro has a noteworthy dependence of χ_1 on ϕ and ψ (Figure 3(c) and (d)). Residues predominantly have a positive χ_1 (positive:negative ≈6:1). Notably, however, when ψ is less than $60°$,

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