ANALYSIS OF SHORT INTERPROTON DISTANCES IN PROLINE PEPTIDES AS A GUIDE IN THE INTERPRETATION OF NUCLEAR OVERHAUSER EFFECTS

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Dedicated to the memory of Dr Karel Bláha.

The conformational dependence of interproton distances in model proline peptides has been investigated in order to facilitate interpretation of the results of Nuclear Overhauser Effect (NOE) studies on such peptides. For this purpose two model systems, namely, Ac-Pro-NHMe and Ac-Pro-X-NHMe have been chosen and used. In the former, short interproton distances detectable in NOE experiments permit a clear distinction between conformations with Pro $\psi = -30°$ (helical region) and those in which ψ is around 120° (polyproline region). For the latter, the variation of distances between the protons of methyl amide and the Pro ring have been studied by superimposing on the Ramachandran map in the (ϕ_3, ψ_3) plane. The results show that β-turns and non-β-turn conformations can be readily distinguished from NOE data and such long range NOEs should be detectable for specific non-β-turn conformations. NOEs involving C^β and C^γ protons are particularly sensitive to the state of pyrrolidine ring puckering.

Interproton nuclear Overhauser effects (NOEs)[1] provide a powerful means of probing the conformations of peptides in solution[2-10]. Indeed identification of secondary structure elements in proteins has been greatly aided by the analysis of short interproton distances in regular polypeptide structures[11,12] and extensions of this approach promise to revolutionize the determination of the three-dimensional structure of proteins in solution[13]. The use of NOE distance constraints, together with stereochemical contact criteria[14] or energy minimization techniques[15-18] can be of value in the conformational analysis of peptides in solution. In this report we describe an analysis of interproton distances in model proline peptides, which should be of utility in analyzing NOE data on proline containing systems.

METHODS

The various interporton distances are calculated by generating the molecule for the different ranges of conformational parameters ψ_2, ϕ_3 and ψ_3. For this purpose, the

standard P–C dimensions[19] have been used for the peptide unit and it is kept rigid and planar. For the proline ring, the dimensions are the same as used in other studies[20]. The two hydrogen atoms at C_2^β, C_2^γ and C_2^δ are fixed tetrahedrally and those that are disposed *cis* with respect to carboxyl are superscripted with 1 ($H_2^{\beta 1}$, $H_2^{\gamma 1}$ and $H_2^{\delta 1}$).

RESULTS AND DISCUSSION

For L-Pro residues the constraints of pyrrolidine ring formation largely restrict the range of ϕ values to $\sim -60° \pm 20°$ (ref.[21]). Conformational variability is consequently primarily restricted to changes in ψ values. Theoretical calculations yield three main energy minima centered at $\psi \sim -50°$ (α-helical conformation), $\psi \sim +70°$ (C_7 or γ-turn) and $\psi \sim +120°$ (semi-extended or poly-proline like)[22]. Experimental evidence for the occurence of Pro residues in all these conformations has been obtained from X-ray diffraction studies in the solid state. In acyclic peptides, conformations in the region $\psi \sim 120°$ have been widely observed[21], while the $\psi \sim +70°$ (C_7) conformations are clearly characterized in cyclic peptides[23]. Pro residues occur in the $\psi \sim -50°$ region mostly in the case of peptides containing additional stereochemical constraints like the strongly helix promoting residue, α-aminoisobutyric acid (Aib)[24]. Proline ring geometries are also variable and two major types C^γ_{exo} and C^γ_{endo} have been identified[25,26]. The ^3J(H, H) coupling constants have proved to be an useful tool in distinguishing between these types[27]. The detailed characterization of conformations of peptides having Pro residues, in solution, is, in principle, possible by the analysis of interproton NOEs. In the subsequent section an analysis of relevant interproton distances is presented for two cases, Ac-Pro-NHMe and Ac-Pro-X--NHMe. The Ac-Pro bond has been restricted to the *trans* ($\omega = 180°$) geometry in both cases.

Ac-Pro-NHMe

The conformationally useful interresidue NOEs are those between the NH proton (H_3) and the pyrrolidine ring protons. Figures 1 and 2 summarize the variation of the various relevant interproton distances with ψ_2 for the C^γ_{endo} and C^γ_{exo} proline ring geometries. For purposes of NOE interpretation an interproton distance cutoff of 3 Å may be applied. The various short distances which can lead to observable interresidue NOEs are represented by zones in Fig. 3 in the stereochemically allowed regions of ϕ, ψ space[28] for a range of ϕ values up to $-80°$. The actual ranges of ψ_2 that will correspond to two representative ranges of the NOE observable interproton distances, namely 2·0–2·5 Å and 2·5–3·0 Å are given in Table I. Clearly the $H_3 \leftrightarrow H_2^\gamma$ NOE will be characteristic of conformations in the regions $\psi_2 \sim 120°$

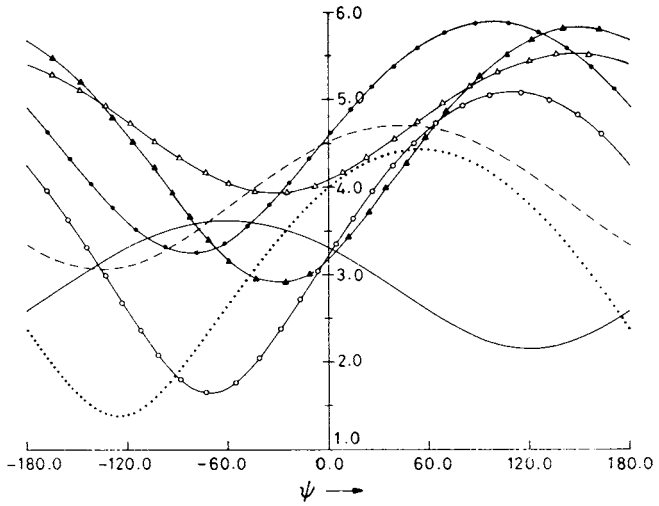

FIG. 1

Variation of interproton distances with ψ_2 when proline ring is in C^{γ}_{endo} conformation: (———) $H_3...H^{\alpha}_2$; (.....) $H_3...H^{\beta 1}_2$; (– – –) $H_3...H^{\beta 2}_2$; ○ $H_3...H^{\gamma 1}_2$; ● $H_3...H^{\gamma 2}_2$; ▲ $H_3...H^{\delta 1}_2$; △ $H_3...H^{\delta 2}_2$

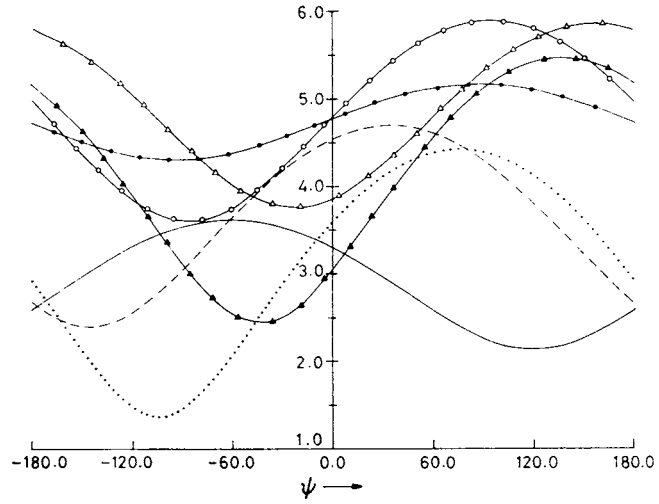

FIG. 2

Variation of interproton distances with ψ_2 when proline ring is in C^{γ}_{exo} conformation: (———) $H_3...H^{\alpha}_2$; (.....) $H_3...H^{\beta 1}_2$; (– – –) $H_3...H^{\beta 2}_2$; ○ $H_3...H^{\gamma 1}_2$; ● $H_3...H^{\gamma 2}_2$; ▲ $H_3...H^{\delta 1}_2$; △ $H_3...H^{\delta 2}_2$

TABLE I

Range of ψ_2 for the NOE observable interproton distances for the C^{γ}_{endo} and C^{γ}_{exo} conformations

Proton[a] pairs	Range of ψ_2 (deg)			
	C^{γ}_{endo}		C^{γ}_{exo}	
	2·0—2·5 Å	2·5—3·0 Å	2·0—2·5 Å	2·0—3·0 Å
$H_3...H^{\alpha}_2$	65 to 170	170 to 180 −180 to −145 30 to 65	65 to 170	170 to 180 −180 to −145 30 to 65
$H_3...H^{\beta 1}_2$	175 to 180 −180 to −165 −85 to −65	−65 to −45 155 to 175	−165 to −145 −60 to −45	−180 to −165 −45 to −25
$H_3...H^{\beta 2}_2$	—	—	−165 to −125	160 to 180 −180 to −165 −125 to −90
$H_3...H^{\gamma 1}_2$	−115 to −100 −40 to −25	−130 to −120 −25 to −5	—	—
$H_3...H^{\delta 1}_2$	—	−50 to −10	−55 to −30	−88 to −55 −30 to 0

[a] $H_3...H^{\gamma 2}_2$ and $H_3...H^{\delta 2}_2$ interproton distances do not fall in the permissible values for NOE for all values of ψ_2. 1 Å = 10^{-10} m.

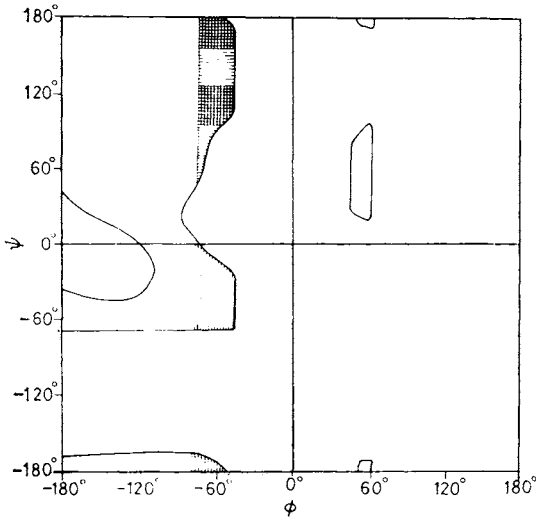

FIG. 3

Ramachandran steric map for an L-amino acid residue showing allowed L-proline values. Zones marked with vertical lines correspond to regions where the interproton distances $H_3...H^{\beta 1}_2$, $H_3...H^{\beta 2}_2$, $H_3...H^{\gamma 1}_2$ and $H_3...H^{\delta 1}_2$ are <3 Å. Zones marked with horizontal lines correspond to regions where interproton distance <3 Å occur for $H_3...H^{\alpha}_2$. Cross hatched regions correspond to regions where $H_3...H^{2}_{\alpha}$, $H_3...H^{\beta 1}_2$ and $H_3...H^{\beta 2}_2$ interproton distances are <3 Å

and $\psi_2 \sim 70°$, with a distance $<2{\cdot}5$ Å over almost the entire range of allowed values in the positive region for ψ_2. The $\psi_2 \sim -50°$ region is characterized by the short distances $H_3 \leftrightarrow H_2^{\beta 1}$, $H_3 \leftrightarrow H_2^{\gamma 1}$ and $H_3 \leftrightarrow H_2^{\delta 2}$. Thus strong NOEs between the $C^{\delta}H_2$, $C^{\beta}H_2$ and $C^{\gamma}H_2$ resonances of Pro and the NH proton of the succeeding residue are diagnostic of conformations in the helical region. Indeed, the distinction between Pro residues at the $i + 1$ position of Type I (III) β-turns ($\psi_2 \sim -30°$) and Type II β-turns ($\psi_2 \sim 120°$)[29,30] may be made uing these criteria.

Ac-Pro-X-NHMe

For determining the conformation of Pro-X sequences three conformational angles ψ_2(Pro) and ϕ_3, ψ_3(X) need to be estimated. Studies in the literature have largely focussed on β-turn conformations[30,31], which are recognized by the involvement of the NH group of the residue succeeding X (residue 4) in intramolecular hydrogen bonding. Short $N_{i+2}H \leftrightarrow N_{i+3}H$ distances are obtained in both Type I (III) and Type II β-turns, leading to an observable $H_3 \leftrightarrow H_4$ NOE[12]. A distinction between the β-turn types is then made using the $H_2^{\alpha} \leftrightarrow H_3$ NOE as a diagnostic[8]. In this study the possibility of a long range NOE between H_4 and the Pro ring protons was explored by conducting a systematic search for these short interproton distances. The ψ_2 value of Pro was fixed as either $-30°$ or 120°, corresponding to the two major energy minima. The distance between H_4 and the Pro ring protons is then a function of ϕ_3, ψ_3. Contour maps, representing the various interproton distances on the ϕ_3, ψ_3 surface, for the seven sets of interproton distances were generated corresponding to Pro (2) ψ values of $-30°$ and 120° for both C^{γ}_{exo} and C^{γ}_{endo} ring

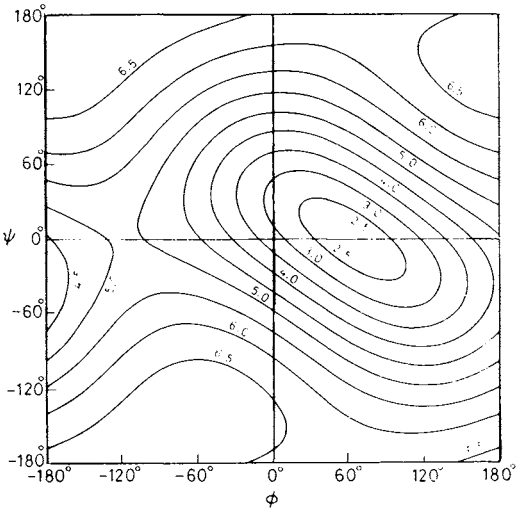

FIG. 4

Representative contour plot on the (ϕ_3, ψ_3) plane showing the variation of the $H_4 \ldots H_2^{\beta 1}$ distance. A fixed value of $\psi_2 = -30°$ and a C^{γ}_{endo} ring conformation has been chosen

geometries. A representative map is shown in Fig. 4. The contours representing distances $\leqq 3$ Å are superimposed on the Ramachandran (ϕ, ψ) map in Fig. 5.

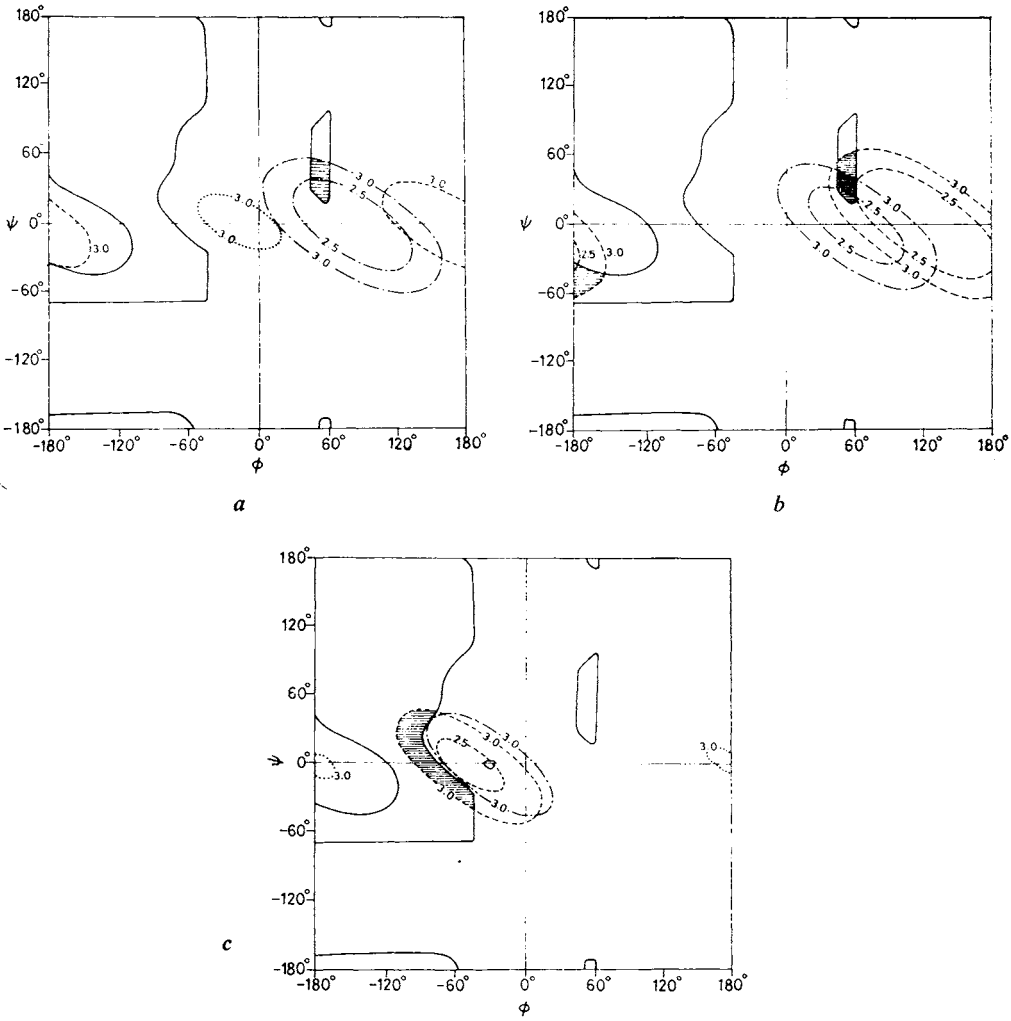

FIG. 5

Superposition of the 2·5 Å and 3·0 Å contours taken from plots such as those shown in Fig. 4, on the Ramachandran ϕ, ψ steric map. Shaded regions correspond to allowed conformations which maybe characterized by long range NOEs between H_4 (methylamide NH) and proline ring protons.

a ····· $H_4 \ldots H_2^{\alpha}$ ($\psi_2 = -30°$); —·—· $H_4 \ldots H_2^{\beta 1}$ ($\psi_2 = -30°$ C^{γ}_{exo}); ——— $H_4 \ldots H_2^{\delta 1}$ ($\psi_2 = -30°$ C^{γ}_{exo}). *b* —·—· $H_4 \ldots H_2^{\beta 1}$ ($\psi_2 = -30°$ C^{γ}_{endo}); ——— $H_4 \ldots H_2^{\gamma 1}$ ($\psi_2 = -30°$ C^{γ}_{endo}). *c* ····· $H_4 \ldots H_2^{\delta 1}$ ($\psi_2 = -30°$ C^{γ}_{endo}); —·—· $H_4 \ldots H_2^{\beta 1}$ ($\psi_2 = 120°$ C^{γ}_{exo}); ——— $H_4 \ldots H_2^{\beta 1}$ ($\psi_2 = 120°$ C^{γ}_{endo})

The shaded regions of sterically allowed (ϕ, ψ) space then encompass conformations in which NOEs may be observed between H_4 and the Pro ring protons. For a Pro ψ_2 value of $-30°$, such long range interresidue NOEs ($H_2^\beta \leftrightarrow H_4$ or $H_2^\gamma \leftrightarrow H_4$) are observable when the X residue falls in the *left* handed helical region (positive ϕ, ψ). In the C^γ_{exo} geometry only a short $H_2^\beta \leftrightarrow H_4$ distance is observed, whereas both $H_2^\beta \leftrightarrow H_4$ and $H_2^\gamma \leftrightarrow H_4$ distances are short in the C^γ_{endo} conformation. For the X residue in the *right* handed helical region the long range NOE, $H_2^\beta \leftrightarrow H_4$, is expected only for $\psi_2 \sim 120°$, with a C^γ_{endo} geometry (Fig. 5*c*).

The above analysis leads to the following conclusions for Pro-X sequences. Long range NOEs between H_4 and the H^β and H^γ protons of the Pro ring are expected in the following cases: Conformation A, $\phi_{Pro} \sim -60°$, $\psi_{Pro} \sim -30°$, $\phi_X \sim 60°$, $\psi_X \sim 30°$; Conformation B, $\phi_{Pro} \sim -60°$, $\psi_{Pro} \sim 120°$, $\phi_X \sim -60°$, $\psi_X \sim -30°$. These conformations can be generated from idealized Type I (III) or Type II β-turn structures by performing an approximate 180° rotation about either ψ_2(Pro) or ϕ_3(X), with small accompanying changes about ψ_3(X). Such non-β-turn structures are indeed stereochemically favourable for Pro-X sequences (Fig. 6). Such conformations also have calculated energies which compare favourably with those for β-turn structures, if intramolecular hydrogen bonding is not considered (unpublished). One may then expect that such conformations are likely in strongly solvating (hydrogen bonding) solvents. The long range NOEs between H_4 and the Pro ring

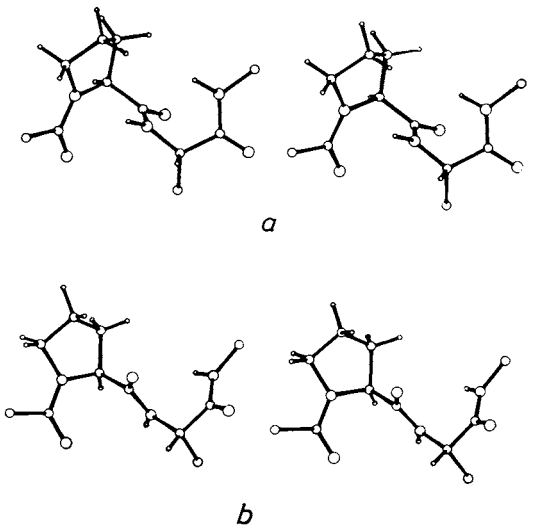

FIG. 6

Stereopair of non -β-turn conformations of -Pro-X-sequences.
a $\phi_2 = -60° \psi_2 = -30° \phi_3 = 60° \psi_3 = 30°$. *b* $\phi_2 = -60° \psi_2 = 120° \phi_3 = -60° \psi_3 = -30°$

protons should provide evidence for their occurrence in solution. Indeed, recent experimental studies in this laboratory on peptides of the type pivaloyl-Pro-X--NHMe (X = L-Ala, L-Leu) have permitted observation of these NOEs, although the observed magnitudes are small (unpublished). Since these sequences have been shown to possess an appreciable population of β-turn conformations in solution[8,32,33] the additional observation of the $H_4 \leftrightarrow H_2^\beta$, H_2^γ NOEs provides definitive proof of conformational heterogeneity. The detection of "mutually exclusive" NOEs is invariably a clear indicator of the presence of multiple conformations in solution.

The results presented above provide a convenient framework for considering NOE studies of Pro containing peptides. The use of a 3 Å cutoff limit is valid for small peptides in the positive NOE region ($\omega\tau_c < 1$)[34]. In macromolecules, NOEs may be observed over longer distances depending on the experimental conditions used[13]. Transient NOE experiments may permit filtering of NOEs such that only very short distances are probed[35,36]. In such cases NOE experiments may serve as a particularly sensitive diagnostic for specific conformations.

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