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# Backbone side-chain interactions in peptides

## IV. β-Turn conformations of Asp and Asn-containing dipeptides in solute and solid states

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**Abstract.** IR, $^1$H-NMR and X-ray experiments have been carried out on dipeptides with the Pro-Asp and Pro-Asn sequences protected on both ends by amide groups. The Pro-Asp dipeptide was investigated for the carboxylic, methyl ester and carboxylate forms of the Asp residue.

In solution, all dipeptides are found to accommodate almost exclusively the βI-turn conformation stabilized by an interaction between the Asp or Asn-NH and C'O bonds. The βI-turn percentage roughly parallels the basicity of the Asp or Asn side substituent, and decreases from $Asp^-$ to Asn, and to Asp or Asp(OMe).

The βI-turn, stabilized by the interaction involving the Asp-C'O site, is retained in the crystal structure of the Pro-Asp(OMe) dipeptide. The Pro-Asp and Pro-Asn dipeptides assume a βII-turn conformation in the solid state and the polar Asp or Asn side-groups are involved in a complex network of intermolecular interactions.

**Key words:** β-bend, crystal structure, hydrogen bond, infrared spectroscopy, NMR spectroscopy, peptide conformation, X-ray diffraction

### Introduction

The preferential incorporation of amino acid residues containing short polar side-chains (Ser, Asn, Asp...) in β-folded sequences is well documented by different statistical analyses of crystallized proteins (Chou and Fasman 1977; Smith and Pease 1980; Kolaskar et al. 1980; Rose et al. 1985). The question then arises as to the reason for this preference: is it an intrisic characteristic of these short sequences, due to a local interaction involving the polar side function, or is it merely the consequence of the accessibility to the surrounding water molecules of these hydrophilic residues, the adjacent hydrophobic regions being solvent-shielded?

This question cannot be answered by the above mentioned statistical analyses, due to the restricted dataset for each dipeptide sequence (Kolaskar et al. 1980). We believe that the model dipeptides with the general formula RCO-Yaa-Yaa-NHR′ can give us some information about the intrinsic conformational properties of dipeptide sequences (Boussard and Marraud 1985), and about the existence of an eventual intramolecular hydrogen bond involving the polar side functions (Marraud and Aubry 1984).

We have recently investigated, by IR, $^1$H-NMR and X-ray diffraction studies, the model dipeptides with Pro-Xaa sequences in which Xaa is an apolar (Ala, Leu, Val) or weakly polar (Cys, Met, Phe, Tyr) residue (Aubry et al. 1985), or contains a hydroxyl group (Ser, Thr) (Aubry et al. 1984; Marraud and Aubry 1984; Aubry and Marraud 1985). We have shown that the existence, when possible, of an intramolecular interaction of the Xaa-NH bond with the Xaa side accepting group greatly increases the propensity to the βI-turn formation in solution[1]. In the solid state there is competition between inter and intramolecular interactions, and the molecular conformation depends on the nature of Xaa. The βI-folded conformer is retained for Xaa = Ser (Aubry et al. 1984) or Thr (Aubry and Marraud 1985), but for all the other cases investigated so far (Xaa = Ala, Leu, Cys(Me), Phe, Tyr), the βII-folded conformer turns out to be the stable conformation (Aubry et al. 1985).

We have already mentioned the frequent occurrence of the Asp and Asn residues in β-folded regions, and this has been the subject of theoretical (Peters and Peters 1984a and b) and experimental

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1 For the definition of the βI and βII-turn conformations in peptides, see Lewis et al. (1973)

(Ishii et al. 1984) investigations, all concluding in favour of the possible existence of side chain interactions.

The present paper deals with the Pro-Asp and Pro-Asn model dipeptides, together with some of their derivatives. The aspartic residue was investigated in the carboxylic Asp, carboxylate $Asp^-$, $Me_4N^+$ and ester Asp(OMe) states, using IR and $^1$H-NMR spectroscopy. The three Asn, Asp and Asp(OMe)-containing dipeptides grew single crystals suitable for X-ray diffraction.

## Experimental

### *Materials*

The dipeptides obey the general formula:

$$\text{tBuCO-Pro-Xaa-NH}\begin{cases}\text{Me}\\ \text{iPr}\end{cases}.$$

They were obtained by classical routes of peptide synthesis (Bodanski 1984).

The pivaloyl tBuCO group was preferred to the more common Boc or Ac groups because it prevents the occurrence of the *cis* disposition of the tBuCO-Pro link (Nishihara et al. 1975). This allows an easier interpretation of IR and $^1$H-NMR data.

The carboxylate form of the Asp derivative was obtained by direct lyophylisation of a stoichiometric mixture of the Pro-Asp model dipeptide with tetramethylammonium hydroxide pentahydrate. The $Me_4N^+$ cation was selected because it probably gives rise to very weak interactions with the peptide part, and because it permits better solubility in organic solvents than the more common $Na^+$ cation.

In the following text, the model dipeptides are represented in abbreviation by their dipeptide sequence. They were investigated in chlorinated ($CH_2Cl_2$, $CHCl_3$) and aprotic solvents (MeCN, $Me_2SO$).

### *IR spectroscopy*

IR spectra were run at room temperature on a Fourier Transform Brüker IFS-85 apparatus with a resolution of 2 $cm^{-1}$. The most informative frequency domains are those of the N–H (3,200 – 3,500 $cm^{-1}$) and C=O (1,550 – 1,750 $cm^{-1}$) stretching vibrations. The absence of solute-solute interaction for concentrations of $5 \times 10^{-3}$ mol/l was confirmed by the fact that unmodified spectra were obtained after further dilution.

### *$^1$H-NMR spectroscopy*

$^1$H-NMR spectra were scanned at room temperature on a Jeol FX-100 apparatus equipped with a $^{14}$N heterodecoupling unit, delivering sharp NH proton signals. Chemical shifts are reported downfield from an internal tetramethylsilane reference. The NH and the Asp or Asn-$C^\beta H_2$ proton signals proved to be the most sensitive to the experimental conditions and the most informative for conformational purposes.

### *X-ray diffraction*

Single crystals of tBuCO-Pro-Asp(OMe)-NHiPr, tBuCO-Pro-Asp-NHMe and tBuCO-Pro-Asn-NHMe were obtained by slow evaporation of ethylacetate solutions. X-ray data were collected at room temperature on an Enraf Nonius CAD 4 automatic diffractometer, with a graphite monochromator and the Cu $K\alpha$ radiation ($\lambda = 1.54051$ Å), in the $\theta - 2\theta$ scanning mode ($\theta \leq 70°$). Intensity data were corrected for decay when three standard reflections decreased by more than 5%, and for Lorentz and polarization effects. Due to the small size (< 0.3 mm) of the crystals, the absorption was neglected. Cell dimensions, obtained by refinement from a set of 25 high angle reflections, are indicated in Table 1, together with other experimental parameters.

The structures were solved by direct methods using the computer program MULTAN-80 (Main et al. 1980) and refined using a full matrix least-squares procedure (Sheldrick 1976). Atom scattering factors used were those listed in the International Tables for X-ray Crystallography (1974).

E-maps revealed the overall structures. After a full-matrix least-squares refinement using individual anisotropic thermal parameters, we noticed a large temperature factor associated with the Pro-$C^\gamma$ atom for all three dipeptides and with the N and C-terminal methyl groups for tBuCO-Pro-Asp(OMe)-NHiPr. However, inspection of the Fourier map revealed a single but elongated peak for these atoms. Therefore, the Pro cycle probably experiences a rapid inversion of the $C^\gamma$ atom, corresponding to a dynamic disorder (Nair and Vijayan 1981). Because of this disorder, the Pro hydrogen atoms were not located for all three dipeptides. The other hydrogen atoms, excepting the Asp carboxylic proton, appeared on difference maps. Refined parameters were calculated using anisotropic temperature factors for the non-hydrogen atoms and fixed isotropic thermal factors for the hydrogen atoms. The final factors

$$R = \Sigma K \,||F_0| - |F_c||/\Sigma K |F_0|$$
$$R_w = \Sigma w |K|F_0| - |F_c||/\Sigma w K |F_0|$$

are given in Table 1.

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2 According to the usual attribution (Tanimura et al. 1984), the low field signal was attributed to the pro-R proton of the Asn and Asp residues

**Table 1.** Unit cell dimensions and other crystal data

| | tBuCO-Pro-Asp(OMe)-NHiPr | tBuCO-Pro-Asp-NHMe | tBuCO-Pro-Asn-NHMe |
|---|---|---|---|
| Space group | $P2_12_12_1$ | $P2_12_12_1$ | $P2_1$ |
| $Z$ | 4 | 4 | 2 |
| $a$ (Å) | 10.466(1) | 9.312(1) | 9.510(1) |
| $b$ (Å) | 11.472(1) | 9.632(4) | 7.867(2) |
| $c$ (Å) | 17.513(1) | 19.529(5) | 11.861(3) |
| $\beta$ (deg) | 90 | 90 | 101.73(2) |
| $d$ (calc) | 1.17 | 1.24 | 1.25 |
| Independent reflections | 1,994 | 1,951 | 1,814 |
| Unique reflections [a] | 1,171 | 1,402 | 1,688 |
| Final $R$ | 0.066 | 0.061 | 0.038 |
| Final $R_w$ | 0.069 | 0.065 | 0.043 |
| $W$ | $1.344/(\sigma^2(F) + 0.0002F^2)$ | $2.68/(\sigma^2(F) + 0.0007F^2)$ | $1.64/(\sigma^2(F) + 0.0012F^2)$ |

[a] $I > 1.5\sigma(I)$

**Table 2.** N–H and C=O stretching frequencies ($cm^{-1}$) for the tBuCO-Pro-Asp(OMe)-NHMe, tBuCO-Pro-Asp-NHMe, tBuCO-Pro-Asp-NHMe, $Me_4N^+$, and tBuCO-Pro-Asn-NHMe dipeptides and the isovaleric acid derivatives [a] modelling the Asp or Asn side-chain ($CH_2Cl_2$, 0.001 mol/l)

| Compound | *NH*Me | Asp or Asn-*NH* | Asn-$N^{\delta}H_2$ | tBu*CO* | Pro-*CO*, Asp-*CO* | Asp or Asn-$C^{\gamma}O$ |
|---|---|---|---|---|---|---|
| Pro-Asp(OMe) | 3,357 | 3,420 | | 1,610 | 1,675 | 1,725; 1742 [b] |
| R-$CO_2$Me [a] | | | | | | 1,741 |
| Pro-Asp | 3,353 | 3,421 | | 1,610 | 1,678 | 1,740 |
| R-$CO_2$H [a] | | | | | | 1,746; 1,707 [c] |
| Pro-$Asp^-$, $Me_4N^+$ | 3,350 | 3,400 [b] | | 1,608 | 1,663 | 1,595; 1,487 [e] |
| R-$CO_2^-$, $Me_4N^+$ [a] | | | | | | 1,570; 1,487 [e] |
| Pro-Asn | 3,353 | [d] | 3,520; 3,402 | 1,609 | 1,681 | 1,665 |
| R-$CONH_2$ [a] | | | 3,526; 3,408 | | | 1,676 |

[a] R = $(CH_3)_2CH-CH_2$
[b] Shoulder of a strong absorption band
[c] This absorption is probably due to a small amount of the cyclic dimer
[d] Not visible and embedded in the Asn-$N^{\delta}H_2$ absorption
[e] These frequencies correspond to the stretching vibrations of the Asp-$C^{\gamma}O_2^-$ carboxyl group

For a reliable comparison of the inter and intramolecular hydrogen bond dimensions, and following the Taylor and Kennard method (1983), NH hydrogen atoms were placed at 1.03 Å from N in the direction obtained by refinement. Fractional coordinates, anisotropic and equivalent isotropic thermal parameters, bond lengths and bond angles are available on request from the authors.

## Solute state

*Intramolecular interations.* Intramolecular hydrogen bonds were characterized by IR and $^1$H-NMR spectroscopy.

N–H and C=O stretching frequencies in $CH_2Cl_2$ solution are listed in Table 2 and attributed by comparison with previously investigated dipeptides (Boussard et al. 1979; Aubry and Marraud 1986), and with simple acid, ester and amides mimicking the Asp or Asn side-chain.

The NH stretching frequency near 3,360 $cm^{-1}$, sensitive to the Me or iPr nature of the C-terminal group, is assigned to the C-terminal NH bond involved in a $(i + 3) \rightarrow i$ hydrogen bond typical of a $\beta$-turn. The absence of any significant absorption near 3,450 $cm^{-1}$, typical of a free NH vibrator (Boussard and Marraud 1985) shows the great predominance of $\beta$-folded conformers.

The low frequency of the middle NH bond is that of an intramolecularly hydrogen bonded vibrator engaged in a rather weak interaction for Pro-Asp(OMe) and Pro-Asp, or a stronger interaction for Pro-$Asp^-$ or Pro-Asn. The Asn-$N^{\delta}H_2$ frequencies (3,520 and 3,402 $cm^{-1}$) are, in contrast, those of free vibrators.

This interpretation is corroborated by the C=O stretching absorption domain although some absorption bands overlap completely. All dipeptides exhibit a strong absorption at 1,610 $cm^{-1}$, attributed to the pivaloyl carbonyl bond engaged in the $(i + 3) \rightarrow i$

interaction (Aubry et al. 1985), whereas both Pro and Xaa-CO contributions have the frequency typical of free vibrators.

The assignment of the Asp(OMe), Asp and Asn-C$^{\gamma}$O absorption is quite easy and we note that its frequency is systematically lower than that of the corresponding isovaleric derivative modelling the side-chain. This confirms the existence of intramolecular bonding of the middle NH bond to the side-chain as accepting site. The case of $Asp^-$ is more complicated because of the complex vibration modes of a $CO_2^-$ group, and is better solved by $^1$H-NMR spectroscopy.

The progressive addition of $Me_2SO$ to a solution of peptide in chloroform has a deshielding effect on the NH protons, and their NMR signals are shifted to an extent depending on their free or bonded character. Hydrogen bonded NH proton signals are less rapidly shifted than free NH protons (Urry et al. 1975).

Figure 1 a reveals a small shift for the C-terminal NH proton signal, confirming its inclusion in a $(i+3) \rightarrow i$ hydrogen bond. In a previous paper (Boussard and Marraud 1985), we have shown that the $\beta$-turn percentage, % [$\beta$-turn], in $CH_2Cl_2$ is related to the difference, $\Delta\delta$, of the C-terminal NH proton chemical shifts when measured in $Me_2SO$ and then in $CHCl_3$:

$$\% [\beta\text{-turn}] = 100 - 39\,\Delta\delta .$$

Table 3 shows that $Asp^-$ induces the maximum $\beta$-turn ratio, followed by Asn, Asp and Asp(OMe). These values are higher than for the homologous Pro-Leu dipeptide (56%, Aubry et al. 1985) and this

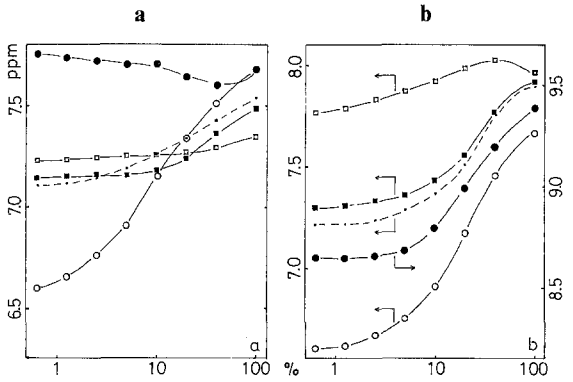


**Fig. 1.** Influence of $Me_2SO$ content in $Me_2SO/CHCl_3$ mixtures on the C-terminal (**a**) and Xaa (**b**) NH proton NMR signals for the tBuCO-Pro-Xaa-NHMe dipeptides (Xaa = Leu (○), Asp(OMe) (·), Asp (■), $Asp^-$, $Me_4N^+$ (●) and Asn (□)). The observed shift of a signal on addition of $Me_2SO$ indicates the progressive solvation of the corresponding NH bond by the strong aprotic $Me_2SO$ molecule. An NH bond protected against solvation by its participation in an intramolecular interaction gives rise to a rather flat variation

increase is probably due to the interaction of the side-chain already demonstrated by IR spectroscopy. Figure 1 b confirms the low solvent accessibility of the middle NH proton when compared to Pro-Leu. All three Asp-containing dipeptides give similar variations as a function of $Me_2SO$ content and this shows that the carboxylate group in $Asp^-$ behaves exactly as the ester and carboxylic groups in Asp(OMe) and Asp respectively, and is engaged in the same type of interaction with the middle NH bond.

*$\beta$-folded conformation.* The spectroscopic data leads us to the conclusion that the Asp or Asn-containing dipeptides are predominantly $\beta$-folded by a classical $(i+3) \rightarrow i$ hydrogen bond. Moreover, an additional intramolecular interaction between the middle NH bond and the side C$^{\gamma}$O group contributes significantly to the stability of this $\beta$-turn which must be of the $\beta$I type (Fig. 2). A $\beta$II conformation would not be compatible with this additional interaction and would correspond to a smaller $^3J_{N\alpha}$ coupling constant (expected value 4.5 Hz, experimental values in Table 3) and to a higher Pro-CO stretching fre-

**Table 3.** $^1$H-NMR data[a], $\beta$I-turn ratio[b], and percentage of the preferential rotamers of the Asp or Asn $C^{\alpha}-C^{\beta}$ bond for the tBuCO-Pro-Xaa-NHMe dipeptides ($CHCl_3$, 0.005 mol/l)

| Xaa | Asp(OMe) | Asp | $Asp^-$, $Me_4N^+$ | Asn |
|---|---|---|---|---|
| $\Delta\delta$ (NHMe)[c] | 0.45 | 0.33 | −0.03 | 0.16 |
| % [$\beta$I-turn] | 83 | 87 | 100 | 94 |
| $^3J_{N\alpha}$[d] | 9.5 | 8.5 | 7.8 | 8.8 |
| $C^{\alpha}H-C^{\beta}H_RH_S$ bond[e] | | | | |
| $\delta$ ($C^{\beta}H_S$) | 2.52 | 2.65 | 2.45 | 2.41 |
| $\delta$ ($C^{\beta}H_R$) | 3.31 | 3.21 | 2.87 | 3.16 |
| $^3J_{\alpha\beta S}$ | 5.0 | 5.4 | 6.2 | 4.5 |
| $^3J_{\alpha\beta R}$ | 3.9 | 4.0 | 2.2 | 3.9 |
| $^2J_{\beta S\beta R}$ | 17.3 | 17.3 | 16.3 | 15.2 |
| % rotamer I ($\chi^1 \simeq -60°$)[f] | 15 | 18 | 0 | 9 |
| % rotamer II ($\chi^1 \simeq -180°$)[f] | 3 | 4 | 0 | 3 |
| % rotamer III ($\chi^1 \simeq -60°$)[f] | 82 | 78 | 100[g] | 88 |

[a] Chemical shifts $\delta$ in ppm and coupling constants $J$ in Hz

[b] % [$\beta$I-turn]

[c] Shift in ppm of the *NH*(Me) proton NMR signal as measured in $CHCl_3$ and $Me_2SO$ pure solutions. This shift is related to the $\beta$I-turn ratio by % [$\beta$I-turn] = 100 − 39 $\Delta\delta$ (NHMe) (Boussard and Marraud 1985)

[d] $^3J$ vicinal coupling constant in the Asp or Asn[H–N–$C^{\alpha}$–H] fragment

[e] Due to the nature of the group in the $\gamma$-position for Asp or Asn residues, the usual pro-R or pro-S assignment for the $C^{\beta}H_2$ protons (Tanimura et al. 1984; Picur and Siemion 1983) must be inverted ($C^{\beta}H_R$ signal shifted downfield)

[f] See Cung and Marraud (1982)

[g] Interpretation in terms of a single conformer corresponding to $\chi^1 \simeq 75°$ (see text)

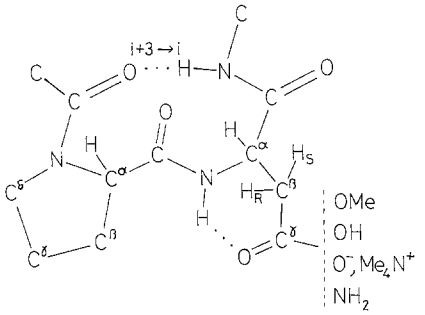


**Fig. 2.** Schematic representation of the $\beta$I-turn characterized by the classical $(i+3) \rightarrow i$ hydrogen bond, and stabilized by an additional interaction involving the middle NH bond and the side C$^{\gamma}$O carbonyl group of an Asp or Asn residue

**Table 4.** Molecular conformation described by the dihedral angles defined by the four atoms in the 1$^{st}$ column[a] for the tBuCO-Pro-Asp(OMe)-NHiPr, tBuCO-Pro-Asp-NHMe, and tBuCO-Pro-Asn-NHMe dipeptides in the solid state

| Atoms | Angle[a] | Pro-Asp(OMe) | Pro-Asp | Pro-Asn |
|---|---|---|---|---|
| $C'_0-N_1-C^{\alpha}_1-C'_1$ | $\phi_1$ | −66 | −57 | −59 |
| $N_1-C^{\alpha}_1-C'_1-N_2$ | $\psi_1$ | −20 | 134 | 138 |
| $C_1-N_2-C^{\alpha}_2-C'_2$ | $\phi_2$ | −91 | 59 | 66 |
| $N_2-C^{\alpha}_2-C'_2-N_3$ | $\psi_2$ | 6 | 26 | 11 |
| $C^{\alpha}_0-C'_0-N_1-C^{\alpha}_1$ | $\omega_0$[b] | −179 | 177 | 173 |
| $C^{\alpha}_1-C'_1-N_2-C^{\alpha}_2$ | $\omega_1$[b] | −179 | −177 | −179 |
| $C^{\alpha}_2-C'_2-N_3-C^{\alpha}_3$ | $\omega_2$[b] | −177 | −178 | 175 |
| $N_2-C^{\alpha}_2-C^{\beta}_2-C^{\gamma}_2$ | $\chi^1_2$ | 68 | −159 | −69 |
| $C^{\alpha}_2-C^{\beta}_2-C^{\gamma}_2-X^{\delta}_2$[c] | $\chi^2_2$ | 158 | −150 | 143 |
| $C^{\beta}_2-C^{\gamma}_2-O^{\delta}_2-C^{\varepsilon}_2$ | $\chi^3_2$ | 174 | | |

[a] According to the recommendations from IUPAC-IUB 1970. Dihedral angles are in degrees. Due to the disordered Pro-C$^{\gamma}$ atom, the values for the Pro cycle are not reported
[b] This dihedral angle is characteristic for a trans ($\omega \simeq 180°$) or cis ($\omega \simeq 0°$) disposition of the amide groups
[c] X = O for Asp and Asp(OMe); X = N for Asn

**Table 5.** Hydrogen bond distances (Å) for the tBuCO-Pro-Asp(OMe)-NHiPr, tBuCO-Pro-Asp-NHMe, and tBuCO-Pro-Asn-NHMe dipeptides in the solid state

| Peptides | Atoms | Symmetry code | Distance |
|---|---|---|---|
| Pro-Asp(OMe) | $N_3-H\ldots O_0$[a] | $x, y, z$ | 3.20 |
| | $N_2-H\ldots O^{\gamma}_2$[b] | $x, y, z$ | 2.94 |
| Pro-Asp | $N_3-H\ldots O_0$[a] | $x, y, z$ | 2.92 |
| | $N_2-H\ldots O_1$[c] | $1-x, -1/2+y, 3/2-z$ | 2.83 |
| | $O^{\delta}-H\ldots, O_2$[c] | $-1/2+x, 5/2+y, 1-z$ | 2.65 |
| Pro-Asn | $N_3-H\ldots O_0$[a] | $x, y, z$ | 2.91 |
| | $N_2-H\ldots O^{\gamma}_2$[c] | $-x, y, 1-z$ | 2.87 |
| | $N^{\delta}-H_t\ldots O_0$[c,d] | $x, -1+y, z$ | 2.91 |
| | $N^{\delta}-H_c\ldots O^{\gamma}_2$[c,d] | $-x, -1+y, 1-z$ | 3.13 |

[a] Intramolecular hydrogen bond of the $(i+3) \rightarrow i$ type ($\beta$-turn)
[b] Intramolecular Asp(OMe)-NH to Asp(OMe)-C$^{\gamma}$O hydrogen bond involving the Asp(OMe) side-chain
[c] Intermolecular hydrogen bond between two molecules related by the symmetry code
[d] $H_t$ is trans and $H_c$ is cis to $O^{\gamma}_2$ in the Asn residue

quency (expected value 1,690 cm$^{-1}$, experimental values in Table 2) (Aubry et al. 1985).

The orientation of the Asp or Asn side chain is related to the proton coupling constants in the C$^{\alpha}$H−C$^{\beta}$H$_2$ fragment (Cung and Marraud 1982). However, the vicinal coupling constants, $^3J_{\alpha\beta R}$ and $^3J_{\alpha\beta S}$, can be interpreted either in terms of a single conformational state of the C$^{\alpha}$−C$^{\beta}$ bond, or in terms of three interconverting staggered conformations corresponding to $\chi^1 \simeq -60°$ (rotamer I), 180° (rotamer II), and 60° (rotamer III) in the usual conventions (IUPAC-IUB 1970). The former interpretation only applies for Pro-Asp$^-$ (Cung and Marraud 1982), giving a Asp$^-$-$\chi^1$ value (Table 3) corresponding to a slightly distorted rotamer III. In the other three cases, the second interpretation gives a large predominance of rotamer III over the other two conformations (Table 3).

Considering now the C$^{\beta}$−C$^{\gamma}$ bond contiguous to a carbonyl C$^{\gamma}$O group, its conformational state ($\chi^2$ dihedral angle) is related to the geminal proton coupling constant $^2J_{\beta R\beta S}$ (Montecalvo and St. Jacques 1975; Barfield et al. 1976). The larger value for Pro-Asp and Pro-Asp(OMe) (Table 3) corresponds to a $\chi^2$ value of nearly 180° whereas the smaller coupling for Pro-Asn corresponds to a lower angle of nearly 150°. This is consistent with a stronger Asn-NH to Asn-C$^{\gamma}$O interaction in the latter case, as can be inferred from Fig. 1 b.

## Solid state

Excepting the disordered Pro-C$^{\gamma}$ atom in all three dipeptides investigated, and iPr or tBu-Me groups in tBuCO-Pro-Asp(OMe)-NHiPr, the molecular conformations of Pro-Asp(OMe), Pro-Asp and Pro-Asn could be determined with a good degree of accuracy. The usual dihedral angles giving the conformational states of the bonds (IUPAC-IUB 1970) are specified in Table 4.

All amide bonds are very near the trans planar conformation ($\omega_i \simeq 180°$, $i = 0-2$) and all three molecules are $\beta$-folded by an $(i+3) \rightarrow i$ hydrogen bond (Table 5). However, this fold is of the $\beta$I-type for Pro-Asp(OMe) (Fig. 3 a) and of the $\beta$II-type for both Pro-Asp and Pro-Asn (Fig. 3 b and c). In the former case, the molecular conformation is stabilized

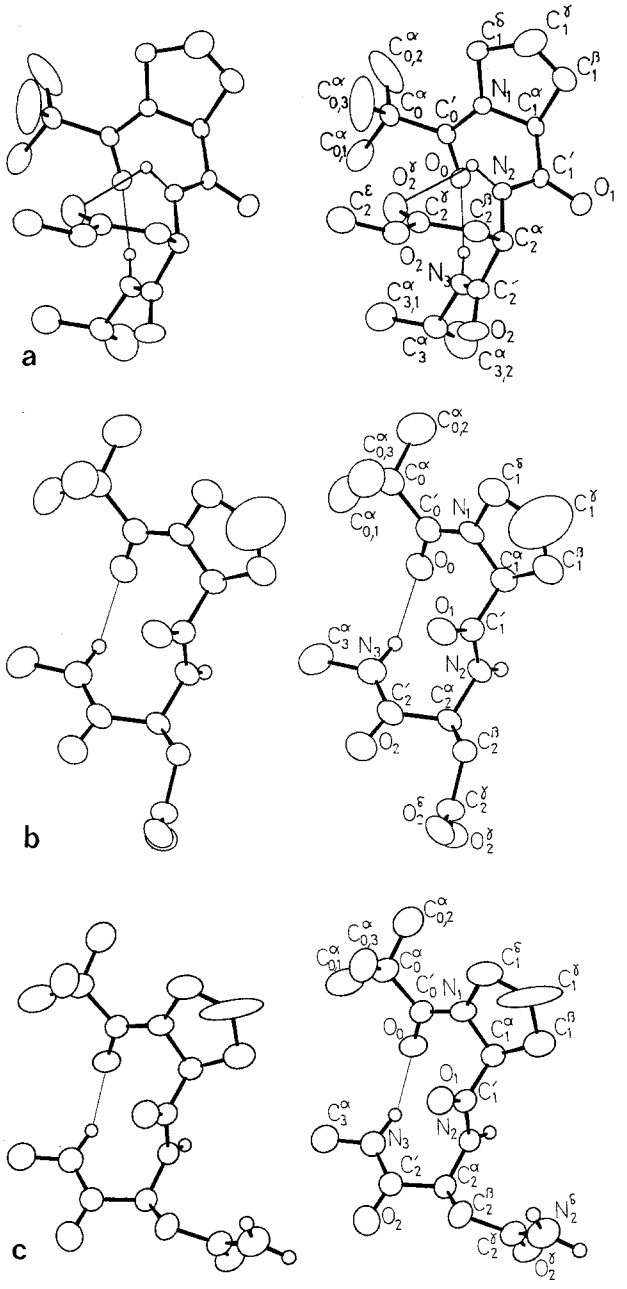

**Fig. 3.** Stereoviews of the molecular conformations for the tBuCO-Pro-Asp(OMe)-NHiPr (**a**, $\beta$I-turn), tBuCO-Pro-Asp-NHMe (**b**, $\beta$II-turn) and tBuCO-Pro-Asn-NHMe (**c**, $\beta$II-turn) dipeptides in the solid state. The intramolecular $(i+3) \rightarrow i$ and Asp(OMe)-NH to Asp(OMe)-C$^{\gamma}$O hydrogen bonds are indicated by thin lines. The ellipsoids correspond to a 50% occupancy for the atomic positions

by an additional intramolecular Asp(OMe)-NH to Asp(OMe)-C$^{\gamma}$O interaction. The other two dipeptides are characterized by a complex network of intermolecular interactions involving the Asp or Asn side chains (Table 5), and each molecule is hydrogen bonded to four neighboring molecules by one or two hydrogen bonds (Fig. 4).

## Discussion

The crystal structure of Pro-Asp(OMe) gives direct evidence for the existence of the Asp-NH to Asp-C$^{\gamma}$O interaction and for its importance as a stabilizing factor of the $\beta$I-turn. However, the different $\beta$-folding modes of both Pro-Asp and Pro-Asn dipeptides in solution ($\beta$I-turn) and in the solid state ($\beta$II-turn) illustrate the conformational flexibility of homochiral sequences (Aubry et al. 1985). This suggests that long-range interactions involving the polar side function are sufficient to induce a transition from the usual $\beta$I-turn to the $\beta$II-turn conformation.

In agreement with results reported earlier (Ishii et al. 1984), deprotonation of the Asp residue increases the $\beta$-turn ratio in solution. Because of the increased basic character of $CO_2^-$ with respect to $CO_2H$, deprotonation induces a stronger interaction with the peptide backbone.

In all four cases investigated, the Asp or Asn $C^{\alpha}-C^{\beta}$ bond preferentially accommodates (in solution) the so-called rotamer III conformation ($\chi^1 \simeq 60°$). This conformation is generally less favored than the other two staggered orientations (Janin et al. 1978; Benedetti et al. 1983), but it is most favorable for the existence of the backbone side chain interaction. The progressive solvation of the middle NH bond by $Me_2SO$ (Fig. 1 b) releases this interaction and the Asp or Asn $C^{\beta}H_2$ protons become nearly magnetically equivalent, whereas the percentage of the $C^{\alpha}-C^{\beta}$ rotamer III decreases (Fig. 5). This effect is less visible for Pro-Asn than for Pro-Asp(OMe), probably because of the stronger basicity of the amide side-function.

The preference for the rotamer III of the $C^{\alpha}-C^{\beta}$ bond in a $\beta$I-turn is confirmed by the crystal structure of Pro-Asp(OMe) (Fig. 3a). On the other hand, the $\beta$II-folded forms of Pro-Asp (Fig. 3b) and Pro-Asn (Fig. 3c) correspond to the $C^{\alpha}-C^{\beta}$ rotamer II and I conformations respectively.

When considering the $\beta$-folded dipeptide sequences encompassing two chiral L-amino acid residues in crystallized proteins, nearly 10% are of the unusual $\beta$II-type (Chou and Fasman 1977). The distribution of the corresponding $C^{\alpha}-C^{\beta}$ bond conformations for the Asp, Asn, polar (Asp, Asn, Ser, Thr, His), and apolar residues (Val and Ile excluded) in the $(i+2)$th position of a $\beta$-turn are quoted in Table 6. It appears that rotamer III is more favored for polar residues in $\beta$I-turns, and this result is probably not fortuitous. Although crystallized proteins are essentially in an aqueous environment the present experiments have been carried out in non-aqueous solvents, the difference between polar and apolar residues (Table 6) suggests that the backbone side-chain interaction is retained in many $\beta$I-turns of

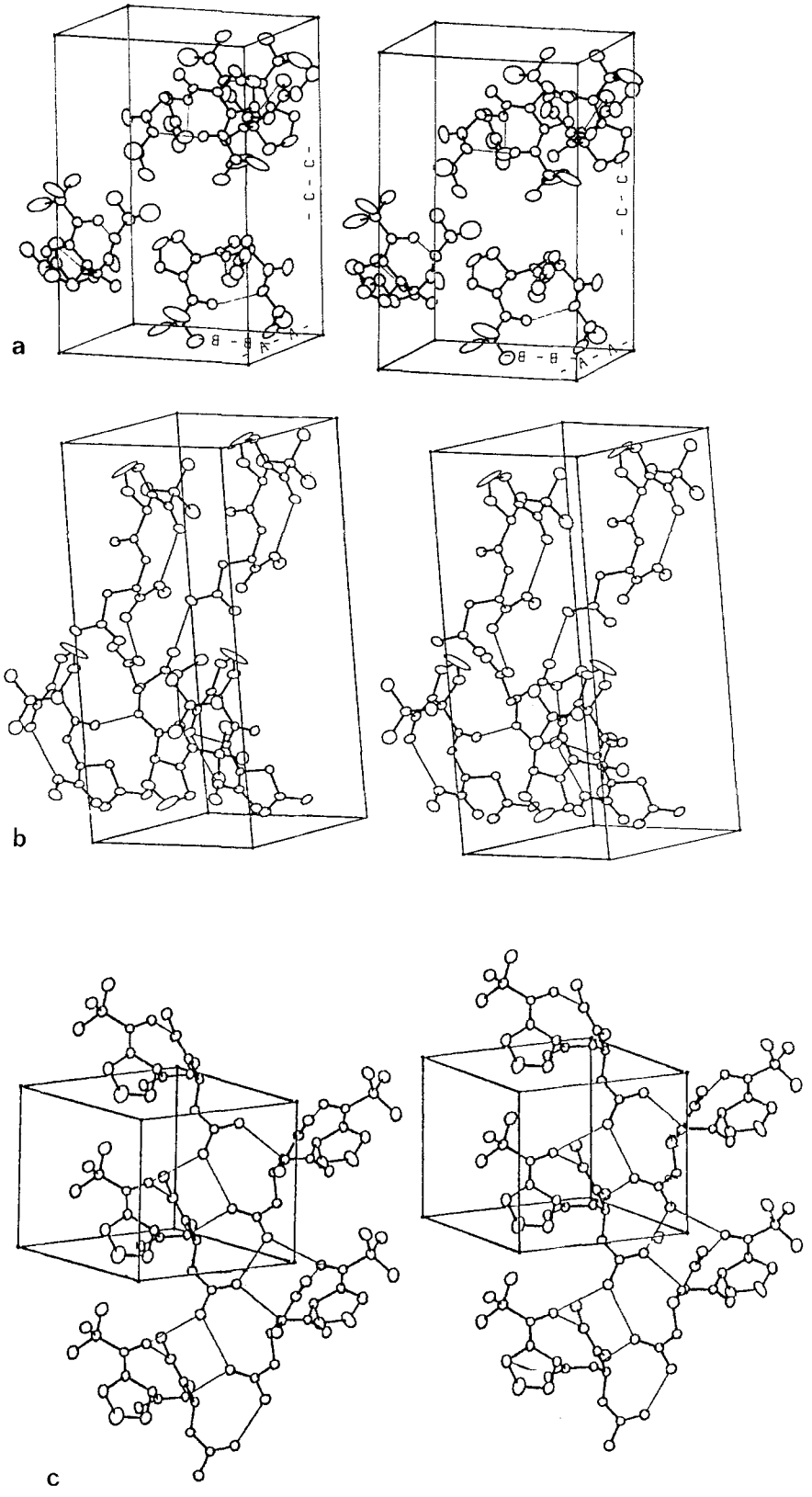


**Fig. 4.** Stereoviews of the molecular packing modes in the crystals for the tBuCO-Pro-Asp(OMe)-NHiPr (**a**), tBuCO-Pro-Asp-NHMe (**b**) and tBuCO-Pro-Asn-NHMe (**c**) dipeptides. The intra and intermolecular hydrogen bonds are indicated by thin lines

**Table 6.** Occurrence ($n$) and percentage (%) of the three staggered rotamers I, II and III[a] for the $C^{\alpha}-C^{\beta}$ bond of the residue in the $(i+2)^{th}$ position of $\beta$I and $\beta$II-turns in the crystallized proteins[b]

| Residue | $\beta$I-turns | | | | | | $\beta$II-turns | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | I | | II | | III | |
| | $n$ | % | $n$ | % | $n$ | % | $n$ | % | $n$ | % | $n$ | % |
| Asp | 21 | 50 | 10 | 24 | 11 | 26 | 1 | [e] | 1 | [e] | 0 | [e] |
| Asn | 13 | 43 | 5 | 17 | 12 | 40 | 6 | 67 | 2 | 22 | 1 | 11 |
| Polar[c] | 56 | 42 | 19 | 14 | 59 | 44 | 8 | 53 | 4 | 27 | 3 | 20 |
| Apolar[d] | 45 | 67 | 12 | 19 | 10 | 14 | 12 | 80 | 2 | 13 | 1 | 7 |

[a] Rotamers I: $\chi^1 \simeq -60°$; II: $\chi^1 \simeq 180°$; III: $\chi^1 \simeq 60°$
[b] Chou and Fasman (1977)
[c] Residues: Asn, Asp, Ser, Thr, His
[d] Residues: Cys, Phe, Leu, Met, Trp, Tyr
[e] Insignificant due to the restricted dataset

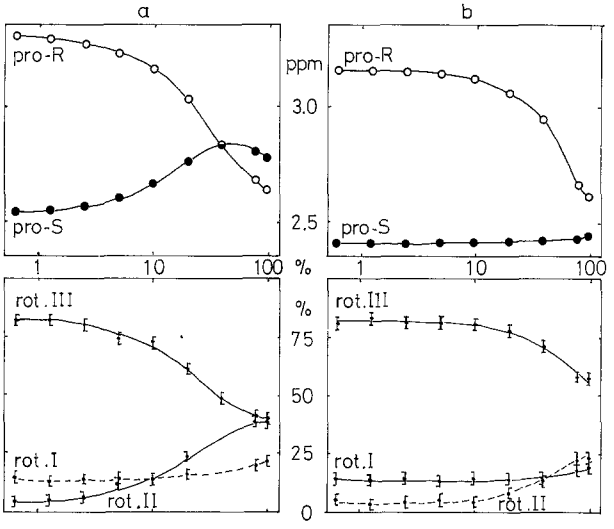


**Fig. 5.** Influence of $Me_2SO$ content in $Me_2SO/CHCl_3$ mixtures on the Asp- or Asn-$C^{\beta}H_2$ proton NMR signals (upper part) and on the Asp or Asn $C^{\alpha}-C^{\beta}$ rotamer distribution (lower part) for the tBuCO-Pro-Asp(OMe)-NHMe (**a**) and tBuCO-Pro-Asn-NHMe (**b**) dipeptides. Introduction of $Me_2SO$ induces loss of the rotamer III, allowing a quasi-free rotation for the Asp or Asn $C^{\alpha}-C^{\beta}$ bond. As a consequence, the Asp or Asn-$C^{\beta}H_2$ protons tend towards magnetical equivalence

the proteins. In contrast, the $\beta$II-turn does not favour rotamer III, whatever the polar or apolar character of the residue. In this case, the backbone side chain interaction does not exist, and the $C^{\alpha}-C^{\beta}$ bond preferentially accommodates the more common rotamer I and II conformations.

Spectroscopic and X-ray diffraction experiments on dipeptides having both N and C-terminal amide functions confirm that these molecules are well adapted to the characterization of the different $\beta$-turn conformations.

When considering Pro-Asp and Pro-Asn sequences in solution, the existence of an attractive interaction between the middle NH bond and the accepting part of the Asp or Asn side-chain is clearly deduced from IR and $^1$H-NMR data, and this interaction greatly contributes to the stability of the $\beta$I-turn. The different basic properties of the side function result in a decreasing propensity for $\beta$I-turn formation when going from $Asp^-$ (ionic carboxylate group) to Asn (amide group), to Asp (neutral carboxylic group) or Asp(OMe) (ester group).

The retention of the above $\beta$I-folded form in the crystal structure of Pro-Asp(OMe) demonstrates the validity of the above interpretation. However, the $\beta$II-folded forms of Pro-Asp and Pro-Asn in the solid state indicate that the difference of free energy between these two forms can be compensated for by long-range interactions. This conformational transition should be taken into consideration when peptide molecules are involved in molecular contacts in the course of biological processes.

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## References

Aubry A, Marraud M (1985) Backbone side-chain interactions in peptides. III. Structure of pivaloyl-L-prolyl-N-methyl-L-threoninamide. Acta Crystallogr Ser C 41:65–67

Aubry A, Marraud M (1986) Stabilization of $\beta$I-turn by polar residues. In: Castro B, Martinez J (eds) Proceedings of the peptide forum, Groupe Français des Peptides, pp 280–283

Aubry A, Ghermani N, Marraud M (1984) Backbone interactions in peptides. I. Crystal structures of model dipeptides with the Pro-Ser sequence. Int J Peptide Protein Res 23:113–122

Aubry A, Cung MT, Marraud M (1985) $\beta$I and $\beta$II-turn conformations in model dipeptides with the Pro-Xaa sequences. J Am Chem Soc 107:7640–7647

Barfield M, Hruby VJ, Meraldi JP (1976) The dependence of geminal H–H spin-spin coupling constants on $\phi$ and $\psi$ angles of peptides in solution. J Am Chem Soc 98: 1308–1314

Benedetti E, Morelli G, Némethy G, Scheraga HA (1983) Statistical and energetic analysis of side-chain conformations in oligopeptides. Int J Peptide Protein Res 22:1–15

Bodanski M (1984) Principles of peptide synthesis. Springer, Berlin Heidelberg New York

Boussard G, Marraud M (1985) $\beta$-turns in model dipeptides. An infrared quantitative analysis with NMR correlation. J Am Chem Soc 107:1825–1828

Boussard G, Marraud M, Aubry A (1979) Experimental investigations on the backbone folding of proline-containing model tripeptides. Biopolymers 18:1297–1331

Chou PY, Fasman GD (1977) $\beta$-turns in proteins. J Mol Biol 115:135–175

Cung MT, Marraud M (1982) Conformational dependence of the vicinal coupling constant for the $C^{\alpha}-C^{\beta}$ bond in peptides. Biopolymers 21:953–967

International Tables for X-Ray Crystallography (1974) Kynoch Press, Birmingham, England, Vol IV

Ishii H, Inoue Y, Chujo R (1984) $\beta$-turn like conformation and side-chain interactions of aspartic acid-containing dipeptides. Polymer J 16:9–21

IUPAC-IUB Commission on Biochemical Nomenclature (1970) Abbreviations and symbols for the description of the conformation of polypeptide chains (1969). Biochemistry 9: 3471–3479

Janin J, Wodak S, Levitt M, Maigret B (1978) Conformation of amino acid side-chains in proteins. J Mol Biol 125: 357–386

Kolaskar AS, Ramabrahmam V, Soman KV (1980) Reversals of polypeptide chain in globular proteins. Int J Peptide Protein Res 16:1–11

Lewis PN, Momany FA, Scheraga HA (1973) Chain reversals in proteins. Biochim Biophys Acta 303:211–229

Main P, Fiske SJ, Hall SE, Lessinger L, Germain G, Declerc JP, Woolfson MM (1980) MULTAN-80. A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data. Universities of York, England, and Louvain, Belgium

Marraud M, Aubry A (1984) Backbone side-chain interactions in peptides. II. Solution study of serine-containing model dipeptides. Int J Peptide Protein Res 23:123–133

Montecalvo D, St.-Jacques M (1975) On the use of geminal coupling constants of methylene protons adjacent to carbonyl groups for structural and conformational assignments. J Org Chem 40:940–942

Nair CMK, Vijayan M (1981) Structural characteristics of prolyl residues. A review. J Indian Inst Sci Ser C 63: 81–103

Nishihara H, Nishihara K, Uefuji T, Sakota N (1975) Conformation and circular dichroism of several N-acyl-L-prolines. Bull Chem Soc Jpn 48:553–555

Peters D, Peters J (1984a) Structure and bonding in proteins. Part 16. The Asn dipeptide. J Mol Struct 109:137–148

Peters D, Peters J (1984b) Structure and bonding in proteins. Part 17. The unionized aspartic acid dipeptide. J Mol Struct 109:149–159

Picur B, Siemion Z (1983) Rotational isomerism about the $C^{\alpha}-C^{\beta}$ bonds in S-containing amino acids and their non sulphur containing isosters. Org Magn Res 21:271–274

Rose GD, Gierasch LM, Smith JA (1985) Turns in peptides and proteins. Adv Protein Chem 37:1–109

Sheldrick GM (1976) SHELX76. Programs for crystal structure determination. University of Cambridge, England

Smith JA, Pease LG (1980) Reverse turns in peptides and proteins. CRC Crit Rev Biochem 8:315–399

Tanimura K, Kato T, Waki M, Lee S, Kodera Y, Izumiya N (1984) Facile synthesis of (2R,3R)-phenylalanine-2,3-$d_2$ and NMR study on deuterated gramicidin-S. Bull Chem Soc Jpn 57:2193–2197

Taylor R, Kennard O (1983) Comparison of X-ray and neutron diffraction results for the N–H ... O=C hydrogen bond. Acta Crystallogr Ser B39:133–138

Urry DW, Long MM, Mitchell LW, Okamoto K (1975) Utilization of proton and carbon-13 magnetic resonance in the evaluation of polypeptide secondary structure in solution. In: Walter R, Meienhofer J (eds) Peptides: Chemistry, structure, biology. Ann Arbor Science, Ann Arbor Mich, pp 113–126