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Registry No. 1, 132018-86-1; **2**, 132018-87-2; **3**, 95433-50-4; **4**, 132018-88-3; **5**, 7663-85-6; **6**, 132018-89-4; NVOC-Cl, 42855-00-5; NV-OH, 1016-58-6; pdCpA, 127067-28-1; $ClCH_2CN$, 107-14-2; Cbz-His(CH_2Ph)-OH, 21929-66-8; Cbz-Ser(CH_2Ph)-OH, 20806-43-3; Cbz-Phe-OH, 1161-13-3; Cbz-Lys(Cbz)-OH, 405-39-0; NVOC-Phe-OH, 30034-25-4; Cbz-Phe-OPh, 4865-47-8; H-Ser(CH_2Ph)-OH, 4726-96-9; H-Phe-OH, 63-91-2; BOC-Asp-OBu-*t*, 34582-32-6; BOC-Asp(O-NV)-OBu-*t*, 132018-90-7; BOC-Asp(O-NV)-OBu-*t*, 132018-91-8; pdCpA[3′-*O*-(Cbz-Phe)], 132018-92-9; pdCpA[2′-*O*-(Cbz-Ser(CH_2Ph))], 132046-22-1; pdCpA[3′-*O*-(Cbz-Ser(CH_2Ph))], 132018-93-0; pdCpA[2′-*O*-(Cbz-His(CH_2Ph))], 132018-94-1; pdCpA[3′-*O*-(Cbz-His(CH_2Ph))], 132018-95-2; pdCpA[2′-*O*-(Cbz-Lys(Cbz))], 132018-96-3; pdCpA[3′-*O*-(Cbz-Lys(Cbz))], 132018-97-4; pdCpA[2′-*O*-(NVOC-Asp(O-NV))], 132018-98-5; pdCpA[3′-*O*-(NVOC-Asp(O-NV))], 132018-99-6; pdCpA[2′-*O*-(NVOC-D-Phe)], 132019-00-2; pdCpA[3′-*O*-(NVOC-D-Phe)], 132019-01-3; pdCpA[2′-*O*-(NVOC-Phe)], 132076-56-3; pdCpA[3′-*O*-(NVOC-Phe)], 132076-57-4; NVOC-D-Phe-OCH_2CN, 132019-02-4; pdCpA[2′-*O*-(H-Phe)], 132019-03-5; pdCpA[3′-*O*-(H-Phe)], 132019-04-6; pdCpA[2′-*O*-(H-asp)], 132019-05-7; pdCpA[3′-*O*-(H-Asp)], 132019-06-8.

Involvement of Side Functions in Peptide Structures: The Asx Turn. Occurrence and Conformational Aspects

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Abstract: The Asx turn is a local conformation in peptide chains that is topologically equivalent to the β turn. It is characterized by an interaction closing a 10-membered cycle and involving the $C^{\gamma}O$ group of an Asn or Asp residue and the peptide NH group two residues ahead in the sequence. Its conformational features have been deduced from its statistical occurrence in crystallized proteins, and from theoretical and experimental analysis of model peptides reproducing this local conformation. Four Asx-turn types have been considered and their relative stability is discussed in relation with the sequence and the existence of longer range interactions.

In a statistical analysis of the intramolecular contacts in proteins with accurate crystal structure, Baker and Hubbard have emphasized the fact that 10% of the peptide nitrogens and 15% of the peptide carbonyls are hydrogen-bonded to side chains.[1] In return, about a quarter of the hydrogen-bonded side chains interact with peptide atoms. Furthermore, nearly half of the main-chain side-chain contacts are short- and medium-range interactions involving one of the four residues on both sides of the residue considered. These observations confirm that side chains play an important role in protein and peptide folding and could stabilize local conformations.

Among the short-range side-chain main-chain interactions, the high occurrence of Asn and Asp residues deserves to be noted, and the contact between the Asn $C^{\gamma}O$ carbonyl or the Asp $C^{\gamma}O_2^-$ carboxylate and the peptide NH two residues ahead is particularly frequent (see Table 7 in ref. 1). The resulting hydrogen bond closes a 10-membered cycle topologically similar to that characterizing the well-known β turn[2] (Figure 1). Hence it has been called Asx turn (Asx standing for Asn or Asp). It was identified in the crystal structures of α-chymotrypsin, prealbumin, and carboxypeptidase A,[3,4] and also more recently in the crystal structures of some Asn-containing oligopeptides.[5-8]

Although the Asx turn is much less frequent than the β turn, it concerns ~18% of the Asn and Asp residues, and 50 Asx turns have been found in the crystal structures of 13 proteins.[1] It is often involved in β-turn- and α-helix-inducing sequences,[1,9] and possibly in posttranslational modifications such as Asn deamidation[10] and N glycosylation.[11]

No conformational analysis of the Asx turn has been reported up to now. We therefore decided to study the conformational properties of this local structure by combining a 3-fold approach: (i) statistical analysis of the Asx turns listed by Baker and Hubbard,[1] (ii) Monte Carlo analysis of Gly-Asn-Xaa-Gly (Xaa = Gly, Ala), which is the shortest sequence reproducing the Asx turn, (iii) experimental study (IR and ^1H NMR spectroscopies and X-ray diffraction) of model di- and tripeptides.

Due to the low precision of the data on most side chains in the crystal structures of the proteins, and the low occurrence of a given sequence in the available data set, a precise analysis of the local conformations resulting from side-chain main-chain interactions is hardly feasible with proteins. Model oligopeptides are much

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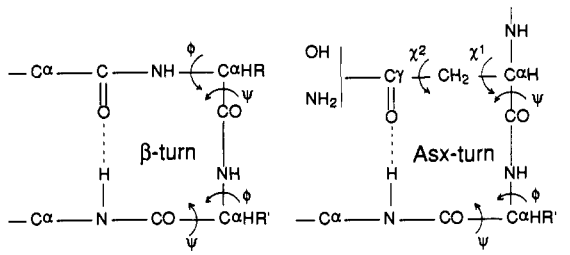

Figure 1. Schematic representation of the β turn (left) and Asx turn (right) stabilized by a hydrogen bond closing a 10-membered cycle in both cases. Nature of the torsional angles defining the two turns.

more adapted to this study because their primary structure can be easily adjusted to reproduce the expected effect, and they can be thoroughly investigated by different techniques under various experimental conditions.

Two types of peptide models have been selected: Boc-Asx-Xaa-NHMe (Asx = Asn, Asp, Asp(OBzl); Xaa = Pro, Phe) and Boc-Asn-Xaa-Ser-NHMe (Xaa = Pro, Ala).

The dipeptide models are the simplest molecules capable of reproducing the Asx turn. The *tert*-butyloxycarbonyl group, which is less nucleophilic than the amide group, and proline in the Xaa position,[2] have been introduced in order to minimize in the Asx-Xaa sequence the β-turn formation, which could compete with the Asx turn. The tripeptides derive from the marker sequence for N glycosylation of peptides and proteins.[12] A preliminary study of their conformational preferences in solution[13] and the resolution of four crystal structures[7,8] have demonstrated that they are also to some extent good models of the Asx turn.

Experimental Section

Synthesis. The model dipeptides Boc-Asx-Xaa-NHMe have been obtained by classical procedures:[14] (i) N methylamidation of Boc-Xaa-OH by the mixed-anhydride method and elimination of the *tert*-butyloxycarbonyl group by gaseous HCl in ethyl acetate, (ii) coupling of Boc-Asn-OH by the *p*-nitrophenyl activated ester method, or Boc-Asp(OBzl)-OH by dicyclohexylcarbodiimide followed by the hydrogenolysis of the benzyl protection. The synthesis of the model tripeptides Boc-Asn-Xaa-Ser-NHMe has been reported elsewhere.[7]

Carboxylic groups are deprotonated at physiological pH. We therefore have studied the Boc-Asp-Xaa-NHMe dipeptides in both the neutral and ionic states of the Asp residue, respectively abbreviated by Asp and Asp$^-$ in the following. In the latter case, the counter cation Me_4N^+ was introduced via its hydroxide pentahydrate (Fluka 87741) in aqueous solution, and lyophilization.

Infrared Spectroscopy. Infrared spectra were run on a Brüker IFS-85 apparatus, working in the Fourier transform mode, and attention was focused on the most informative C=O (1600–1750 cm^{-1}), N—H (3200–3500 cm^{-1}), and O—H (3200–3650 cm^{-1}) stretching vibrations. The solvents used were CH_2Cl_2, MeCN, and Me_2SO at a peptide concentration of 5×10^{-3} M in order to exclude intermolecular aggregation, which was verified by further dilution. The residual water contribution in Me_2SO was corrected if necessary by cancellation of the O–H stretching absorption bands in the 3500–3600-cm^{-1} domain.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were run on Brüker AC-200P and AM-400 instruments. Chemical shifts were assigned by COSY experiments and measured with reference to internal Me_4Si. The solvent accessibility of the NH protons was followed by the solvent titration procedure when $CHCl_3/Me_2SO$ mixtures were used, and by the temperature effect in Me_2SO on the NH proton signals.

Monte Carlo Analysis. The conformational analysis of the neutral Gly^1-Asn^2-Xaa^3-Gly^4 fragments (Xaa = Gly, Ala) has been carried out by a Monte Carlo procedure. The Monte Carlo analysis was not used in the present study in order to get averages, but rather as a means for extracting molecular conformations of minimal energies from samples realized according to an importance sampling algorithm.[15,16] The conformational energy was calculated as the sum of van der Waals and electrostatic (with a dielectric constant equal to 3.5) interactions between nonbonded atoms, hydrogen bonding, and torsional contributions. The bond lengths, bond angles, electrostatic charges, and energy parameters, including hydrogen bond functions, were taken from Momany et al.[17]

We have considered the four identified Asx turns (see below) and the Iβ and IIβ turns for comparison. In the Asx turns, 5 of the 10 torsional angles were kept constant (Gly^1 ϕ, ψ = −70°, 180°; Asn^2 ϕ = −70°; Gly^4 ϕ, ψ = −70°, −70°) while the other torsional angles were allowed random variations of 1° within 60° domains centered on preselected secondary minima of energy. In the β turns, the Asn^2 ϕ angle was also allowed random variations.

The calculation procedure started from a preselected conformation corresponding to one of the six turns in consideration. The optimization was carried out by random perturbations of the torsional angles, and in a first stage, the 10 conformations of lowest energy were stored. The procedure was automatically restarted from these 10 conformations until more than 800 new conformations had been explored in order to finally obtain the one of minimum energy for each of the turn types.

Table I. Occurrence of the Three Asx-Turn Types for the Asx-Xaa Sequences in 11 Proteins[a]

Asx turn	Asx		Xaa						
	D	N	P	G	A,C,I,L,V	F,W,Y	K,R	D,E,N	S,T
I_t	19	11	1		8	2	2	5	12
I_g	2	3				1		1	3
II_g	7	2		6			1	1	1

[a] See refs 1 and 18.

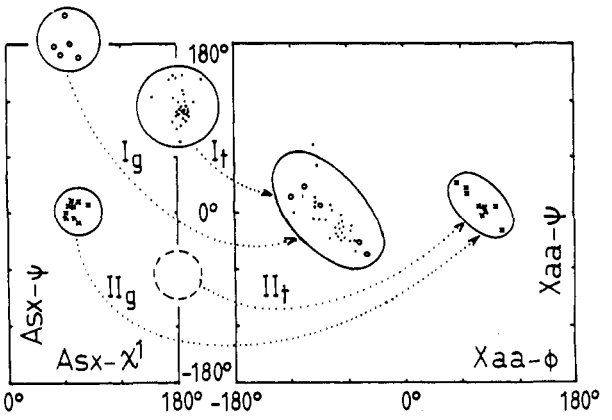

Figure 2. Representation on Ramachandran-like maps of the Asx-folded Asx-Xaa sequences listed in Table I. No example of the II_t conformation is found in the present data set.

Results and Discussion

Statistical Analysis. We have considered 44 Asx turns listed by Baker and Hubbard in 11 proteins (see Table 8 in ref 1) available from the protein data bank.[18] The occurrence of the residues in these Asx-Xaa sequences shows that short polar residues are frequently present in position Xaa (Table I). This is probably related to the fact that, in 24 cases, the Xaa residue is also involved in a β-turn structure[1] and the frequent occurrence of short polar residues in β-folded sequences is well documented.[2]

We have also considered the dihedral angles of both residues in these Asx-Xaa sequences. From this point of view, an Asx turn is defined by five dihedral angles (Figure 1), three for the Asx residue (ψ, χ^1, χ^2) and two for the Xaa residue (ϕ, ψ). In most cases, the Asx χ^2 values equals 120–180°, and we have only considered the other four angles.

When the points corresponding to the two pairs of angles Asx ψ, χ^1 and Xaa ϕ, ψ are plotted on Ramachandran-like maps three different conformational families appear (Figure 2). They differ by the trans ($\chi^1 \simeq 180°$) or gauche ($\chi^1 \simeq 60°$) orientation of

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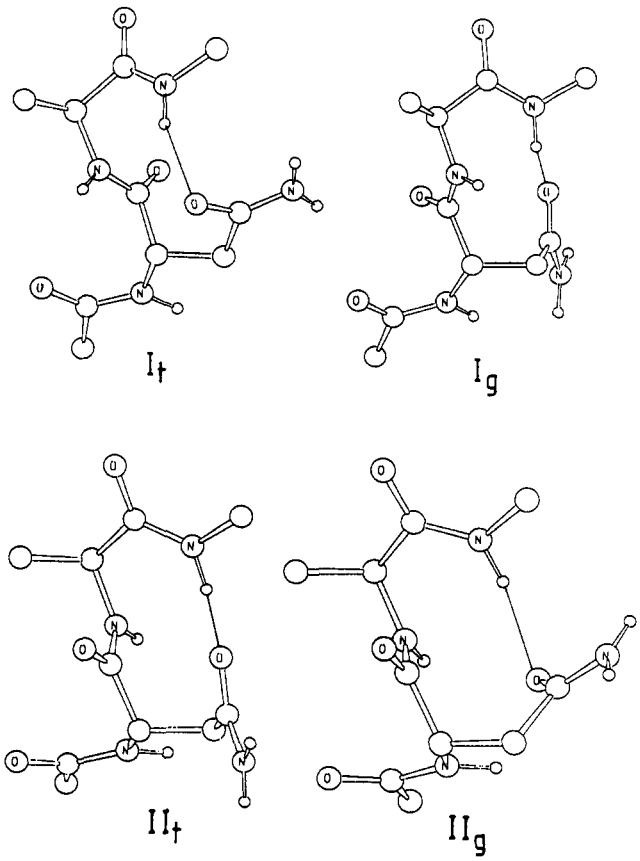

Figure 3. Representation of the four Asx-folded conformations of Ac-Asn-Ala-NHMe deduced from a Monte Carlo conformational analysis.

the Asx side chain and/or by a rotation of nearly 180° of the Asx-Xaa amide plane (Figure 3) exactly as in the well-known I and II dispositions of the β turn:[2] (a) The most frequent conformation, denoted I_t, concerns 30 cases and is characterized by an Asx χ^1 angle of 180° and a negative Xaa ϕ angle around −80°. (b) The less frequent conformation (five cases) mainly differs from I_t by the Asx χ^1 value of 60° and is noted I_g. (c) The II_g family (nine cases) differs from I_g by a 180° rotation of the Asx-Xaa amide plane. It follows that the Xaa ϕ angle accommodates a positive value of 80°, which is not frequent for L-amino acid residues. In fact, six out of these nine II_g Asx turns concern Asx-Gly sequences, in which glycine can act as a D-amino acid residue. (d) A fourth family, denoted II_t, could also be imagined, deriving from II_g by a 120° rotation of the Asx C^α–C^β bond, exactly as for I_g and I_t. However, no example is found in the present survey, and it corresponds to a less stable conformation, as we will see later.

It is interesting to note that there is no example in the statistical analysis of Baker and Hubbard of a Glu or Gln residue involved in a structure similar to the Asx turn.[1] This seems to indicate that the additional methylene in Glu or Gln greatly destabilizes this type of side-chain main-chain contact.

Monte Carlo Conformational Analysis. The study of the crystallized proteins has shown the existence of at least three types of Asx turns with different occurrences. We have carried out a Monte Carlo search for the most stable Asx-folded conformations of the Gly^1-Asn^2-Xaa^3-Gly^4 tetrapeptides with Xaa = Gly or Ala. The conformational constraint was the existence of the Gly^4 NH to Asn^2 $C^\gamma O$ hydrogen bond. We have also considered for comparison the Iβ and IIβ turns with the existence of the Gly^4 NH to Gly^1 CO hydrogen bond as the conformational constraint. The results are reported in Table II.

It appears first that the Iβ turn is the most stable structure for both Asn-Gly and Asn-Ala sequences, and that the I_t Asx turn with an energy difference of ~2 kcal/mol is actually as favored as the IIβ turn. For the Asn-Ala sequence, the I_t Asx turn is followed by the I_g and II_g forms, which are also present in the proteins. The fourth II_t form, which is not observed in the protein data set, is effectively predicted to be the least stable Asx-folded conformer. These four Asx turns are reproduced in Figure 3 for the Ac-Asn-Ala-NHMe dipeptide.

Table II. Dihedral Angles (deg), Hydrogen Bond Distance (Å), and Enthalpy (kcal/mol) for the Most Stable Asx- and β-Folded Conformations of the Gly-Asn-Xaa-Gly Sequences with Xaa = Gly or Ala

	Asn				Xaa			
turn type	ϕ	ψ	χ^1	χ^2	ϕ	ψ	N···O	enthalpy
Xaa = Gly								
Iβ	−57	−37	67	122	−72	−26	2.76	0[a]
IIβ	−61	99	61	121	71	30	2.83	1.8
I_t	−70	112	176	−153	−66	−22	2.90	1.8
II_g	−70	−11	66	152	81	29	2.82	2.6
II_t	−70	−47	−176	100	104	−30	2.81	2.9
I_g	−70	165	56	−98	−97	21	2.74	3.1
Xaa = Ala								
Iβ	−57	−37	67	122	−72	−26	2.76	0[a]
I_t	−70	121	176	−152	−69	−29	2.94	2.2
IIβ	−57	113	63	122	57	30	2.80	2.6
I_g	−70	166	65	−98	−105	12	2.70	3.6
II_g	−70	−14	66	150	59	30	3.13	3.7
II_t	−70	−37	−175	101	70	17	2.83	5.0

[a] This most stable conformation was taken as reference for each sequence considered.

The above order of stability is somewhat modified for the Asn-Gly sequence. In particular, both II_t and II_g forms corresponding to a positive Gly ϕ angle are more stable than for the Asn-Ala sequence. This result agrees with the observation that six out of nine II_g Asx-folded sequences in the protein data set concern an Asx-Gly sequence (Table I).

Experimental Conformational Analysis. The conformational properties of the Asx turn have also been studied by ^{1}H NMR and IR spectroscopies for two families of dipeptides (Boc-Asx-Xaa-NHMe: Asx = Asn, Asp, Asp(OBzl); Xaa = Pro, Phe) and tripeptides (Boc-Asn-Xaa-Ser-NHMe: Xaa = Pro, Ala) in solution (CH_2Cl_2, $CHCl_3$, MeCN, and Me_2SO).

Contrary to the general observation for Pro-containing linear oligopeptides, a single set of ^{1}H NMR signals is observed for the present peptides. It indicates that the Asx–Pro amide bond assumes a single conformation, which proves to be trans on the basis of the following arguments: (i) The small nonequivalence (~5 ppm) of the Pro $^{13}C^\beta$ and $^{13}C^\gamma$ resonances is typical of the trans conformer;[19] (ii) The N*H*(Me) and Ser N*H* proton signals for the dipeptides and tripeptides, respectively, are observed below 7.5 ppm in $CHCl_3$ whereas the corresponding resonances are shifted to lower fields (above 8 ppm) in the cis conformers of the Boc-Xaa-Pro-NHMe dipeptides with Xaa = Ala, Phe, or Pro.[20-22]

Figure 4 shows the variation of the amide proton signals for the four Boc-Asx-Pro-NHMe dipeptides as a function of the solvent composition in $CHCl_3/Me_2SO$ mixtures. A small variation corresponds to a hydrogen-bonded and solvent-protected NH proton whereas a free and solvent-exposed proton is readily solvated by Me_2SO and undergoes a rapid shift to low fields.[23] These experiments reveal the free state of the Asx NH and Asn $N^\delta H_2$ protons on the one hand, and the bound state of the C-terminal N*H*(Me) proton on the other hand. Moreover, the sensitivity of this N*H*(Me) proton to the nature of the Asx residue suggests a NH(Me) to Asx $C^\gamma O$ contact.

This is confirmed by the IR N—H and C═O stretching frequencies, which are listed in Table III. Previous studies on similar dipeptides and related molecules in $CHCl_3$ solution[13,20-23] have

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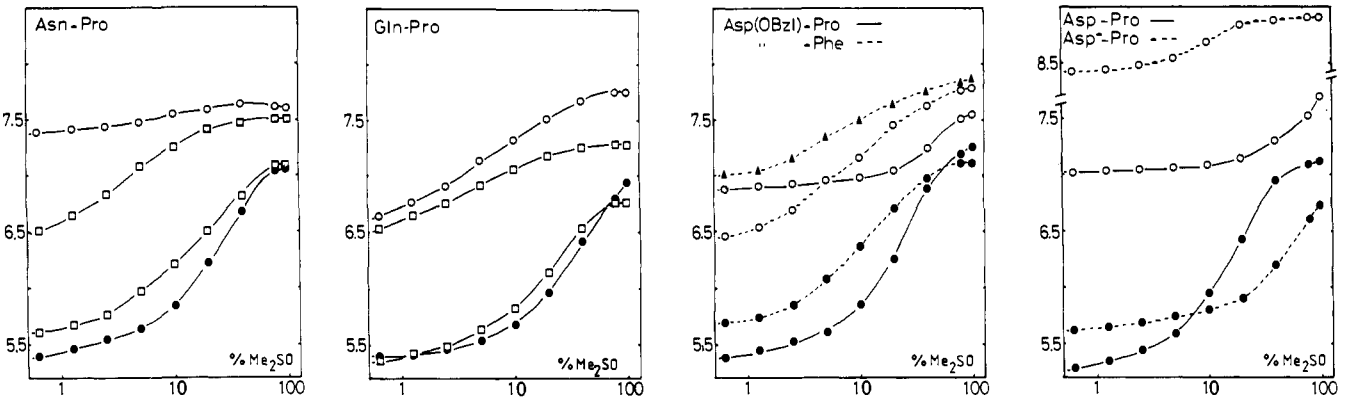

Figure 4. Influence of Me_2SO content in $CHCl_3/Me_2SO$ mixtures on the amide proton chemical shifts (ppm) for the model dipeptides with various sequences: ○, N*H*Me; ●, Asx or Gln N*H*; □, Asn $N^{\delta}H_2$ or Gln $N^{\delta}H_2$; ▲, Phe N*H*.

Table III. N—H and C=O Stretching Frequencies (cm^{-1}) for the Boc-Asx-Pro-NHMe Dipeptides and Model Compounds Mimicking the Asx Side Chain

compound[a]	Asn $N^{\delta}H_2$	Asx NH	NH(Me)	Boc CO	Asx $C^{\gamma}O$	Asx CO	Pro CO
Boc-Asn-Pro-NHMe	3520–3405	3425	3357	1711	1675	1651	1665
Boc-Asn-Pro-OMe	3520–3405	3423		1711	1688	1651	1743
Boc-Asn-NMe_2	3519–3405	3435		1712	1690	1650	
$(CH_3)_2CHCH_2CONH_2$	3524–3406				1686		
Boc-Asp(OBzl)-Pro-NHMe		3411[b]	3411[b]	1716[b]	1716[b]	1655	1668
$(CH_3)_2CHCH_2CO_2Me$					1741		
Boc-Asp-Pro-NHMe		3422	3356	1714	1736	1654	1667
$(CH_3)_2CHCH_2CO_2H$					1746		

[a] Solvent: CH_2Cl_2, 5×10^{-3} M. [b] Broad absorption embedding two close contributions.

shown that (i) free C=O bonds absorb at ~1740 (ester and monomeric carboxyl), 1710 (Boc group), 1685 (standard peptide bond), and 1650 cm^{-1} (tertiary Xaa–Pro peptide bond); (ii) free N–H bonds give a single sharp absorption at 3430–3450 (standard peptide bond) and 3420–3430 cm^{-1} (Boc group), or two sharp peaks at 3400–3410 and 3520–3530 cm^{-1} for the carboxamide group; (iii) their participation in an intramolecular hydrogen bond results in a shift to low frequencies depending on the strength of the interaction, and as a rule it is equal to 10–20 cm^{-1} for carbonyls and 70–150 cm^{-1} for N–H bonds in the peptide series.

Let us consider first Boc-Asn-Pro-NHMe (Figure 5 and Table III), which reveals the following: (a) The Asn $N^{\delta}H_2$ vibrations are typical of a free carboxamide NH_2 group as in isovaleramide mimicking the Asn side chain. (b) All the amide carbonyls except Asn $C^{\gamma}O$ also have normal free frequencies. This rules out the possibility of a β-turn structure that would result in a lower Boc carbonyl frequency. (c) The Asn NH stretching vibration is moderately shifted, which suggests its participation in a weak $i \rightarrow i$ interaction.[2] (d) The low frequency of the C-terminal N–H bond, and the shift by 10 cm^{-1} of the Asn $C^{\gamma}O$ stretching frequency with reference to Boc-Asn-NMe_2 prove their participation in a strong hydrogen bond typical of the Asn turn.

Similar observations are valid for the other Pro-containing dipeptides (Figure 4 and Table III). In comparison, the spectroscopic data for the Asx-Phe dipeptides are indicative of more flexible molecules, in which all the amide NH protons are practically solvent-protected to the same extent (Figure 4). In particular, the relative protection of the NH(Me) proton from the solvent suggests a partial participation in an interaction either with the Asx $C^{\gamma}O$, typical of the Asx turn, or with the Boc carbonyl, typical of the β turn. Effectively, the weaker free Boc carbonyl absorption at 1711 cm^{-1}, compared to that of the homologous Pro-containing dipeptide (Figure 6), indicates the existence of noticeable amounts of β-folded conformers.

We have also studied for comparison the Boc-Gln-Pro-NHMe dipeptide in order to examine the perturbation induced by the additional methylene in the side chain. The C-terminal NH(Me) proton is much more accessible to the solvent (Figure 4), and the Gln $C^{\delta}O$ and NH(Me) absorptions at 1685 and 3450 cm^{-1}, respectively, are typical of free vibrators. These results demonstrate

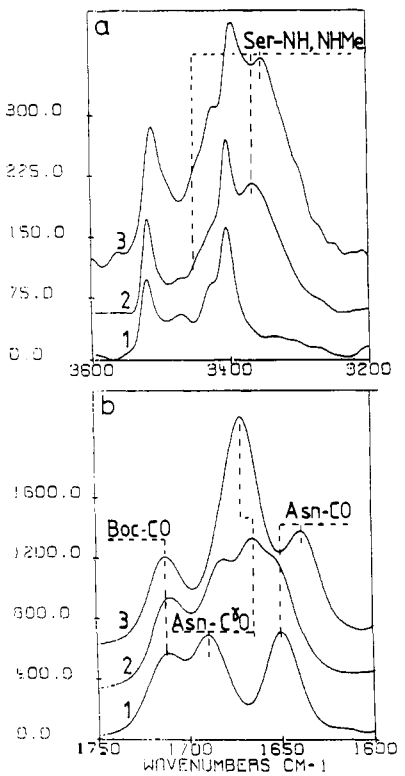

Figure 5. N—H (a) and C=O (b) stretching absorptions for Boc-Asn-NMe_2 (1), Boc-Asn-Pro-NHMe (2), and Boc-Asn-Pro-Ser-NHMe (3). Solvent: CH_2Cl_2, 5×10^{-3} M.

the destabilizing influence of a Gln residue on the side-chain main-chain interaction, typical of the Asx turn, and are in agreement with the observation that "Glx turns" are absent from the crystallized proteins.[1]

A preliminary spectroscopic study of the Boc-Asn-Xaa-Ser-NHMe tripeptides with Xaa = Pro or Ala has been reported

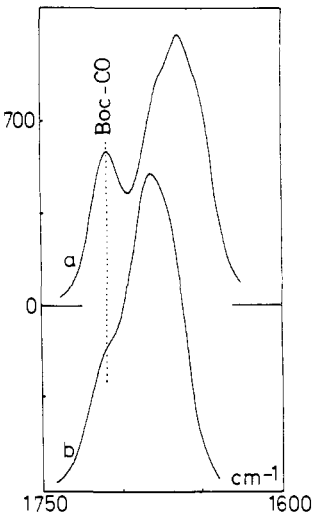

Figure 6. Compared C=O stretching absorptions for Boc-Asn-Pro-NHMe (a) and Boc-Asn-Phe-NHMe (b). Solvent: CH_2Cl_2 with 5% Me_2SO, 5×10^{-3} M.

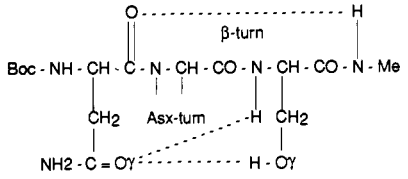

Figure 7. Schematic representation of the intramolecular interactions in the Boc-Asn-Xaa-Ser-NHMe tripeptides in solution.

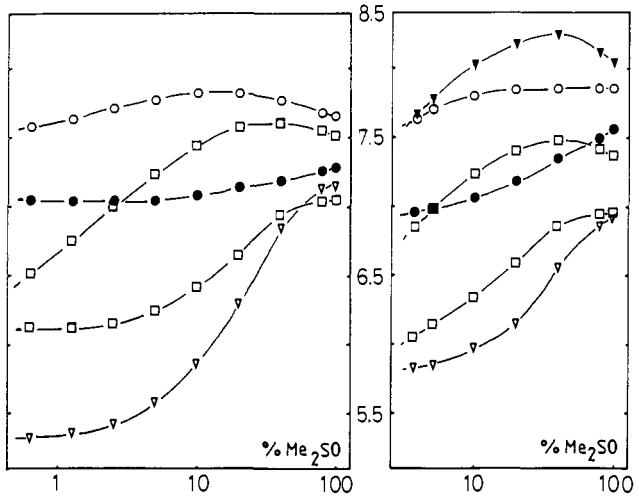

Figure 8. Influence of Me_2SO content in $CHCl_3/Me_2SO$ mixtures on the amide proton chemical shifts (ppm) for the Boc-Asn-Xaa-Ser-NHMe tripeptides with Xaa = Pro (left) and Ala (right): ○, N*H*Me; ●, Ser N*H*; ▼, Ala N*H*; ▽, Asn N*H*; □, Asn $N^{\delta}H_2$.

elsewhere,[13] and we concluded the existence of a tightly folded structure in poorly polar solvents with an Asx-folded Asn-Xaa sequence and a Iβ-folded Xaa-Ser sequence. Moreover the Ser $O^{\gamma}H$ hydroxyl is bonded to the Asn $C^{\gamma}O$ carbonyl, which is therefore involved in a double interaction (Figure 7).

This result was fully corroborated by further experiments. In the Pro-containing tripeptide, the solvent protection of both Ser NH and NH(Me) protons contrasts with the solvent accessibility of the other amide protons (Figure 8). The absence of a sharp IR absorption at 3450 cm^{-1} confirms the participation of the former protons in strong intramolecular interactions, and the same is true for the Ser $O^{\gamma}H$ hydroxyl proton because of the absence of a free absorption at 3600 cm^{-1} (Figure 5). Considering the C=O stretching absorptions, both Asn CO and Asn $C^{\gamma}O$ contributions are shifted by nearly 10 and 20 cm^{-1}, respectively, with reference to Boc-Asn-NMe_2. All the other carbonyls have normal free stretching frequencies.

Table IV. NH and OH Proton Temperature Coefficients ($\times 10^3$ ppm/°C) for the Boc-Asn-Xaa-Ser-NHMe Tripeptides in Me_2SO

	Asn			Ala	Ser		
Xaa	$N^{\delta}H$(cis)	$N^{\delta}H$(trans)	NH	NH	NH	$O^{\gamma}H$	NH(Me)
Pro	4.2	4.5	8.7		2.6	4.5	2.1
Ala	5.8	3.9	5.8	4.5	4.2	5.5	3.5

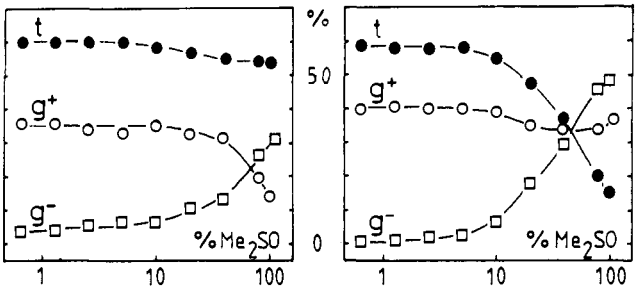

Figure 9. Influence of Me_2SO content in $CHCl_3/Me_2SO$ mixtures on the percentages of the Asn C^{α}–C^{β} rotamers for Boc-Asx-Pro-NHMe with Asx = Asn (left) or Asp (right).

Boc-Asn-Ala-Ser-NHMe differs from Boc-Asn-Pro-Ser-NHMe by less typical variations of the amide proton signals with the solvent, indicating more conformational flexibility (Figure 8). However, the smaller variation for both Ser NH and NH(Me) protons denotes a persistent major folded conformation in poorly polar solvents.

In the more active Me_2SO solvent, Boc-Asn-Pro-Ser-NHMe retains a folded conformation on the basis of the shifted Asn CO and Asn $C^{\gamma}O$ stretching frequencies, and of the small temperature coefficients for the Ser NH and NH(Me) proton signals, whereas Boc-Asn-Ala-Ser-NHMe appears to be essentially flexible, with higher NH proton temperature coefficients (Table IV).

Asx-Turn Conformation in Solution. The four Asx turns predicted to occur on the basis of theoretical considerations and statistical analysis of the crystallized proteins mainly differ by the trans or gauche orientation of the Asx side chain and/or the disposition of the Asx-Xaa amide plane. Therefore they can be characterized by the vicinal proton coupling constants in the Asx $C^{\alpha}H$–$C^{\beta}H_2$ and Xaa NH–$C^{\alpha}H$ fragments, which are related to the Asx χ^1 and Xaa ϕ angles, respectively.[24,25] The NMR data for the Boc-Asx-Pro-NHMe dipeptides and Boc-Asn-Xaa-Ser-NHMe tripeptides are listed in Tables V and VI.

The vicinal coupling constants in the Asx $C^{\alpha}H$–$C^{\beta}H_2$ fragment have been interpreted as usual in terms of percentages of the three staggered C^{α}–C^{β} rotamers denoted t ($\chi^1 = 180°$), g^+ ($\chi^1 = 60°$), and g^- ($\chi^1 = -60°$) (Table V).[24] In principle, the assignment of the t and g^- percentages requires the assignment of the *pro-R* and *pro-S* $C^{\beta}H_2$ protons,[26,27] whereas the g^+ percentage is unambiguously obtained by difference from unity. In most cases, the *pro-R* $C^{\beta}H$ proton in Asx residues, or the *pro-S* $C^{\beta}H$ proton in the non β- or γ-functionalized residues, is situated at lower field in $CHCl_3$.[23,27-30] In the present cases, it corresponds to the smallest $^3J_{\alpha\beta}$ coupling constant and, therefore, to small percentages of the g^- rotamer, in good agreement with the fact that the major Asx turn is only compatible with the other two t and g^+ rotamers.

In the Asx-Pro sequences, the pyrrolidine ring imposes a Pro ϕ angle of nearly −60°, which rules out the possibility of the II_t

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Table V. Percentages of the Asx and Ser C^{α}–C^{β} Rotamers[a]

peptide	Asx $\chi^1 = -60°$ g^-	Asx $\chi^1 = 180°$ t	Asx $\chi^1 = 60°$ g^+	Ser $\chi^1 = -60°$ g^-	Ser $\chi^1 = 180°$ t	Ser $\chi^1 = 60°$ g^+
Boc-Asn-Pro-NHMe[b]	5	60	35			
Boc-Asp-Pro-NHMe[b]	0	60	40			
Boc-Asp(OBzl)-Pro-NHMe[b]	10	60	30			
Boc-Asp$^-$-Pro-NHMe[b]	5	60	35			
Boc-Asn-Pro-Ser-NHMe[b]	5	65	30	15	5	80
Boc-Asn-Ala-Ser-NHMe[c]	20	10	70	0	10	90

[a] See ref 24. [b] Solvent, $CHCl_3$. [c] Solvent, $CHCl_3$ with 5% Me_2SO.

Table VI. $^3J_{N\alpha}$ Vicinal Coupling Constants (Hz) and Corresponding ϕ Angles (deg)[a]

peptide	Asx $^3J_{N\alpha}$	Asx ϕ[d]	Pro, Ala $^3J_{N\alpha}$	Pro, Ala ϕ	Ser $^3J_{N\alpha}$	Ser ϕ
Boc-Asn-Pro-NHMe[b]	8.8	−90; −150		−60		
Boc-Asp-Pro-NHMe[b]	9.3	−95; −145		−60		
Boc-Asp(OBzl)-Pro-NHMe[b]	8.7	−90; −150		−60		
Boc-Asp$^-$-Pro-NHMe[b]	8.3	−85; −155		−60		
Boc-Asn-Pro-Ser-NHMe[b]	8.2	−85; −155		−60[f]	8.6	−90[f]; −150
Boc-Asn-Ala-Ser-NHMe[c]	7.3	−80; −160	5.0	80[e]; 40 −70[e,f]; −170	8.3	−85[f]; −155

[a] See ref 25. [b] Solvent, $CHCl_3$. [c] Solvent, $CHCl_3$ with 5% Me_2SO. [d] Both values are equally probable. [e] Value compatible with the Asx turn. [f] Value compatible with the C-terminal Iβ turn.

Table VII. Conformational Angles (deg) and Hydrogen Bond Distance (Å) for Asx-Folded Sequences in Crystallized Oligopeptides

peptide (sequence)	Asx ϕ	Asx ψ	Asx χ^1	Asx χ^2	Xaa ϕ	Xaa ψ	N···O	Asx-turn type	ref.
Boc-Asn-Pro-Tyr-NHMe I[a]	−101	106	175	−170	−58	−27	3.12	I_t	5
Boc-Asn-Pro-Tyr-NHMe II[a]	−108	108	−173	157	−60	−28	3.05	I_t	5
Boc-Asn(Me)-Pro-Ser(Bzl)-NHMe[a]	−105	108	168	−170	−67	−20	2.93	I_t	7
Boc-Asn-Pro-Ser(Bzl)-NHMe	−93	128	177	−164	−66	−23	3.03	I_t	7
Boc-Asn(Me)-Pro-Ser-NHMe[a]	−97	107	−178	172	−59	−27	2.88	I_t	8
Boc-Asn(Me)-Ala-Ser-OMe[b]	−102	99	177	−176	−112	24	3.10	I_t	8
β-amanitin[c] (Asp-HyPro)	−172	179	61	−152	−60	−37	3.31	I_g	32
S-deoxo[Ile^3]amaninamide[c] (Asn-HyPro)	−172	179	64	−134	−68	−17	2.93	I_g	33
S-deoxo[γ(*R*)-hydroxy-Ile^3]amaninamide[c] (Asn-HyPro)	−163	180	64	−137	−73	−14	2.94	I_g	6

[a] The Asx turn overlaps a Iβ turn (Pro-Tyr or Pro-Ser sequences). [b] The Asn $C^{\gamma}O$ is also intramolecularly hydrogen-bonded to the Ser $C^{\gamma}H$. [c] Asx assumes an extended conformation with an $i \rightarrow i$ interaction. The Asx $C^{\gamma}O$ is also the acceptor site from the Trp NH, three residues ahead in the sequence.

and II_g Asx turns. In fact, the t rotamer of the Asx C^{α}–C^{β} bond is the most frequent (60–70%) and corresponds to the type I_t Asx turn. The g^+ rotamer, corresponding to the type I_g Asx turn, is less frequent (30–40%), in good agreement with the relative occurrence of these folded structures in the crystallized proteins (Table I) and their predicted relative stability (Table II). The third g^- rotamer, only compatible with open conformers, is by far the least populated in $CHCl_3$ (<10%), but by addition of Me_2SO, its percentage increases to an extent depending on the relative basicity of the Asx side function (Figure 9).

The situation is different for the Asn-Ala sequence (Table V). The most frequent Asn C^{α}–C^{β} rotamer is the g^+ orientation corresponding to both I_g and II_g Asx turns, which are equally compatible with the small Ala $^3J_{N\alpha}$ coupling constant (Table VI). However, the latter, with a positive Ala ϕ angle (Table II), is hardly compatible with a contiguous β-folded Ala-Ser sequence and is probably less frequent than the former. The minor t rotamer likely corresponds to a small amount of I_t Asx turn.

The large Asx $^3J_{N\alpha}$ coupling constant corresponds to two equally possible values of the Asx ϕ angle (Table VI). One notes also that the Ser C^{α}–C^{β} bond in both tripeptides (Table V) almost exclusively adopts the g^+ orientation, which is generally favored for Ser residues, especially when they are included in β turns.[31]

This study shows the different behavior of the Asx-Pro and Asx-Ala sequences, for which the conformational preferences are Asx-Pro, $I_t > I_g$, and Asx-Ala, $I_g > II_g \gg I_t$.

However, the only Asx-Ala sequence we have examined is included in a tripeptide containing additional intramolecular interactions (Figure 7), which could perturb the intrinsic stability of the different Asx-turn types. Actually, most of the Asx turns in the proteins are of the I_t type, although in the pool there is only one example containing proline (Table I).

Crystal Structures. To our knowledge, only eight crystal structures of peptides containing an Asx turn have been reported in the literature, and four of them are derivatives of the model tripeptides investigated above.[5-8,32,33] In Table VII are listed the conformational angles of the sequence involved in the Asx turn, together with the N···O distance of the main-chain to side-chain interaction typical of the Asx turn. This N···O distance ranges from 2.88 to 3.31 Å and is similar to that in β turns.[2]

In one case (Boc-Asn-Pro-Ser(Bzl)-NHMe), the Asx turn is the sole intramolecular interaction, which demonstrates its intrinsic stability for the Asn-Pro sequence. In all other cases, additional intramolecular interactions more or less overlap the Asx turn. Considering the Asx-folded Asx-Pro-Yaa sequence, a Iβ-folded Pro-Yaa sequence is observed in the three linear tripeptides, and an extended Asx residue with an $i \rightarrow i$ interaction is found in the three cyclic peptides of the amanitin series (Table VII). In one case (Boc-Asn(Me)-Ala-Ser-OMe), the Asx turn is complemented by a contact, closing a 13-membered cycle, between the Ser $O^{\gamma}H$ hydroxyl and the Asn $C^{\gamma}O$ carbonyl, which is therefore engaged in a double interaction.[8] It is to be noted that two of the above three additional interactions, which are each compatible with the Asx turn, are found to coexist in the tripeptide models of the

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N-glycosylation marker sequence on the basis of spectroscopic experiments in the solute state (Figure 7).

Among the nine Asx turns listed in Table VII, six I_t and three I_g Asx-turn types are found. They differ essentially by a 120° rotation of the Asx χ^1 angle, and a 60° rotation of the Asx ψ angle. The I_t Asx turn concerns the linear tripeptides, while the I_g Asx turn is found in the cyclopeptides of the amanitine series. In this latter case, the Asx $C^\gamma O$ carbonyl is also engaged in a second hydrogen bond closing a 13-membered cycle and involving the NH group three residues ahead in the sequence. It is therefore topologically equivalent to the Ser $O^\gamma H$ to Asn $C^\gamma O$ contact in Boc-Asn(Me)-Ala-Ser-OMe.

Eight of the nine Asx turns in Table VII concern an Asx-Pro sequence. However, it is worth noting that the Pro residue is not absolutely required for the Asx turn. In fact, there is only one Asn-Pro sequence among the 44 Asx turns found in the 11 crystallized proteins we have considered in this analysis (Table I).

The only Ala-containing tripeptide assumes in the solid state an Asx turn of the same I_t type as the other four Pro-containing tripeptides, but both Ala ϕ and ψ angles differ by more than 50° from their counterpart values for proline. This demonstrates the flexibility of the Asx turn, which moreover exists under three, and possibly four, different conformations.

Compared Stability of the Asx and β Turns. Since the β turn is compatible with all types of dipeptide sequences, it is obviously more frequent in the proteins than the Asx turn, which only concerns the Asx-Xaa dyads. However, the percentage of Asx-folded Asx-Xaa dyads (18%) is nearly equal to that of the β-folded dipeptide sequences, which suggests similar intrinsic energies for the two turns.

When considering the simplest peptides (Boc-Asx-Xaa-NHMe) capable of reproducing the Asx turn, it appears that Pro in the Xaa position is the most propitious to Asx-turn formation. In fact, these dipeptides can also accommodate the β turn, except when proline is in the so-called $(i + 2)$ position.[2,20] Therefore it is not surprising that the highest percentage of Asx-folded conformers is observed for the Asx-Pro dipeptide. In the other cases, the competition with β-folded conformers results in more flexible molecules.

In the proteins, and contrary to the model dipeptides, other medium- and long-range interactions are possible whatever the sequence considered, and proline is not particularly favored in the Xaa position of the Asx turn. In the present data set of 11 crystallized proteins, the Asp^{31}-Pro^{32} sequence in tuna cytochrome is the only one to be Asx-folded out of six Asx-Pro sequences, which corresponds to the above-average percentage of 18%.

The conformational properties of the Boc-Asn-Xaa-Ser-NHMe tripeptides corroborate the above observation. The percentage of Asx-folded conformers is slightly higher for Xaa = Pro, but the difference with Xaa = Ala is much less notable than for the Boc-Asn-Xaa-NHMe dipeptides. The interaction between the Asn and Ser side chains, which is not compatible with a β-folded Asn-Xaa sequence, contributes to the stability of the Asx turn whatever the nature of Xaa.

Therefore, the apparent positive influence of proline on the Asx turn in small peptide models is the consequence of two favorable factors: (i) the relative conformational rigidity of the Pro pyrrolidine ring with reference to the other amino acid residues (ii) the low tendency toward β-turn formation for Xaa-Pro sequences.

Conclusion

When combined with the statistical data from crystallized proteins, the study of very simple peptides is a convenient way of obtaining information on the local conformations that are present in the proteins. These particular conformations can obviously exist in bioactive peptides, and it is important to know more about their conformational properties and their stability.

The Asx turn is characterized by a hydrogen bond closing a 10-membered cycle and involving the $C^\gamma O$ group of an Asn or Asp residue and the NH site of the second residue ahead in the chain. It is therefore topologically similar to the β turn, but the accepting carbonyl belongs to a side substituent instead of the main chain. The fact that this structure is not retained when Glu or Gln is substituted for Asp or Asn shows how subtle the effects are that govern the conformation of peptides, and how very similar residues can result in different conformational properties.

The Asx turn appears to be an intrinsically stable conformation, as is the β-turn structure. Moreover, additional interactions, such as a contiguous β turn or a contact between side chains can contribute to the stability of this local structure. Nearly 18% of the Asx-Xaa sequences in the crystallized proteins occur in Asx turns, which is about the same relative occurrence as for the β-folded sequences.

From the conformational point of view, three Asx-turn types are present in the proteins examined, and a fourth one could also exist. They differ by the orientation of the Asx-Xaa amide plane, exactly as in the Iβ and IIβ turns, and/or by the trans or gauche conformation of the Asx C^α–C^β bond. Their relative occurrence depends on the sequence. The I_t form is generally favored, especially by the Asx-Pro sequence, but other forms are also observed when alanine or glycine are substituted for proline. It follows that the Asx turn is a flexible structure, which can be modulated by long-range interactions. This flexibility could be of importance for molecular recognition in biological processes.

The Asx turn illustrates the role played by functional side chains in influencing the whole conformation of the peptide backbone. They can give rise to local conformations capable of inducing particular secondary structures such as β turns and α helices. This possibility should be more systematically taken into consideration in the conformational analysis of Asn- and Asp-containing peptides.

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