FOR THE RECORD

Mimicry by asx- and ST-turns of the four main types of β -turn in proteins

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Abstract

Hydrogen-bonded β -turns in proteins occur in four categories: type I (the most common), type II, type II', and type I'. Asx-turns resemble β -turns, in that both have an NH $\cdots$ OC hydrogen bond forming a ring of 10 atoms. Serine and threonine side chains also commonly form hydrogen-bonded turns, here called ST-turns. Asx-turns and ST-turns can be categorized into four classes, based on side chain rotamers and the conformation of the central turn residue, which are geometrically equivalent to the four types of β -turns. We propose asx- and ST-turns be named using the type I, II, I', and II' β -turn nomenclature. Using this, the frequency of occurrence of both asx- and ST-turns is: type II' > type I > type II > type I', whereas for β -turns it is type I > type II > type I' > type II'. Almost all type II asx-turns occur as a recently described three residue feature named an asx-nest.

Keywords: asx-turn; β -turn; hydrogen-bond; nest; ST-turn

Hydrogen-bonded β -turns are considered to have four residues, i , $i + 1$, $i + 2$, and $i + 3$, with the defining hydrogen bond between the main chain CO group of residue i and the main chain NH group of residue $i + 3$. The division into four categories (Venkatachalam 1968) for peptides with *trans* peptide bonds has been confirmed (Richardson 1981; Baker and Hubbard 1984; Wilmot and Thornton 1988, 1990; Hutchinson and Thornton 1994, 1996; Gunasekharan et al. 1998).

Richardson (1981) pointed to the frequent occurrence of asx-turns and their resemblance to β -turns. The term asx means either aspartate or asparagine, which behave simi-

larly in that both have side chain γ -carbonyl groups. Both asx-turns and β -turns form a 10-atom, hydrogen-bonded ring. In asx-turns, the main chain atoms in residue i and the NH group of residue $i + 1$ in the β -turn are replaced by the side chain of an asx residue. Hence the asx side-chain atoms mimic the main-chain ones of the β -turn. Asx-turns have three residues, i (asx), $i + 1$, and $i + 2$, so the residue numbering differs from that of homologous β -turns, such that $i + 1$ of asx-turns corresponds to $i + 2$ of β -turns. Several authors (Tainer et al. 1982; Rees et al. 1983; Richardson and Richardson 1989; Eswar and Ramakrishnan 1999; Wan and Milner-White 1999a; Chakrabarti and Pal 2001) discuss the asx-turn and others (Presta and Rose 1988; Richardson and Richardson 1988; Bordo and Argos 1994; Doig et al. 1997; Aurora and Rose 1998; Pal et al. 2003) show it is common at the N terminus of α -helices.

The side chain oxygen atoms of serine and threonine residues (i) often form a hydrogen bond with the main chain NH groups of the residue two ahead ($i + 2$). Such features are like asx-turns except that they are 9-atom, instead of

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10-atom, hydrogen-bonded rings. Data have been assembled (Baker and Hubbard 1984; Eswar and Ramakrishnan 1999, 2000) showing their common occurrence and pointing to their similarity with asx-turns. We refer to these collectively as ST-turns (Wan and Milner-White 1999b). Sequence comparisons of homologous proteins (Vijayakumar et al. 1999; Wan and Milner-White 1999b) show that, within and between asx- and ST-turns, the four residues in question often substitute each other.

Previous work on ST-turns has noted their prevalence and grouped them into three geometrically distinct categories, but their similarity to β -turns has not been explicitly discussed. Asx-turns have been more extensively studied, and of particular relevance is the work of Eswar and Ramakrishnan (1999) who categorized asx-turns into four geometrically distinct classes noting some similarities with the β -turn classes. However, their insights regarding the four classes were not presented in summary form and careful reading of the entire paper is required to absorb them. There is a need for making these important findings more accessible so that the general reader has a realistic opportunity to appreciate them. We also expand on them by noting that there are four categories of ST-turns, which also, despite having one less carbon atom, clearly resemble the four types of β -turns. It therefore seems appropriate that both asx- and ST-turns should in future be named using the type I, I', II, and II' β -turn nomenclature.

A commonly occurring anion-binding motif has been described (Watson and Milner-White 2002a,b) as a nest because it consists of a concavity made from the main chain NH groups of three successive amino-acid residues. The anionic group bound is often a single oxygen atom with either a whole or partial negative charge. A recurring variant of this was called an asx-nest because the first nest residue is either aspartate or asparagine and its side chain oxygen is the anion in the nest. A similar distribution is found for ST-nests, which are like asx-nests except that either serine or threonine is the first residue and its side chain oxygen occupies the nest. They are relevant because it emerges that asx- and ST-turns mimicking type II β -turns are the same as asx- and ST-nests.

Results

In a database of 500 proteins, Table 1 shows the numbers of each N, D, S or T residue and of those involved in asx- or ST-turns. Both asx-turns and β -turns consist of a 10-atom, hydrogen-bonded ring closed by a $\text{CO} \cdots \text{H-N}$ hydrogen bond. ST-turns are similar but the ring is 9-membered. Figure 1 provides a comparison of the relevant torsion angles of the two types of turn. To facilitate this, a new set of angles called ω_e , ϕ_e , and ψ_e are defined in Figure 1, A–C. These are angles in the asx- or ST-turn equivalent to corresponding angles in the β -turn. If there is mimicry, the angles ϕ_e

Table 1. Proportions of residues involved in β -turn mimics

Residue type	Asn	Asp	Ser	Thr	N/D/S/T
Number in database	5096	6617	6728	6345	24,786
Number forming asx- or ST-turns	569	1071	491	367	2498
Percentage forming asx- or ST-turns	11.2	16.2	7.3	5.8	10.1

The total numbers of aspartate, asparagine, serine, and threonine residues in the dataset of 500 proteins are given, along with the numbers and percentages of each of that form of asx- and ST-turns (i.e., occupy position i of an asx- or ST-turn).

and ψ_e in asx- and ST-turns should resemble ϕ and ψ of residue $i + 1$ in β -turns, whereas ϕ and ψ of residue $i + 1$ of asx- and ST-turns should resemble ϕ and ψ of residue $i + 2$ of β -turns.

Figure 1, D and F, reveals that the ϕ_e, ψ_e values for asx- and ST-turns cluster into four groups placed symmetrically on the Ramachandran plot, making allowance for one of the groups being sparsely populated. In Figure 1, E and G, the ϕ, ψ values for residue $i + 1$ are plotted; here two clusters are observed, which is consistent with the four groupings. Finding four groups leads to a consideration of the extent to which they are related to the four known groups of β -turns. Torsion angle values were used to group the asx- and ST-turns into types equivalent to the four types of hydrogen-bonded β -turn. The data points in Figure 1, D–G, have been colored to allow comparison of distributions of different types across the asx- and ST-turns.

Table 2 provides a comparison of the average values of the ϕ, ψ angles of residues $i + 1$ and $i + 2$ of types I, I', II, and II' β -turns with those of the corresponding ϕ_e, ψ_e angles, and residue $i + 1$ ϕ, ψ angles, of asx- and ST-turns. It is evident that the four groups of both asx- and ST-turns have average torsion angle values that resemble those of β -turns. For asx-turns the similarity is most striking whereas slight differences are observed between the ST-turns and β -turns. The average ψ_e angles of the type II and II' ST-turns differ from ψ of the type II and II' β -turns by 18° and 27° , respectively. However, the similarity is still apparent and for this reason it is appropriate that the nomenclature for the four β -turn types be used for asx- and ST-turns.

The numbers of different types of asx- and ST-turns in the database are shown in Table 2. In β -turns the most common occurrence is of type I; this might be regarded as a 3/10-helix with only one hydrogen bond; such a conformation is energetically favorable. In asx and ST-turns the most common is type II', whereas the next most common is type I. The structures of type II asx- and ST-turns are the same as those previously identified as asx-nest or ST-nest motifs (Watson and Milner-White 2002a). The tight clustering of ϕ_e and ψ_e angles in type II asx- and ST-turns, seen in Figure 1, D and F, may result from the constrained nest

Figure 1. Distributions of torsion angles of asx- and ST-turns. (A–C) Diagrams of the β -turn, asx-turn (with aspartate side chain), and ST-turn (with serine side chain), respectively. ϕ_e , ψ_e , and ω_e are the angles equivalent to ϕ , ψ , and ω of β -turns; they are also defined in B and C. Residue positions are indicated, and it should be noted that position $i + 1$ of an asx- or ST-turn is equivalent to $i + 2$ of a β -turn. ψ_e , ϕ_e , and ω_e have a fixed approximate relationship to ψ , χ_1 , and χ_2 : $\psi_e \approx \psi + 120^\circ$; $\phi_e \approx \chi_1 - 120^\circ$; $\omega_e \approx \chi_2 + 180^\circ$. (D–E) Turns of different types are represented by data points of different colors, as indicated by the legend. D and E show asx-turn angles whereas F and G show ST-turn angles. D and F plot the asx- or ST-turn angles ϕ_e vs. ψ_e , equivalent to ϕ and ψ of residue $i + 1$ of a β -turn; E and G plot ϕ against ψ for asx- or ST-turn residue $i + 1$, equivalent to residue $i + 2$ of a β -turn.

conformation. One factor allowing asx-turns (but not ST-turns) to exhibit minor conformational differences from β -turns is the fact that ω_e , corresponding to ω of residue $i + 1$ of β -turns, is a side chain angle (χ^2), so is not restrained to the *trans* conformation of ω . The flexibility of ω_e provides extra conformational possibilities for asx-turns compared to β -turns.

Discussion

Four types of asx- and ST-turns are observed that are geometrically equivalent to the four main types of β -turns: I, I', II, and II'. We propose they be named in the same way. The most common of the asx- and ST-turn conformations is type II', characterized by a side chain χ_1 value around 180°

Table 2. Comparing torsion angles between β -turns and their mimics

Turn type	No.	Torsion angle values									
		ω	ω_e	ϕ	ϕ_e	ψ	ψ_e	$\phi_{(i+2)}$	$\phi_{(i+1)}$	$\psi_{(i+2)}$	$\psi_{(i+1)}$
Type I β -turn		<i>trans</i>		-60		-30		-90		0	
Type I asx-turn	302		-143		-58		-55		-72		-13
Type I ST-turn	205		N/A		-44		-62		-75		-9
Type I' β -turn		<i>trans</i>		60		30		90		0	
Type I' asx-turn	5		102		53		70		98		-18
Type I' ST-turn	0		N/A		—		—		—		—
Type II β -turn		<i>trans</i>		-60		120		80		0	
Type II asx-turn	203		-176		-62		128		80		5
Type II ST-turn	207		N/A		-57		102		80		7
Type II' β -turn		<i>trans</i>		60		-120		-80		0	
Type II' asx-turn	1130		155		65		-123		-73		-14
Type II' ST-turn	445		N/A		53		-93		-89		-5

Numbers and average torsion angles of four different types of asx- and ST-turns are given, along with ideal β -turn torsion angle values (Lewis *et al.* 1973; Wilmot and Thornton, 1990). The widely quoted so-called ideal values, from the original β -turn analysis of Venkatachalam (1968), are included. More recent β -turn definitions have often involved distance criteria rather than hydrogen bonding so comparison with such values might be misleading. Ideal torsion angle values of β -turns and values of equivalent torsion angles of asx- and ST-turns share the same columns. The shorter side chains of ST-turns do not have ω_e angles.

(angle interconversion is discussed in the legend to Fig. 1). Types I and II are less common. Type I has χ_1 of about 60° and mimics the main chain part of a 3/10-helix. Type II also has χ_1 in the region of 60° and has a central residue ($i + 1$) with the α_L or γ_L conformation. The rarest is type I'.

Considering that ST-turns consist of a hydrogen-bonded ring of 9, rather than 10, atoms, it is at first sight surprising they occur in the same classes as asx-turns with similar structures. However, the chemical properties of the four residues (D, N, S, and T) are not dissimilar and they exhibit a tendency in such situations to substitute each other over evolutionary time (Vijayakumar *et al.* 1999; Wan and Milner-White 1999b).

The greater flexibility of asx-turns compared with β -turns, conferred by the side chain χ^2 angle, suggests the asx-turn can adopt a conformation with a more favorable hydrogen bond. On the other hand, ϕ_e of the asx-turn (χ^1 of the side chain) is more constrained than ϕ of the β -turn, as it must adopt a rotamer conformation. Considering this, it is interesting to reverse our perspective on how β -turns are mimicked by asx- and ST-turns and instead ponder mimicry of the asx side chain rotamer angle by ϕ of the β -turn. It could be said that this ϕ angle mimics the rotamer conformation of the asx-turn.

A common motif incorporating asx- or ST-turns is the asx- or ST-nest. This is a recently identified, yet by no means uncommon, protein motif (Watson and Milner-White 2002a). The observation that almost all type II asx- and ST-turns occur as asx- or ST-nest motifs is an unexpected finding of the present work. Conversely, all asx- or ST-nests are also type II asx- or ST-turns so they are nearly synonymous.

Materials and methods

A data set of 500 PDB files was obtained from the kinemage Web site (<http://kinemage.biochem.duke.edu>). The proteins are at a resolution $<1.8 \text{ \AA}$ and homology is $<50\%$. Selection emphasizes data quality by removing structures not meeting criteria such as B-factor, clash score, and main chain torsion angles (Lovell *et al.* 2003). Hydrogen atoms were added as needed using the Reduce program (Word *et al.* 1999a) and the program was also used to correct side-chain orientations (Word *et al.* 1999b). Hydrogen bond definition is based on the method recommended by Baker and Hubbard (1984). Asx- and ST-turns are identified by a single hydrogen-bond, that between the side chain oxygen of an aspartate, asparagine, serine, or threonine residue (i) and the main chain NH group of a residue two ahead ($i + 2$). β -Turns are identified by a hydrogen bond between the main chain CO of residue i and the main chain NH of residue $i + 3$. Dihedral angles used are defined in Figure 1. In asx- and ST-nests the first residue is D, N, S, or T and its side chain oxygen atom is within $4.0 \text{ \AA}$ of at least two of the main chain nitrogen atoms of residues i , $i + 1$, or $i + 2$.

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