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A one-variable topographical descriptor for the β-turns of peptides and proteins

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The β-turn is a common secondary structure in biologically active peptides and globular proteins, where it is widely thought to serve as a molecular recognition site for many biological processes. Although the primary β-turn recognition requirements are thought to be straightforward, relating mainly to the relative positions of the peptide sidechains, current classifications of β-turns are complex and are based solely upon the very variable geometry of the peptide backbone. We demonstrate here that β-turns can be described in terms of a single dihedral angle, which we have called β, which provides a complete description of the spatial relationship between the entry and exit peptide bonds as well as the relative orientations of the intervening sidechains for any β-turn. This description should prove particularly useful in the development and application of novel peptide mimetic drugs, compounds for which a classification based on a peptide backbone geometry may be entirely irrelevant.

β-Turn classification; Molecular recognition; Peptide mimetic

1. INTRODUCTION

The β-turn, also known as the β-bend, constitutes a well-studied subset of the reverse turns and is a common feature in biologically active peptides and globular proteins (for a review, see [10]). Unlike the α-helix and the β-sheet, the backbone conformation of the β-turn is highly variable (Fig. 1, panel A). This is partly a result of the selection criteria generally adopted for β-turns, which state that any tetrapeptide sequence in which the $\alpha C_{(1)}$–$\alpha C_{(4)}$ distance is less than or equal to 7 Å and which occurs in a non-helical region, is a β-turn [10]. The specific type of β-turn is then classified according to the geometry of the peptide backbone, as described by the ϕ and ψ backbone torsion angles in residues 2 and 3 (Fig. 1, panel A). Although there are slight variations in the classification of β-turns based on the ϕ and ψ peptide backbone torsion angles (Table I), all workers [14–18] have found similar distributions of β-turn conformers, as well as large numbers (30–50%) of non-ideal β-turns (generally defined as those turns which have a single torsion angle differing by more than 45–50° from the ideal) and one or more ill-defined categories (types IV, VI and VII in Table I).

It is generally accepted that sidechains provide a greater energy contribution than the peptide backbone in peptide hormone-receptor interactions [13]. From a molecular recognition viewpoint, therefore, the most important features of the β-turn are the relative dispositions of bonds 1, 2, 3 and 4 (Fig. 1, panel A). Bonds 2 and 3 are important because they govern the placement of the sidechains of residues 2 and 3, whose exposed nature make a logical recognition site. Bonds 1 and 4 are important because they determine the position of any binding groups which might occur before and after the β-turn. None of these features is clearly defined by the current classification which does not lend itself to clarifying the poorly understood physicochemical properties of β-turns [10]. We show here that β-turns can be described in terms of a single dihedral angle, which we have called β, which provides a complete description of the spatial relationship between the entry and exit peptide bonds (bonds 1 and 4 in Fig. 1) and the relative orientations of the intervening sidechains (bonds 2 and 3 in Fig. 1) for any β-turn.

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2. EXPERIMENTAL AND RESULTS

From Dreiding models with backbones set to the ideal β-turn types as defined in Table I (except IV, VI and VII), we observed that despite large changes in the geometry of the peptide backbone, the relative positions of bond 1, bond 2 and atom $\alpha C_{(3)}$ remained similar. This is because of the planar, *trans* nature of the intervening peptide bonds. In other words, these 3 components comprise a single conformational unit across all the β-turn types in the dataset. Likewise, we observed that the relative positions of atom $\alpha C_{(2)}$, bond 3 and bond 4 varied only slightly between the different types of β-turn and these therefore also constitute a single conformational unit. In fact, the only significant difference between the various conformations of the β-turns, with respect to bonds 1, 2, 3 and 4, appeared to be in the dihedral

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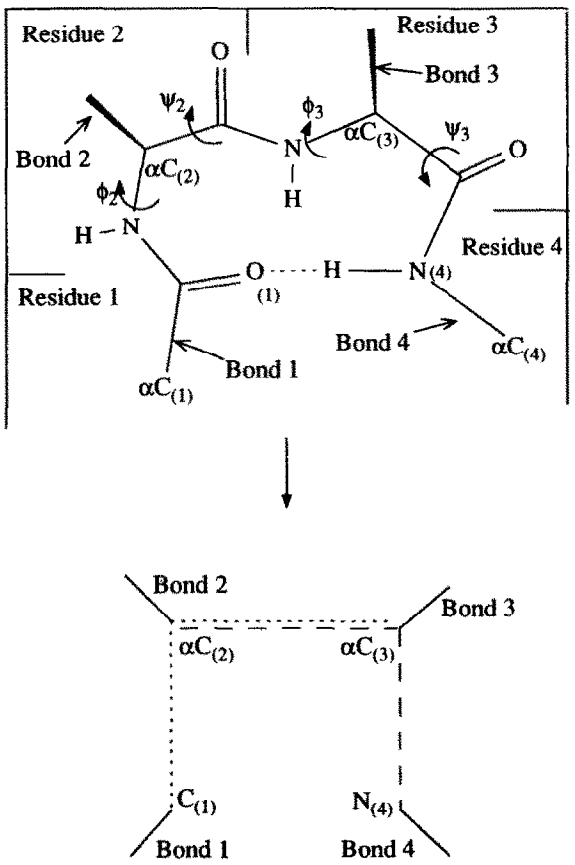

Fig. 1. The structure of the β-turn (panel A). The projections of the exposed sidechains of residues 2 and 3 are governed by bonds 2 and 3 respectively, while the angle at which the peptide chain enters and leaves the turn is determined by bonds 1 and 4, respectively. The hydrogen bond shown from $N_{(4)}$ to $O_{(1)}$ is not present in all β-turns. The β-turn is classified by the ϕ and ψ peptide backbone torsion angles shown (see Table I). We observed that β-turns could be described in terms of only two conformational units with respect to bonds 1, 2, 3 and 4 (panel B). The first unit is defined by bond 1, bond 2 and atom $\alpha C_{(3)}$, and the second by atom $\alpha C_{(2)}$, bond 3 and bond 4.

Table I

Classification of turn types according to the torsion angles of residues 2 and 3 in Fig. 1 [14,15,17]

Turn type	ϕ_2 (deg.)	ψ_2 (deg.)	ϕ_3 (deg.)	ψ_3 (deg.)
I	−60	−30	−90	0
I′	60	30	90	0
II	−60	120	80	0
II′	60	−120	−80	0
III	−60	−30	−60	−30
III′	60	30	60	30
IV	A turn with 2 or more angles differing by at least 40° from those given above			
V	−80	80	80	−80
V′	80	−80	−80	80
VI	A *cis* Pro at position 3			
VII	A kink in the protein chain created by $\Psi_2 \sim 180°$ and $\lvert\phi_3\rvert < 60°$ or $\lvert\Psi_2\rvert < 60°$ and $\phi_3 \sim 180°$			

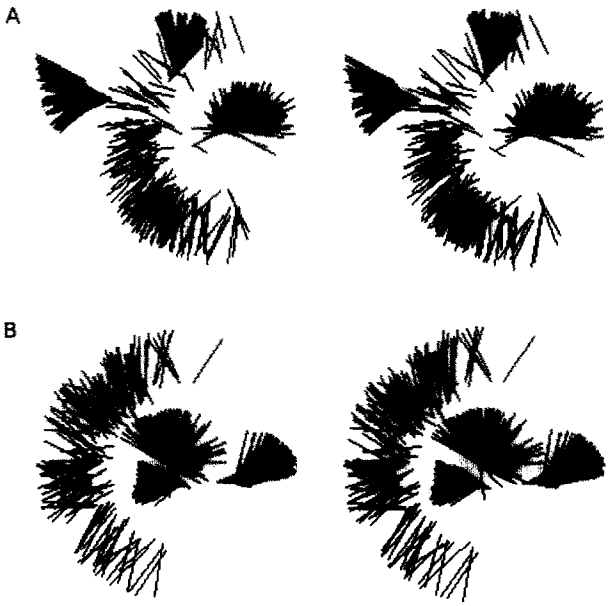

Fig. 2. Stereoview of computer-aided superimpositions of 140 examples of β-turns (20 each of types I, II, III, IV and II′, 13 each of types I′ and III′, 4 of type V, 3 of type V′ and 7 of type VII; no type VI turns are shown). For the sake of clarity, only bonds 1, 2, 3 and 4 (see Fig. 1) are shown. The template used for superimposition is defined in panel A by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ set in the average angle of 93°, and in panel B by $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set in the average angle of 90°. In panel A, bonds 1 and 2 form tight conical clusters at top left and top right, respectively, bond 3 forms the right, more splayed cluster and bond 4 forms the broad, arc-like cluster. In panel B, bond 1 forms the broad, arc-like cluster on the left, bond 2 the splayed, conical cluster in the centre, and bonds 3 and 4 the tighter, conical clusters on the right and left, respectively.

angle between these two conformationally invariant units (Fig. 1, panel B).

To test this observation, we used the program CRYS-X to generate a database of L-alanine tetrapeptides in 146 different β-turn conformations using data listed in the literature [14]. We selected the first 20 sequential examples of each type of β-turn, except for those types for which there was not this much data (namely I′(13), III′(13), V(4), V′(3), VI(6) and VII(7)). Through the use of computer graphics, we superimposed all except for the *cis*-proline-containing type VI β-turns, as shown in stereo in Fig. 2. All superimpositions involved a rigid, least-squares fit and were performed using the program A-LOOK (CRYS-X and A-LOOK are programs within the MORPHEUS [19] software package). For the sake of clarity, only bonds 1, 2, 3 and 4 are illustrated. In Fig. 2 (panel A), the template of superimposition is defined by atoms $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ using the average angle for all examples of 93°. Immediately obvious are the relatively tight conical clusters formed by bonds 1 and 2 which result from slight variations in the shape of the first conformational unit. The variation in the *projection* of bonds 3 and 4, which are part of the second conformational unit, relative to bonds 1 and 2, is clearly very large.

The fact that bond 4 is similarly projected and positioned relative to bond 3 in all β-turns is illustrated in Fig. 2 (panel B). The template here is defined by atoms $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ using the average angle for all examples of 90°. The tight conical clusters of bonds 3 and 4 illustrate the similar shape of the second conformational unit across all the β-turns. Obvious again is the great variation in the dispositions of both bonds 1 and 2 relative to bonds 3 and 4.

Based on these observations, we then defined the variable dihedral angle β ($C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$), which describes the twist of one con-

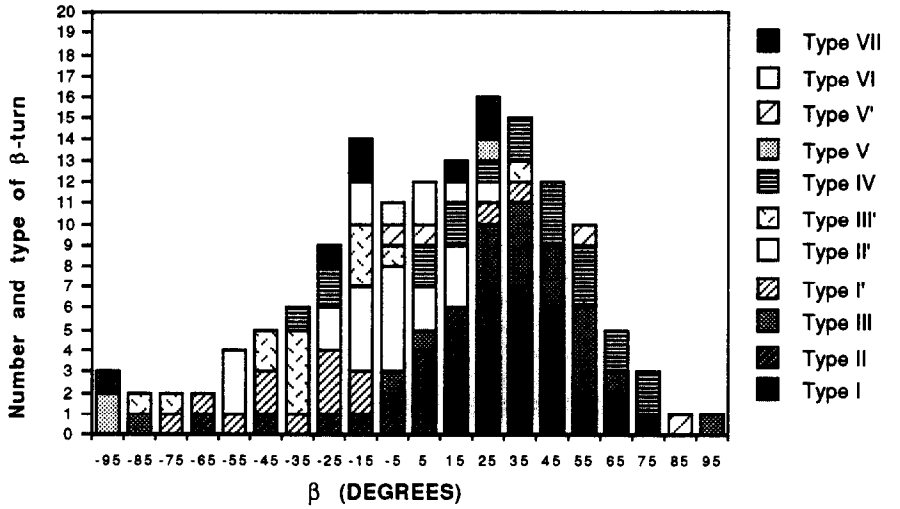

Fig. 3. Correlation of the dihedral angle β ($C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$) with the β-turn type for a series of 146 β-turns. The relative conformation of bonds 1 to 4 are different for type VI β-turns because they contain a *cis*-peptide bond, but the variable β is still useful to describe the degree of planarity of the turn and so these β-turn types are included in this figure. Each column spans 10°, such that, for example, the column at −5° represents the torsional range from β = −9° to 0°.

formational unit relative to the other, and therefore the topographical footprint of any β-turn. The relationship between β and the standard β-turn types is shown in Fig. 3. There is clearly significant topographical overlap between all of the β-turn types and the inadequacy of the peptide backbone classification to provide a description of the twist that a β-turn imparts to the peptide chain is thus clearly brought out.

As a further illustration of this point, Fig. 4 shows a superimposition of the 16 examples of β-turns in which β is 21–30°. The template is defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ with β set to 25° and with angles $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ and $\alpha C_{(2)}$-$C_{(3)}$-$N_{(4)}$ set to the averages of 96° and 90°, respectively. The large variation in the geometry of the peptide backbone is witness to the fact that seven different classical 'types' of β-turn are present, yet the relative dispositions of bonds 1, 2, 3 and 4 are very similar indeed. The untidy problems of ill-defined β-turn types (see Table I), of which there are two in this sample (IV and VII), and of non-ideal-β-turns, of which there are three examples here (one a type V, one a type I′, and the other a type II′), are totally eliminated when β is used as a topographical descriptor.

3. DISCUSSION

The stability of the β-turn has led to the suggestion that it is an important directing force in protein folding [3,4,7,10,11] and molecular recognition [1–3,5,6,8–12].

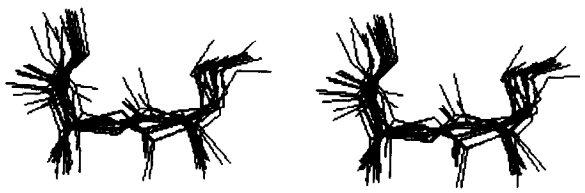

Fig. 4. Stereoview of the superimposition of the 16 examples of β-turns in Fig. 3 in which β is 21 − 30°. The template is defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ with β set to 25° and angles $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ and $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set at the average for these examples of 96° and 90°, respectively. For the sake of clarity, all $O_{(1)}$ and hydrogen atoms have been deleted. The orientation is such that bond 1 lies on the left.

Consequently, there is rapidly increasing interest in the construction of rigid, non-peptide β-turn mimics [20], and a few of these [2,5,6,9] have already given rise to potent peptide mimetics. Dissatisfaction has been expressed [10] about the ability of the unwieldy traditional β-turn classification to reveal aspects important for molecular recognition, and a more concise and informative new nomenclature has very recently been proposed [21]. This is still based on the geometry of the peptide backbone, however, and sidechain disposition remains obscure. We believe that our simplified description of β-turns based on the value of the continuous variable β, which assumes a greater role for sidechains than the peptide backbone, will facilitate the understanding of the physicochemical properties of β-turns and also provide a rapid means of designing or selecting appropriate conformationally constrained analogues for any peptide or protein β-turn.

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