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STEREOCHEMICAL REQUIREMENTS FOR THE EXISTENCE OF HYDROGEN BONDS IN β-BENDS

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SUMMARY: β-bends in proteins are characterized by a range of dihedral angles. They can be classified into eight groups, according to the orientation of the three peptide groups comprising the bend. The possibility of formation of intra-bend hydrogen bonds, involving N-H and C=O groups, depends on the relative orientation of the peptide groups, and hence differs for various types of bends. Therefore, nuclear magnetic resonance, infrared, or Raman spectroscopic data on hydrogen bonding or the shielding of N-H groups can be used in some cases to distinguish between various types of bends.

In a polypeptide chain, a β-bend is formed by three peptide units, extending from the C^{α} atom of residue i to the C^{α} atom of residue i+3 (1-3). Its conformation is characterized by the values of the backbone dihedral angles ϕ and ψ in residues i+1 and i+2. Previous discussions of various types of bends (1-5) dealt with the orientation of the middle peptide group, located between the C^{α} atoms of residues i+1 and i+2. The orientation of this peptide group distinguishes between type I and type II bends (1-6). In this communication, we explore the structural consequences of variation of the orientation of the other two peptide groups in bends.

The "standard" bend types, viz., types I, II, and III, together with their inverses, the type I', II', and III' bends, have been defined (1,3) in terms of specified sets of dihedral angles (Table I). The original definition of bend types (1) was based on the possibility of forming i+3 → i hydrogen bonds in a polypeptide chain (between the N-H of residue i+3 and the C=O of residue i). Bends often are drawn with a linear hydrogen bond of this type. Actually, however, this hydrogen bond can be found only in bends with dihedral angles (6,7) which differ considerably from those of the "standard" bends, especially in the case of type I bends (Table I). If the bend is constructed

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Table I. Dihedral Angles for the "Standard" Bends of Various Types and for Bends with Optimal i+3 → i Hydrogen Bonds

Bend Type	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}
	(degrees)			
"Standard"[a]				
I	-60	-30	-90	0
II	-60	120	80	0
III	-60	-30	-60	-30
With optimal hydrogen bond[b]				
I	50	-100	-145	50
II	-60	110	80	10

a. As defined in refs. 1 and 3. For the inverse bend types I', II', and III', the sign of each dihedral angle is the opposite of that shown for the respective bends in the Table. Bends in proteins have been classified (3) as belonging to the types indicated if at least three of the four dihedral angles are within ±40° of the values shown in the first three lines of the Table.

b. Refs. 6, 7.

with the "standard" dihedral angles, then the hydrogen bond is considerably bent (4), because the C=O group of residue i does not point toward the N-H group of residue i+3 (see, e.g., Fig. 2 of ref. 3, Fig. 7 of ref. 8, and Fig. 4 of ref. 9).

Of course, the "standard" dihedral angles listed in Table I are an idealization. Observed bends in proteins occur with a distribution of dihedral angles (3,5,10). In Fig. 1, we have plotted the (ϕ,ψ) values for residues in bends of type I, II, and III, found in 23 proteins of known structure (10). The wide range of the distributions in Fig. 1 has prompted us to inquire into the stereochemical relationships between bends of various types. These relationships can be expressed in terms of the orientation of the peptide groups, the associated dihedral angles, and the potential for the formation of various kinds of intramolecular hydrogen bonds (only bends with *trans* peptide groups are considered in this paper).

Type I and III bends have similar dihedral angles in residue i+1. The dihedral angles in residue i+2 form one continuous distribution for these two bends (Fig. 1A). The division of this distribution into two subsets (for type I and type III) is therefore arbitrary. If one compares typical type III (and some type I) bends, which have $\psi_{i+2} < 0°$, with the frequently occurring type I

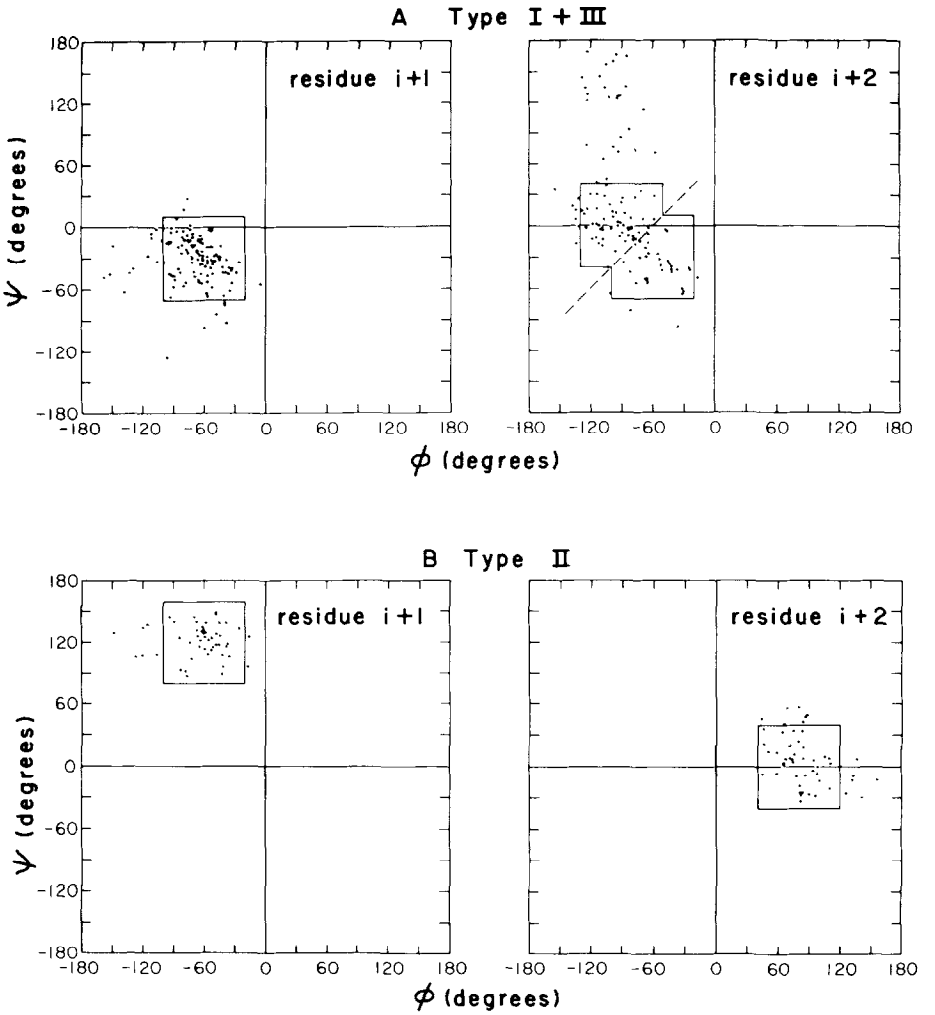

Figure 1. Distribution of dihedral angles for residues i+1 and i+2 in bends (A) of types I and III and (B) of type II, in 23 proteins of known structure (based on a survey in ref. 10). Residues i+1 and i+2 form a bend if they are not part of an α-helical sequence of three or more residues and the distance R_3 between the C^α atoms of residues i and i+3 is ≤7.0 Å (according to the definition in refs. 3, 11). The classification into bend types follows ref. 3. The squares indicate ranges of ±40° (cf. ref. 3) around the "standard" values of (φ,ψ) in each type of bend (cf. Table I). The dashed line indicates the boundary between type I and type III bends for residue i+2.

bends which have $\psi_{i+2} > 0°$, it is seen that they differ from each other mainly by the changed orientation of the third peptide group, i.e., the one between the C^α atoms of residues i+2 and i+3. This is seen in Figs. 2A and 2B.

Two forms of the type II bends can be distinguished in terms of an analogous relationship, viz., according to whether the sign of ψ_{i+2} is nega-

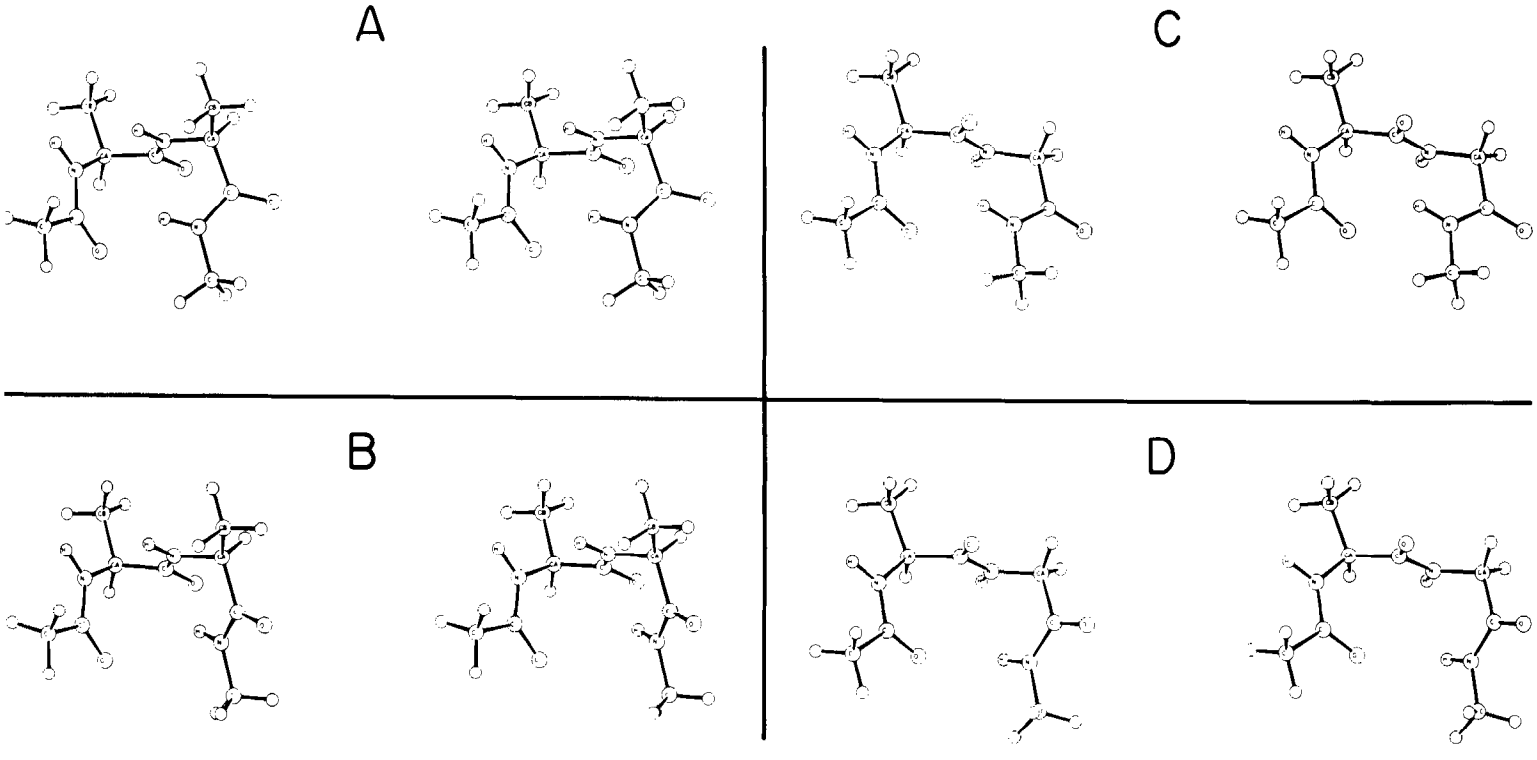

Figure 2. Stereoscopic drawings of terminally-blocked dipeptides in representative conformations corresponding to the four kinds of bends. CH_3CO-L-Ala-L-Ala-$NHCH_3$ is shown in (A) a type III bend and in (B) a type I bend, respectively. CH_3CO-L-Ala-Gly-$NHCH_3$ is shown in (C) a type II_3 bend and in (D) a type II_1 bend, respectively. Note the orientation of the peptide groups. The N-H bond of the second peptide group points up in A and B, down in C and D. The N-H bond of the third peptide group points weakly up in A and C, down in B and D. The acetyl C=O bond does not point directly at the C-terminal nitrogen in any of the bends. The dihedral angles ($\phi_{i+1},\psi_{i+1},\phi_{i+2},\psi_{i+2}$) are as follows: A (-70°,-35°,-70°,-35°); B (-70°,-35°,-70°,35°); C (-60°,130°,85°,-35°); D (-60°,130°,85°,35°). An Ala-Gly dipeptide is shown in the type II bends because an L-Ala-L-Ala sequence would have high energy in the conformations shown.

Table II. Description of the Orientation of the Peptide Groups in Various Types of Bends

Bend type[a]	Dihedral angles				Orientation of N-H bond[b] in residue			Possibility of formation of hydrogen bond[c]		
	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}	i+1	i+2	i+3	i+3→i	i+2→i	i+3→i+1
III	<0	<0	<0	<0	U	U	U	-	-	-
I	<0	<0	<0	>0	U	U	D	+	-	+
II_3	<0	>0	>0	<0	U	D	U	-	+	+
II_1	<0	>0	>0	>0	U	D	D	+	+	-
III'	>0	>0	>0	>0	D	D	D	-	-	-
I'	>0	>0	>0	<0	D	D	U	+	-	+
II_3'	>0	<0	<0	>0	D	U	D	-	+	+
II_1'	>0	<0	<0	<0	D	U	U	+	+	-

a. Defined in refs. 1 and 3. Subscripts 1 and 3 defined in the text.
b. U = up, D = down. The direction of the C^{α}-C^{β} bond of an L-residue in the bend is used as the reference direction of orientation: in the views shown in Fig. 2, this bond is pointing up.
c. Indices in the headings denote the residues which may form an N-H → O=C hydrogen bond. + = a bent hydrogen bond may be formed because both the H and the O atom are on the same side of the plane of the bend. (It may require some adjustment of the dihedral angles, but no change of sign.) - = a bent hydrogen bond cannot be formed with this choice of sign of the dihedral angles, because the H and O atoms are on opposite sides of the plane of the bend.

tive or positive (Fig. 1B). The two subsets of class II bends differ by the changed orientation of the third peptide group (Figs. 2C and 2D). The two subsets are referred to in this paper as type II_3 and type II_1 bends, in order to indicate their structural relation to type III and I bends, respectively.

Type III and I bends form one group, and type II_3 and II_1 bends another group. The two groups differ from each other by having opposite signs of both ψ_{i+1} and ϕ_{i+2}. This difference in signs corresponds to the opposite orientation of the second peptide group (1-6), seen when Figs. 2A and 2B are compared with Figs. 2C and 2D. The correlations between the dihedral angles of the four kinds of bends and the corresponding orientations of the peptide groups are shown in the first half of Table II.

The first peptide group (between the C^{α} atoms of residues i and i+1) has the same orientation in all of the bends discussed so far. Opposite orientation of this peptide group, in combination with all permutations of orientations of the other two peptide groups, gives rise to the inverse bend types (1,3) III', I', II_3', and II_1', as shown in the lower half of Table II. Table

II thus lists the eight possible permutations of the orientation of three consecutive peptide groups.

The classification of peptide group orientations, shown in Table II, is of interest because it is correlated with the potential of the various types of bends to form hydrogen bonds. In addition to a possible i+3 → i bent hydrogen bond, resulting in a ten-membered hydrogen-bonded ring structure (1), there is also the possibility of forming a bent i+2 → i or a bent i+3 → i+1 hydrogen bond in a bend, resulting in seven-membered C_7 ring structures (12). Each of these hydrogen bonds may be formed only if the corresponding N-H and C=O groups point to the same side of the plane of the bend (defined roughly by the four C^{α} atoms). As indicated in Table II, it is not possible to form *all* of the three kinds of hydrogen bonds in a given type of bend. With the aid of the Table, experimental evidence for the involvement of a particular N-H or C=O group in an intra-bend hydrogen bond can be used to eliminate the presence of certain types of bends in a peptide. Such information may be obtained from nuclear magnetic resonance, infrared, or Raman measurements. We have used this analysis recently to confirm the theoretical prediction (13) of the presence of a type II_1 bend in a cyclized dipeptide derivative (14-16).

The potential for hydrogen bonding in a given type of bend, shown in Table II, does not imply that the hydrogen bond necessarily is present in the bend. Small changes in dihedral angles (well within the ranges indicated in Fig. 1) may be sufficient to alter somewhat the direction of an N-H or a C=O group, thereby breaking the hydrogen bond. In fact, only about 31% of the bends observed in proteins have an i+3 → i hydrogen bond with an N···O distance below 3.2 Å (17).

Other observable parameters also may be used to differentiate between some of the structures shown in Fig. 2 and in Table II. For example, the stretching frequency of a peptide N-H group (observed in the infrared spectrum) is shifted not only if the group forms a hydrogen bond, but also by the presence of a nearby alkyl group, e.g., by the side chain of the residue (18,19).

The frequency shift is smaller than in the case of hydrogen bonding (16), and it occurs only when the N-H bond and the C^{α}-C^{β} bond are nearly eclipsed, i.e., when $\phi \approx -60° \pm 20°$ for L-amino acid residues (18). This range of ϕ_{i+1} is found in type III, I, II_3, and II_1 bends, while this range of ϕ_{i+2} is found in type III, I, II_1', and II_3' bends. For D-residues, the shift occurs for $\phi \approx 60° \pm 20°$, i.e., in bends which are the inverses of those listed.

Shielding of N-H groups from the solvent, as observed in nuclear magnetic resonance measurements, does not necessarily arise only from hydrogen bonding. In most bends, those N-H protons which have the potential for hydrogen bonding are also less accessible to the solvent, even if they do not form a hydrogen bond. Thus, the information in the last three columns of Table II can also be used in connection with proton chemical shift data to restrict the choice of possible bends.

The analysis of experimental information, as described here, can supplement or confirm the conclusions reached about the conformation of bends in peptides, obtained from the analysis of coupling constants (8,21-23) and nuclear Overhauser effects (24-26).

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