(M^+), 759 (MH^+); high-resolution FAB mass spectrum, 758.2043 ($C_{32}H_{34}N_6O_{16}$ requires 758.2031 amu).

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Supplementary Material Available: Final atomic positional parameters, anisotropic thermal properties, torsion angles, bond lengths and angles, and graphics from X-ray structure determinations of **7b**, **8b**, **14**, and **15** (121 pages); observed and calculated structure factors (21 pages). Ordering information is given on any current masthead page.

Amide Chemical Shifts in Many Helices in Peptides and Proteins Are Periodic

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Abstract: A survey of the literature data on the NMR chemical shifts of amide hydrogens in peptides and proteins finds many cases in which resonances in helical structures show a periodicity of ca. 3-4 residues/cycle. Some helices do not show such behavior, nor do α carbon hydrogen resonances. The simplest explanation is that the helical curvature found by high-resolution X-ray crystallographic studies for some helices in proteins is a fairly general phenomenon for protein and peptide structures in solution.

Introduction

There have been several reports[1-5] that the mean chemical shift of amide protons is approximately 0.2 ppm upfield in helical structures and approximately 0.3 ppm downfield in β sheet structures. We add the further observation that, in many cases, the helical component is periodic in character, with a dominant repeat near 3.6 residues. The most striking example is a coil–coil peptide: the leucine zipper[6] (Figure 1). The periodicity is obvious for residues 10–31 of the leucine zipper, and, on analysis, accounts for 65% of the sequential variation in chemical shift for these amide protons. Other examples abound in proteins and peptides (Table I; see also ref 5, Figure 1).

Methods and Results

We used three methods to extract the periodic contribution to the chemical shifts. Simple plots of chemical shift vs sequence[5] allow a visual assessment. For such plots, we used the criterion that the spacing of three maximum of three minima corresponded to periodicity of α helices (i; $i + 3$ or $i + 4$; $i + 7$). For sequences that met this standard, we estimated the peak-to-peak value of the sinusoidal component (Table I). The second method used a linear prediction method to calculate the dominant periodicity over an arbitrary window[7] (Table I). Finally, Fourier coefficients can be extracted by using the formula given by Eisenberg et al.[8] In Table I we give the average amplitude (ca. 40% of the peak-to-peak value for sinusoids) and the fraction of the total oscillatory signal that occurs with a period of 3.6 residues/cycle.

On the basis of the data in Table I, we draw the following observations: (1) significant oscillations of the amide proton chemical shift are seen for three-fourths of the helices assigned in the data set; (2) the average helical variation is approximately 0.4 ppm in amplitude, with the maximal variations approaching 1 ppm; (3) in most cases, the downfield protons are associated with hydrophobic side chains; (4) the chemical shifts of α carbon protons do not show much periodicity, although a weak out-of-phase component (<20%) cannot be ruled out; (5) a similar oscillation with a repeat of ca. 2 residues in β sheet regions is occasionally seen (see Figure 1 in Williamson[5]) but is not a general phenomenon; (6) the dominant effect is transverse to the helical axis—there is little periodic chemical shift displacement associated with the ends of helices. Occasional nonhelical features (e.g., reverse turns) can contribute to some helix-like signal but the magnitudes are usually small.

We can also show that the amplitude of the helical variation, calculated as either the average amplitude or the maximum amplitude at 3.6 residues/cycle, is roughly proportional to the circular dichroism signal at $[\Theta]_{222}$ (Figure 2) for a small peptide in water or water/TFE solutions.

Finally, we have made a cursory inspection of ^{15}N amide and ^{13}C carbonyl assignments. Helical variations of ca. 4 ppm in the ^{15}N chemical shifts are observed for three of four helices in thioredoxin:[9] variations of ca. 4 ppm are noted for the ^{13}C carbonyl

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Table I. Chemical Shifts of ^1H, ^{13}C, and ^{15}N of Backbone Amides in Proteins and Peptides

protein	residues[a]	visual signal[b]	LP period[c]	amplitude[d] p-to-p	amplitude[d] av	amplitude[d] %	ref
		^1H Amide Chemical Shifts					
BPTI	47–56	B	3.6				21
C3a	5–14	C	2.2	–	0.11	30	22
	19–28	B[e]	4.1	(1.2)[e]	0.13	20	
	47–70	B	3.4	0.5	0.19	39	
calbindin	4–16	B	3.8	0.6	0.19	35	23
	24–35	A	3.3	1.0	0.23	52	
	44–55	A	4.2	0.8	0.23	50	
	63–74	C	3.2	–	0.19	28	
GHRF	1–22	B	2.7	0.4			24
LamB	10–25	B	2.6	0.4	0.05	21	25
leucine zipper	9–31	A	3.6	0.9	0.30	64	6
$C_\alpha H$ chem shifts		C	3.6	–	(0.03)		
Lysl-cp	4–13	B	5.5	0.2			26
lysozyme	6–15	B	4.0	0.6	0.19	33	27
	25–38	C[e]	6.5[e]	–	0.64[e]	53[e]	
	89–101	B	2.8	0.6	0.12	39	
	41–56	(nonhelical region)			0.06	12	
neuropeptide Y	17–33	B	3.6	0.3	0.10	66	28
staphylococcal nuclease	59–69	C	3.4	–			29, 30
	100–107	C	–	–			
	125–137	A	4.7	0.6			
synthetic peptide	4–14 25 °C	B	4.9		0.07_6	54	19
	15 °C	B	4.7		0.09_7	54	
	5 °C	B	4.5		0.12	54	
	5 °C + TFE	A	3.5		0.18	63	
thioredoxin	7–19[f]	B	2.5	0.6	0.23	44	31, 32
	35–49	C	(3.3)	–	–		
	62–70[f]	B	3.2	0.5	–		
	96–105	B	3.9	0.6			
VIP′ 1–28	C		–				33
ubiquitin	23–36	A	3.4	0.8			34, 35
	54–60	B	–	(0.8)			
zervamicin	1–9	B	3.4	0.5			36[g]
		^{13}C Carbonyl Backbone Chemical Shifts					
BPTI	47–55	A	3.9	4.0			10
		^{15}N Amide Backbone Chemical Shifts					
thioredoxin	7–20	B	3.0	4.0	2.1	63	9
	35–49	B	7.4 (3.7)[c]	6.0	1.3	64	
	62–70	B	2.6	5.0	–		
	94–105	B	3.9	4.0	1.2	34	

[a] NMR assignments are used unless otherwise noted. [b] Direct observation of chemical shift vs sequence plots (e.g., Figure 1): A, strong helical periodicity; B, noticeable helical periodicity with secondary components; C, no obvious helical periodicity. [c] Dominant period (residues/cycle) calculated by using linear prediction methods.[7] A least-squares singular value decomposition routine was used for the windows shown. The filter width was taken as half of the window width. For some sequences, the second most important period is shown in parentheses. [d] Amplitude in ppm. the peak-to-peak amplitudes are estimated visually. The average amplitudes were calculated from the formula in Eisenberg et al.[8] by using an angle of 100° and a window of 11. A small correction was applied to compensate for the lack of an integral number of turns of the helix by subtracting the value obtained by using this formula with the average signal magnitude. The percentage of the alteration in chemical shifts due to the helical component is also reported. [e] Signal influenced by anomalous shifts of a few residues. [f] Helix described as distorted in original paper. [g] In DMSO.

chemical shifts for the C-terminal helix of BPTI.[10]

Discussion

We next consider the origin of the helical periodicity in chemical shifts. It is important to begin with the comment that this periodicity cannot be used to *define* helical structures in peptides and proteins. First, it is clear that not all helices in proteins show such behavior, so periodicity is not a necessary concomitance. Second, contributions to chemical shifts from many sources can generate some signal in nonhelical structures. In fact, if the explanation offered below is correct, the periodicity arises from distortions in the helical structure rather than from the helical state itself. It remains to be seen if it is useful to employ helical periodicity to support assignments of the starting and ending points of helices.

The real question is the origin of these oscillations in chemical shifts when they are unamgibuous. Possible sources are hydrogen bonding,[11,12] electric field effects,[13] or magnetic anisotropies.[14] Any of these explanations can be developed for helices in proteins because of their asymmetrical placement with respect to the solvent. The most plausible explanation is the well-documented alteration in hydrogen bond lengths and angles in some amphipathic helices studied by X-ray crystallography.[15-17] The effect is particularly well documented in the coil–coil protein ColE1 Rop[18] and the peptide zervamicin.[17] The hydrogen bonds to the buried carbonyl groups average about 0.1–0.2 Å shorter than those to the solvent-exposed carbonyl groups. This hydrogen-bonding pattern was reported first by Blundell and co-workers,[15] who

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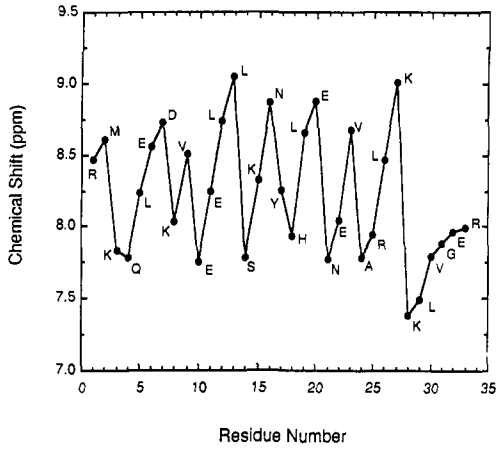

Figure 1. Amide proton chemical shift plotted against residue sequence number for the leucine zipper peptide; data from Oas et al.[6] The chemical shifts are not corrected for random coil shifts or ring current shifts.[5] Such corrections would not alter the observed periodicity.

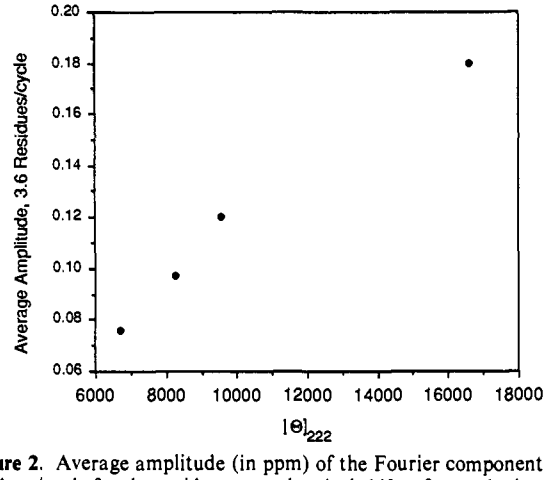

Figure 2. Average amplitude (in ppm) of the Fourier component at 3.6 residues/cycle for the amide proton chemical shifts of a synthetic peptide [ETGTKAELLAKYEATHK] under four conditions plotted against the circular dichroism molar ellipticity $[\Theta]_{222}$. The four conditions for the experimental studies are as follows: 25, 15, 5 °C, all in aqueous solution at pH 2.0; 25% trifluoroethanol (TFE), water at pH 2.0, 5 °C. The helical content increases as the temperature is lowered and as TFE is added.[19]

suggested that helices are often curved rather than strictly linear. Systematic alteration of the length of hydrogen bonds is certainly adequate to explain the magnitude of the oscillation in chemical shifts. The total effect of forming a hydrogen bond is roughly 5–10 ppm. Modulation of this by 3–5% would be sufficient to explain the effects we have noted. The empirical relation developed by Wagner et al.[12] also indicates that such perturbations in hydrogen bond lengths could generate changes ca. 1 ppm in amide proton chemical shifts. Further, this model is consistent with the relative downfield shifts being associated with the more hydrophobic faces of the helices, the lack of sensitivity of α proton chemical shifts (see discussion), and the (limited) observations on the sensitivity of other amide nuclei such as ^{15}N and ^{13}C. Note, however, that alterations in hydrogen bonding cannot explain the *average* shift to high field for helical secondary structures noted by Williamson and others.[1-5] Further, the correlation of downfield shifts with the hydrophobic *face* of the helix cannot be extended to individual hydrophobic or hydrophilic residues—the mean chemical shift on a residue basis is within 0.1 ppm for sets of the common divisions into "polar" and "nonpolar" amino acids.

It is more difficult to understand the (smaller) chemical shift oscillations in the helical peptide studied by Bradley et al.[19] There is evidence from CD and NMR spectra that this peptide forms stable monomers in solution in NMR concentrations.[19] If this observation is correct, then we must postulate that the helix or helices in the peptide have some time-averaged curvature, perhaps induced by protection of the carbonyl groups from solvent by the hydrophobic side chains. The distortions appear to increase in proportion to the total helicity as measured by CD. This system clearly merits more experimentation, possibly along the lines suggested below.

There are several tests of the idea that hydrogen bond length variations are the source of the chemical shift variations. First, there should be a small but measurable variation in the NOE intensities for the α protons of residue i to the amide protons of residue $i + 3$. Second, the periodicity in chemical shifts should be reduced locally with the substitution of less hydrophobic side chains, even when these increase the overall helical character (e.g., the substitution of Ala for Val). Third, the effect should be smaller in nonaqueous solvents if amide solvation is reduced.

In conclusion, the simplest interpretation of the observations made here is that most helical features in globular proteins and peptides in aqueous solution have small but periodic alterations in hydrogen bond lengths and/or angles. It is not possible to say, from NMR data alone, whether these helices are curved. The helices that do not show oscillatory chemical shifts could have more ideal geometry, or they may contain less regular distortions such as kinks and sharp bends. Similar conclusions have been drawn for globular proteins in crystals by Barlow and Thornton.[20]

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