J. Am. Chem. Soc. 1992, 114, 9676–9677

NMR Chemical Shifts: A Tool To Characterize Distortions of Peptide and Protein Helices

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Received June 5, 1992

NMR chemical shifts in proteins are receiving much attention[1-12] because δ deviations from random coil values reflect the secondary structure, thus complementing the traditional method[13] based on NOEs. This observation[1,2] has been confirmed by δ statistics using either small[3] or large[5,6,8,10] data sets. An interesting regularity of proton δ values is the striking helix periodicity detected in the amide and H_α conformational shifts of several peptide and protein helices.[4,14-16] Although the existence of helix curvature originated by its amphipathic character has been suggested[14-16] as the cause of such periodicity, direct experimental evidence was needed.[25] Here we report novel observations on model helices and chemical shift computations which show that minor geometric distortions of peptide and protein helices are directly related to the magnitude of H_α and amide proton δ changes upon helix formation (helix shifts). Analysis of helix shifts in helices of dissimilar amphipathic character also showed that helix shift periods are determined by the hydrophobic–hydrophilic repetition periods of the amino acid sequences.

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(25) After submission of this paper, an interesting study appeared[26] in which helix-axis curvature was concluded also to be the reason for the amide helix shift periodicity detected in a monomeric amphiphatic model peptide.

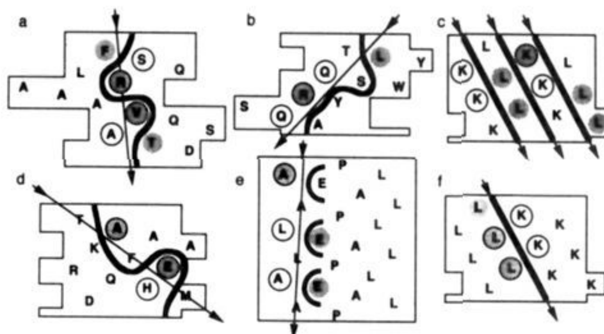

Figure 1. Helical net diagrams[22] showing, for several isolated peptide and protein helices, the distribution of the amino acid residues with either minimum H_α (circled) and/or maximum NH amide (shaded) helix shifts. They appear along a line or lines, marked by arrows, approximately parallel to the thick line (or lines) defining the boundary (or boundaries) between helix surface regions of differing hydrophobic character: (a) thermolysin 281–294 helix,[16] Eisenberg's[23] hydrophobic moment, μ = 0.14; (b) α-amylase inhibitor 13–23 helix,[16] μ = 0.16; (c) Ac-(Leu-Lys-Leu-Lys)$_3$-NH$_2$ model helix, μ = 0.03, period = 2 (see Figure 2); (d) ribonuclease A 3–14 helix,[16] μ = 0.28; (e) Pro-containing model helix,[24] μ = 0.15, period = 7; (f) Ac-(Leu-Lys-Lys-Leu)$_3$-NH-Et model helix,[14] μ = 0.46, period = 4.

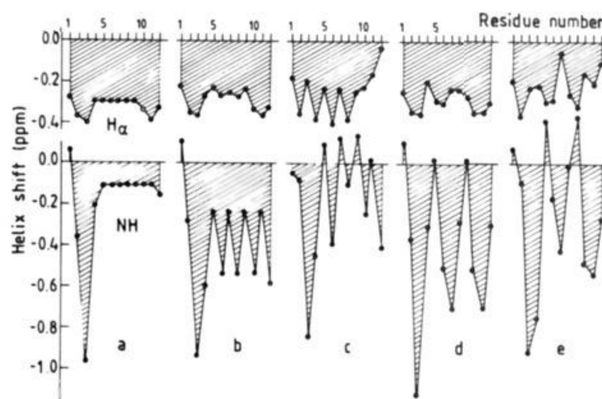

Figure 2. Comparison of the experimental amide and H_α helix shifts shown by two model peptide helices [(c) Ac-(Leu-Lys-Leu-Lys)$_3$-NH$_2$, alternate and nonamphiphatic, this work, and (e) Ac-(Leu-Lys-Lys-Leu)$_3$-NH-Et,[14] fully amphiphatic] and those computed[12] for (a) a regular Arnott's[18] straight helix, (b) a distorted helix showing kinks every two residues, and (d) an average curved helix.[18] See supplementary material for NOE characterization of the alternate helix and other experimental and computing details.

A straightforward way to investigate the relationship between δ periodicity and peptide amphiphilicity is to examine the distribution on the helix surface of the residues that show extreme H_α or amide helix shifts. These shifts are better estimated in isolated peptides undergoing helix transitions than in proteins.[14,16] Figure 1 shows such distributions for various isolated protein and model peptide helices selected because of the dissimilar arrangement of their hydrophobic–hydrophilic surfaces. The amino acid residues showing extreme H_α or amide helix shifts always align themselves along interface boundaries irrespective of the number of boundaries, the boundary shape, or the frequency of repetition of hydrophobic residues (hydrophobic period). Alternate, fully amphiphilic, and Pro-containing helices (Figure 1c,f,e) are specially illuminating because a change in the hydrophobic period of the sequences (2, 4, or 7) originates parallel changes in the helix shift periods. For the peptide with a hydrophobic period of 7, in particular, the extreme values of the helix shifts correspond to points on the helix surfaces were hydrophobicity suddenly changes but not to Pro residues, as might be expected from the propensity of Pro to originate helix kinks in protein crystals.[17] Results shown in Figure 1 strongly support the hypothesis that changes in the periodicity of hydrophobicity along the sequence determine the helix shift periodicity, most probably

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via distortions, also periodic, from regular helix geometries.

Can a small geometric distortion of a helical secondary structure quantitatively explain the experimentally observed fairly large sequence-related variability of helix shifts, and also the different behavior of amide and H_α protons? The magnitude of helix shifts of backbone protons in short distorted helices is unknown, but can be computed using a recent protein chemical shift model.[12] On the basis of average crystal geometries of protein helices,[18] we computed the helix shifts of one regular straight and two distorted peptide helices. On the whole, the computed and the experimental helix shifts of model helices (Figure 2) are in close agreement and coincide in features such as the two helix periods (2 and 4), the relative magnitudes of the amide and H_α signals within each helix, and the magnitudes of helix shifts in the two distorted helices. Other less obvious features also appear in both computed and experimental data; e.g., the amide helix shifts of the first helical turn differ in size from those of the other two turns. This is probably because the first turn lacks the preceding peptide CO groups whose magnetic anisotropy strongly influences the δ values of the subsequent residues.[8,11,12] It is therefore a terminal rather than a distortion effect, and it appears also in the δ values computed for the regular Arnott's helix[18] (Figure 2a) that, interestingly, do not show other sequence dependent amide helix shift variations.

Despite having a hydrophobic moment $\mu = 0.03$, the alternate model helix shows sequence-related variability of its H_α and amide helix shifts in both the experimental and computed shift vs sequence profiles (Figure 2b,c). The experimental amide helix shifts of the second and third helical turns in both model helices are clustered more strongly in the positive range than in the computed ones (Figure 2c,e). These more positive shifts probably reflect the existence of intrahelix hydrogen bonds in the second and third turns that are shorter than the hydrogen bonds between first-turn residues and solvent molecules.

The excellent agreement between observed and computed helix shifts in Figure 2d,e supports that natural amphipathic helices in solution are bent so that the hydrophobic side has shorter hydrogen bonds, as observed in protein crystals.[17-19] Bending seems to be a property of such helices in polar solvents rather than a packing requirement of the tertiary structure, since many isolated amphipathic[20] protein helices show δ periodicity[3,4,15,16] and therefore curvature.

The differences involved in the geometries of distorted helices are extremely small (typically 0.1–0.2 Å) but still detectable by helix shifts. The main reason is the aromatic-like behavior of the peptide CO groups[11,12] whose magnetic anisotropy strongly and very selectively influences the δ values of nearby protons. Also amide δ values are extremely sensitive to hydrogen bond lengths.[2,8,12,21] Although agreement between experimental and computed amide δ values can be obtained[26] using a simple hydrogen bond length model of chemical shifts,[2] hydrogen bonds alone cannot explain the now well established sequence variability of helix shifts of the H_α and H_β protons[16] nor those of amide protons not intramolecularly hydrogen bonded. Such minor geometric differences would be very difficult to detect by conventional NOE-based structure refinements.

Acknowledgment. We thank the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) for financial support and Max Gorman for text revision.

Registry No. Pro, 147-85-3.

Supplementary Material Available: One table and two figures showing the NMR chemical shifts, NOE summary, and NOESY spectrum of the Ac(Leu-Lys-Leu-Lys)$_3$-NH$_2$ model peptide in mixed TFE/H_2O solvent, details on chemical shift computation, experimental procedures, and additional references (6 pages). Ordering information is given on any current masthead page.

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Recognition of Guanine and Adenine in DNA by Cytosine and Thymine Containing Peptide Nucleic Acids (PNA)[1,2]

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Received August 12, 1992

Reagents that recognize specific sequences in RNA or double-stranded DNA may be developed into drugs which can modulate gene expression[3] or they may be used as diagnostic or molecular biological probes. We have recently found that nucleic acid analogues, in which the entire phosphate–sugar backbone has been replaced with a backbone consisting of (2-aminoethyl)glycine units to which thymines are attached through methylenecarbonyl bridges (PNA, **1**, Figure 1), bind very efficiently to complementary DNA.[4] The PNA[5] was found to bind to the complementary oligodeoxynucleotides with a 2:1 stoichiometry, as expected for analogues with neutral backbones.[6] It was also observed that the binding of PNA reagents containing 10 T ligands to a $(dA/dT)_{10}$ target in a 248 base pair double-stranded DNA fragment took place with strand displacement, i.e., binding to the A-strand with displacement of the opposite T-strand.[7]

Obviously, it would be most exciting to extend this recognition to other bases, and we now report the incorporation of a second base, cytosine, which is shown to recognize its complementary base, guanine, with a 2:1 stoichiometry presumably involving both Watson–Crick and Hoogsteen base pairing.

PNA Synthesis. The cytosine monomer (Boc-C[Z]aeg-OH, **3**, Scheme I), was prepared in a manner analogous to the preparation of the thymine monomer, with the exception that it was equipped with a benzyloxycarbonyl (Z) protecting group. The fully protected Boc-T_4C[Z]T_5-Lys(Cl-Z)-benzhydrylamine resin was assembled by stepwise Merrifield synthesis[8,9] utilizing an improved solid-phase procedure, i.e., in situ DCC coupling (0.15 M in 50% DMF/CH_2Cl_2) instead of the previously reported pentafluorophenyl ester activation. All of the coupling steps proceeded with an efficiency of 98–100%. Deprotection and release of the free PNA, H-T_4CT_5-Lys-NH$_2$, from the resin were accomplished with anhydrous HF under standard conditions. The purified product was homogeneous by analytical HPLC and showed the expected molecular weight by fast atom bombardment mass spectrometry (found (calcd), 2792.21 (2792.14)). The positively charged lysine amide at the C-terminus was originally

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