Journal of Biomolecular NMR, 2 (1992) 307–322
ESCOM

J-Bio NMR 067

Relationship between ^{1}H and ^{13}C NMR chemical shifts and the secondary and tertiary structure of a zinc finger peptide

Min S. Lee, Arthur G. Palmer III and Peter E. Wright*

Department of Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.

Received 19 February 1992
Accepted 10 April 1992

Keywords: Zinc finger; 2D NMR; Secondary structure

SUMMARY

Essentially complete assignments have been obtained for the ^{1}H and protonated ^{13}C NMR spectra of the zinc finger peptide Xfin-31 in the presence and absence of zinc. The patterns observed for the ^{1}H and ^{13}C chemical shifts of the peptide in the presence of zinc, relative to the shifts in the absence of zinc, reflect the zinc-mediated folding of the unstructured peptide into a compact globular structure with distinct elements of secondary structure. Chemical shifts calculated from the 3D solution structure of the peptide in the presence of zinc and the observed shifts agree to within ca. 0.2 and 0.6 ppm for the backbone $C^{\alpha}H$ and NH protons, respectively. In addition, homologous zinc finger proteins exhibit similar correlations between their ^{1}H chemical shifts and secondary structure.

INTRODUCTION

The zinc finger represents an important nucleic acid-binding motif in proteins that recognize specific sequences of DNA and RNA. A number of classes of zinc-binding proteins that differ in the nature of the zinc ligands has been identified. The roles of zinc and zinc finger domains in the structure and function of transcriptional regulatory proteins have been reviewed (Klug and Rhodes, 1987; Evans, 1988; Kaptein, 1991). In the TFIIIA class of zinc finger domains, zinc is coordinated by two Cys and two His ligands (Cys_2/His_2) (Miller et al., 1985). Studies by circular dichroism and NMR spectroscopy have been reported for zinc-complexed zinc finger peptides of this type derived from TFIIIA (Frankel et al., 1987; Lee et al., 1989b), ADR1 (Parraga et al., 1988,1990; Klevit et al., 1990; Xu et al., 1991), Xfin (Lee et al., 1989a,b,1990,1991; Palmer et al., 1991), SWI5 (Neuhaus et al., 1990), ZFY (Weiss and Keutmann, 1990; Weiss et al., 1990;

* To whom correspondence should be addressed.

0925-2738/$ 5.00

Kochoyan et al., 1991a,b), EBP-1 (Omichinski et al., 1990; Clore et al., 1991), and a synthetic consensus peptide (Krizek et al., 1991). An investigation by NMR of mKr2 in the absence of zinc has also been reported (Carr et al., 1990). A number of studies have shown that zinc is essential for folding of zinc fingers and for binding to DNA (Frankel et al., 1987; Parraga et al., 1988; Lee et al., 1989a,1991).

Protein structure determination by NMR spectroscopy is based largely on the interpretation of nuclear Overhauser enhancement and scalar coupling constants in terms of distance and dihedral angle constraints (Havel and Wüthrich, 1984; Wüthrich, 1986). While chemical shifts are extremely sensitive to protein conformation, interpretation of chemical shifts in terms of specific structural parameters is not straightforward (Osapay and Case, 1991). In the present work, assignments are reported for ^{1}H and the protonated ^{13}C resonances of Xfin-31 in the presence and absence of zinc and an analysis is presented of the ^{1}H and ^{13}C NMR secondary chemical shifts due to structural changes of the peptide upon binding of zinc. The zinc finger is a unique system in that complete ^{1}H and ^{13}C resonance assignments in both the folded and unfolded states can be obtained and thereby the effects of secondary and tertiary structure on chemical shift can be measured directly. The influence of both secondary and tertiary structure on the ^{1}H chemical shifts of homologous zinc finger proteins also is examined to seek further insights into the relationship between chemical shift and protein structure.

MATERIALS AND METHODS

The Xfin-31 (Ac-YKCGLCERSFVEKSALSRHQRVHKN-NH_2) peptide, a 25-residue synthetic zinc finger peptide of the TFIIIA class from the consensus sequence of the *Xenopus laevis* protein Xfin (Altaba et al., 1987) was synthesized and purified as previously described (Lee et al., 1991). The zinc complex of Xfin-31 was prepared as a 6.6 mM solution at pH 5.5 in 90% H_2O/10% D_2O and at pH 5.5 and 6.1 in D_2O. The zinc-free Xfin-31 was prepared as a 4 mM solution in 90% H_2O/10% D_2O and 100% D_2O at pH 5.5. The pH was measured with a glass electrode and was not corrected for isotope effects. All the solvents used for the sample preparation were purged with argon gas and all manipulations were carried out under argon to prevent oxidation of the two cysteines while preparing the samples for the NMR experiments.

The ^{1}H NMR spectra were recorded at 278 K at pH 5.5 with the transmitter set to the frequency of the $^{1}H_2O$ resonance. Two-dimensional NMR spectra were recorded in the phase-sensitive mode with quadrature detection in the ω_1 dimension using time-proportional phase incrementation (TPPI) (Bodenhausen et al., 1980; Marion and Wüthrich, 1983). Double-quantum-filtered COSY (2QF-COSY) spectra were acquired using the standard pulse sequence and phase cycling (Rance et al., 1983). Spectra were acquired with 64 scans per t_1 value and 512 t_1 values. Spectra were Fourier transformed using a Lorentzian-to-Gaussian weighting function in the ω_2 dimension and a shifted sine bell weighting function for the ω_1 dimension.

Natural abundance ^{13}C heteronuclear NMR spectra of the zinc-complexed and zinc-free Xfin-31 peptide in D_2O solution were measured at 303 K and pH 6.1 and 5.5, respectively. Single-bond heteronuclear correlation (HMQC) spectra were obtained using the standard pulse sequence and phase cycling (Muller, 1979; Bax et al., 1983; Cavanagh and Keeler, 1988). Decoupling of ^{13}C during acquisition was performed using WALTZ-16 (Shaka et al., 1983). Separate spectra were acquired of the aliphatic and aromatic ^{13}C resonances to minimize the decoupling power required.

TABLE 1
^{1}H CHEMICAL SHIFTS FOR Xfin-31 AT 278 K AND pH 5.5

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	$C^{\varepsilon}H$
Zinc-free Xfin-31						
Tyr[1]	8.34	4.39	2.91,2.86		7.04	6.75
Lys[2]	8.28	4.19	1.68	1.24	1.56	2.87
Cys[3]	8.35	4.32	2.86			
Gly[4]	8.61	3.90				
Leu[5]	8.22	4.27	1.59,1.53	1.55	0.86,0.80	
Cys[6]	8.49	4.39	2.85			
Glu[7]	8.54	4.19	1.99,1.89	2.21		
Arg[8]	8.45	4.23	1.75,1.70	1.58,1.52	3.08	
Ser[9]	8.39	4.34	3.82,3.76			
Phe[10]	8.36	4.49	3.05		7.16	7.27 7.22($C^{\varepsilon}H$)
Val[11]	8.04	3.86	1.91	0.87,0.84		
Glu[12]	8.41	4.11	1.98,1.91	2.27,2.22		
Lys[13]	8.51	4.14	1.77,1.73	1.42	1.60	2.89
Ser[14]	8.34	4.28	3.86,3.77			
Ala[15]	8.33	4.18	1.36			
Leu[16]	8.09	4.20	1.60,1.53	1.60	0.86,0.80	
Ser[17]	8.14	4.30	3.86,3.80			
Arg[18]	8.18	4.18	1.74,1.68	1.54,1.48	3.08	
His[19]	8.34	4.57	3.19,3.08		7.16	8.27/8.21[a]
Gln[20]	8.34	4.24	2.01,1.93	2.29		
Arg[21]	8.48	4.25	1.75,1.70	1.58,1.52	3.12	
Val[22]	8.27	3.98	1.94	0.87,0.81		
His[23]	8.69	4.59	3.12,3.05		7.16	8.27/8.21[a]
Lys[24]	8.52	4.21	1.71,1.65	1.33	1.60	2.89
Asn[25]	8.69	4.59	2.78,2.69			
Zinc-complexed Xfin-31[b]						
Tyr[1]	8.85	4.44	2.98,2.74		7.03	6.84
Lys[2]	8.65	4.64	1.76,1.61	1.47,1.27	1.67,1.49	2.91
Cys[3]	8.90	4.29	3.40,2.76			
Gly[4]	8.90	4.18,3.83				
Leu[5]	9.25	4.40	0.83,0.68	1.37	0.69,0.65	
Cys[6]	7.71	4.89	3.32,3.21			
Glu[7]	8.60	4.26	2.19,2.01	2.28,2.13		
Arg[8]	8.42	3.96	1.30,1.23	1.77,1.48	3.08,2.89	7.14(NH)
Ser[9]	7.86	5.13	3.40,3.36			
Phe[10]	8.60	4.65	3.36,2.64		7.22	6.79 6.08($C^{\varepsilon}H$)
Val[11]	8.87	4.01	2.19	1.04,0.99		
Glu[12]	7.83	4.77	2.17,1.99	2.31		
Lys[13]	8.73	3.12	1.40,1.08	0.90,0.84	1.44,1.39	2.77
Ser[14]	8.89	4.05	3.84			
Ala[15]	6.97	4.01	1.63			
Leu[16]	7.04	3.17	1.97,1.29	1.48	1.00,0.90	
Ser[17]	8.13	4.20	3.85			

TABLE 1 (continued)
^{1}H CHEMICAL SHIFTS FOR Xfin-31 AT 278 K AND pH 5.5

Residue	NH	C$^\alpha$H	C$^\beta$H	C$^\gamma$H	C$^\delta$H	C$^\varepsilon$H
Arg^{18}	7.80	3.94	1.77,1.76	1.76,1.47	3.22,3.11	7.27(NH)
His^{19}	7.57	4.26	3.08,2.78		6.98 14.92(HN$^\delta$)	7.86
Gln^{20}	7.94	3.58	2.18	2.82,2.65		
Arg^{21}	7.18	3.98	1.81	1.67	3.16	7.23(NH)
Val^{22}	7.73	3.87	1.91	0.66,0.42		
His^{23}	7.17	4.69	3.20,3.01		6.41 14.77(HN$^\delta$)	7.91
Lys^{24}	7.81	4.23	1.84,1.74	1.37	1.62	2.91
Asn^{25}	8.50	4.63	2.80,2.72			

Shown are the ^{1}H chemical shifts of the zinc-free and zinc-complexed Xfin-31 at a temperature of 278 K and pH 5.5. The chemical shifts are referenced to H_2O at 4.96 ppm and are generally accurate to $\pm$ 0.01 ppm ($\pm$ 0.03 ppm for geminal protons separated by < 0.1 ppm).

[a] The C$^\varepsilon$H of His^{19} and His^{23} for zinc-free Xfin-31 have not been assigned sequence specifically due to overlap of the C$^\delta$H protons as well as lack of NOEs to the C$^\varepsilon$H protons.

[b] Assignments and chemical shifts from Lee et al., 1989b; note that the chemical shifts reported earlier contained several typographical errors which are corrected in the present table (underlined).

Typically, 128–256 scans were acquired for each of 100–450 t_1 values. For all spectra, 2K points were acquired in each quadrature channel during t_2. Typically, the spectral width in ω_2 was 5000 Hz and the spectral width in ω_1 was 7142 Hz for spectra of aliphatic regions and 2778 Hz for spectra of aromatic regions; the ^{1}H transmitter frequency was set to the frequency of the residual HDO resonance. The ^{13}C transmitter frequency was placed at 37.9 ppm for spectra of the aliphatic region and 115 ppm for spectra of the aromatic region. Spectra were recorded in the phase-sensitive mode with quadrature detection in the ω_1 dimension achieved using TPPI. Spectra were Fourier transformed using a Lorentzian-to-Gaussian weighting function in the ω_2 dimension and

TABLE 2
^{13}C CHEMICAL SHIFTS FOR Xfin-31 AT 303 K and pH 5.5 (ZINC-FREE) AND 6.1 (ZINC-COMPLEXED)

Residue	C$^\alpha$	C$^\beta$	C$^\gamma$	C$^\delta$	C$^\varepsilon$	C$^\zeta$
Zinc-free Xfin-31						
Tyr^1	56.3	36.8		131.3	116.3	
Lys^2	53.9	31.2	27.0	22.7	39.9	
Cys^3	56.5	25.9				
Gly^4	43.3					
Leu^5	53.5	40.3	25.1	21.5,22.9		
Cys^6	56.6	25.9				
Glu^7	55.0	28.2	34.3			
Arg^8	54.4	28.9	25.1			
Ser^9	56.7	61.6				
Phe^{10}	56.3	37.5		130.0	129.6	128.0
Val^{11}	60.8	30.9	19.1,19.1			

TABLE 2 (continued)
^{13}C CHEMICAL SHIFTS FOR Xfin-31 AT 303 K and pH 5.5 (ZINC-FREE) AND 6.1 (ZINC-COMPLEXED)

Residue	C^{α}	C^{β}	C^{γ}	C^{δ}	C^{ϵ}	C^{ζ}
Glu[12]	55.0	28.2	34.3			
Lys[13]	55.1	30.7	27.0	22.7	39.9	
Ser[14]	56.7	61.6				
Ala[15]	51.2	16.9				
Leu[16]	53.5	40.3	25.1	21.5,22.9		
Ser[17]	56.7	61.6				
Arg[18]	54.4	28.9	25.1			
His[19]	53.9	27.8		118.0	135.1/135.3[a]	
Gln[20]	54.2	27.6	31.8			
Arg[21]	54.4	28.9	25.1			
Val[22]	60.2	30.9	18.6,19.1			
His[23]	53.4	27.8		118.0	135.1 135.3[a]	
Lys[24]	53.9	31.2	27.0	22.7	39.9	
Asn[25]	51.0	37.0				
Zinc-complexed Xfin-31						
Tyr[1]	55.8	37.6		131.3	116.0	
Lys[2]	53.5	32.5	22.7	26.9	40.0	
Cys[3]	58.6	27.6				
Gly[4]	44.2					
Leu[5]	53.5	40.7	25.3	20.9,23.2		
Cys[6]	56.5	28.6				
Glu[7]	55.5	26.7	34.2			
Arg[8]	55.4	29.4	26.0	41.0		
Ser[9]	54.6	63.6				
Phe[10]	55.2	41.6		130.3	128.6	126.6
Val[11]	62.3	30.4	19.4,19.2			
Glu[12]	52.5	30.2	34.0			
Lys[13]	57.8	29.8	22.9	27.0	39.8	
Ser[14]	58.8	59.6				
Ala[15]	52.5	16.9				
Leu[16]	55.8	38.0	25.4	21.0,24.4		
Ser[17]	59.7	60.3				
Arg[18]	57.4	28.3	25.9	41.4		
His[19]	56.5	26.5		125.6	137.4	
Gln[20]	57.0	26.1	32.9			
Arg[21]	56.6	28.0	25.2	41.4		
Val[22]	61.8	29.1	17.6,18.1			
His[23]	53.2	27.0		125.6	137.8	
Lys[24]	54.7	30.8	22.7	26.9	40.0	
Asn[25]	51.1	37.0				

Shown are ^{13}C chemical shifts of the zinc-free and zinc-complexed Xfin-31 at a temperature of 303 K and pH 5.5 and 6.1, respectively. The ^{13}C chemical-shift frequency scale was referenced indirectly from the ^{1}H resonance frequency of the residual HDO (Live et al., 1984; Bax and Subramanian, 1986).

[a] The C^{ϵ} of His[19] and His[23] have not been assigned because the corresponding protons could not be assigned sequence specifically. The Cys C^{β} resonances at 25.9 ppm provide strong evidence that the two sulfhydryls are reduced in the zinc-free peptide. Minor unidentified resonances at 38.0 ppm may be attributable to an oxidized species (Jung et al., 1970).

a Kaiser function in the ω_1 dimension. The ^{13}C chemical shifts were indirectly referenced to TMS using the frequency of the residual HDO resonance (Live et al., 1984; Bax and Subramanian, 1986). Heteronuclear relay correlation spectra were acquired using 20-ms free-precession COSY (Brühweiler and Wagner, 1986a) or 40-ms TOCSY mixing periods (Lerner and Bax, 1986; Otting and Wüthrich, 1988). WALTZ-16 was used for isotropic mixing. Free-precession relay spectra were processed with a trapezoidal window function in ω_1 (Brühweiler and Wagner, 1986a). Other experimental parameters were similar to those used for HMQC spectra.

All experiments were performed on a Bruker AM500 spectrometer. All data processing was performed on CONVEX C120 and C240 computers using a modified version of FTNMR (Hare Research, Inc.).

Secondary chemical shifts, $\Delta\delta$, for alpha proton, amide proton, and alpha carbon resonances were determined as the differences between the observed shifts for zinc-complexed Xfin-31 and zinc-free Xfin-31. To quantify the influences of the four aromatic residues and the two primary elements of secondary structure in the zinc-complexed Xfin-31, conformation-dependent secondary shifts of the alpha and amide protons were calculated for 13 refined structures of the peptide using a parameterized model that includes the effects of aromatic ring currents, the anisotropy of the peptide group and side-chain electrostatic interactions (Osapay and Case, 1991). The structures were calculated as previously described (Lee et al., 1989a) with additional χ_1 angles, hydrogen bonds, β-methylene stereospecific assignments, and NOE constraints (manuscript in preparation).

RESULTS AND DISCUSSION

Sequence-specific ^1H resonance assignments of the zinc-complexed and zinc-free Xfin-31 peptide were obtained by standard methods, as previously described (Lee et al., 1989b,1991). The assignments of the ^{13}C resonances in the HMQC spectra were based on the ^1H proton assignments at 278 K and were confirmed using heteronuclear correlated relay spectra. The sequence-specific ^1H and ^{13}C resonance assignments for the zinc-complexed and zinc-free Xfin-31 are listed in Tables 1 and 2. The fingerprint regions of the DQF-COSY spectrum of the zinc-complexed and zinc-free Xfin-31 peptides are shown in Fig. 1 and the $^{13}C^{\alpha}$-$^1H^{\alpha}$ regions of the HMQC spectra of both peptides are shown in Fig. 2.

As can be seen, the dispersions of the backbone resonances in the ^1H and ^{13}C NMR spectra of Xfin-31 are substantially increased upon binding of zinc. In the zinc-complexed peptide, the chemical shifts span more than 2 ppm for the NH protons and just under 2 ppm for the $C^{\alpha}H$ protons; in the zinc-free peptide, the dispersion of both the NH and $C^{\alpha}H$ protons is under 1 ppm. The ranges of ^{13}C chemical shifts spanned by the C^{α} carbons are similar in the two peptides; however, the dispersion of particular amino acids, such as the three Ser residues, is greatly increased in the zinc-complexed peptide. Notable changes are also observed in the positions of the methyl resonances and the His and Phe aromatic-ring resonances. The large dispersion of the chemical shifts of the zinc-complexed Xfin-31 is typical of folded proteins with stable tertiary conformations that exhibit unique local environments for individual residues. In contrast, the narrow distribution of chemical shifts observed for the zinc-free peptide (Lee et al., 1991) relative to the random-coil shifts (Richarz and Wüthrich, 1978; Bundi and Wüthrich, 1979) is typical of predominantly unstructured polypeptides.

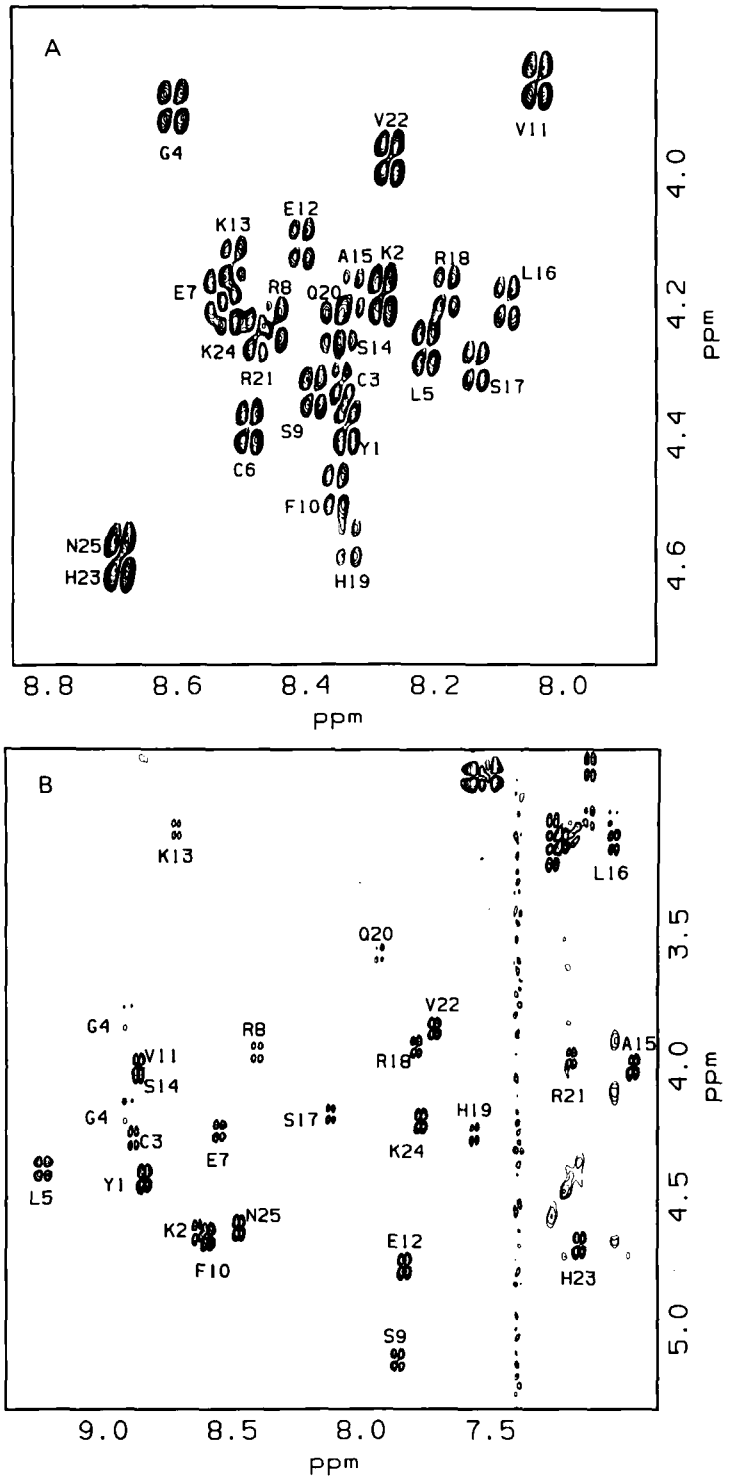

Fig. 1. Fingerprint regions of the ^{1}H NMR DQF-COSY spectra of (A) the zinc-free and (B) the zinc-complexed Xfin-31 peptide at pH 5.5 and 278 K.

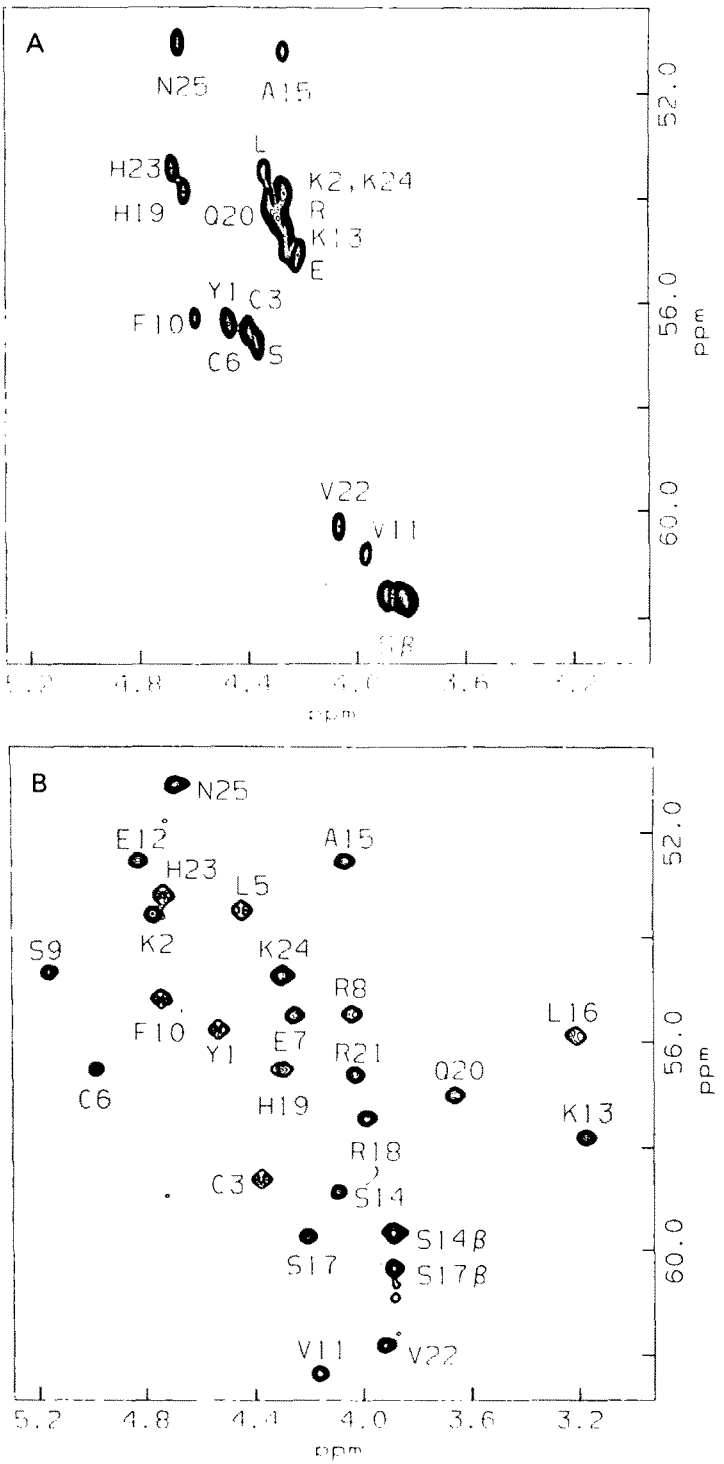

Fig. 2. C^{α} and $C^{\alpha}H$ regions of the ^{13}C-^{1}H HMQC spectra of (A) the zinc-free and (B) the zinc-complexed Xfin-31 peptide at 303 K and pH 5.5 and 6.1, respectively.

The anisotropic magnetic fields generated by aromatic residues are one cause of the chemical-shift dispersions of protons in folded proteins; in addition, previous reports have demonstrated a relationship between elements of secondary structure and the chemical shifts for main-chain protons, relative to the random coil chemical shifts, in which downfield shifts are observed for β-sheets and upfield shifts for α-helices (Veitch et al., 1988; Williams, 1989; Williamson, 1990; Wishart et al., 1991). Although results from solid-state NMR had suggested a correlation between conformation and ^{13}C chemical shifts (for a review see Saito, 1986), the effects of secondary structure on ^{13}C chemical shifts in proteins in solution have not been as extensively documented because the ^{13}C assignments of fewer proteins have been reported (Brühweiler and Wagner, 1986b; Nirmala and Wagner, 1988; Clore et al., 1990; Ikura et al., 1990; Robertson et al., 1990; Wang et al., 1990). Recent studies of the correlation between secondary structure backbone φ and ψ angles from X-ray structures and ^{13}C chemical shifts (Spera and Bax, 1991) concluded that the resonances of alpha carbons are shifted downfield in helices and upfield in β-sheet.

Analysis of secondary chemical-shift differences based on published random coil chemical shifts is subject to systematic errors arising from the dependence of the random-coil shifts on the local amino acid sequence and from differences in experimental conditions. In the present instance, these uncertainties can be avoided by analysis of the conformation-dependent chemical-shift differences, Δδ, between zinc-complexed and zinc-free Xfin-31. Graphs of both Δδ and the calculated average conformation-dependent shifts are shown in Fig. 3 for the NH and $C^{\alpha}H$ protons and Δδ for C^{α} carbons in Fig. 4, respectively. The root-mean-square (rms) deviations between the experimental and calculated secondary chemical shifts are 0.62 and 0.24 for the NH and $C^{\alpha}H$ protons, respectively. The level of agreement between calculated and observed shifts is similar to that observed previously (Osapay and Case, 1991).

In the 3D structure of the zinc-complexed Xfin-31, a β-sheet that encompasses a hairpin turn extends from residues 1-10, and a helix extends from residues 13-23. The mean values of Δδ of residues 1-10 and 13-23 are given in Table 3 for the NH protons, $C^{\alpha}H$ protons, and C^{α} carbons. The rms deviations between experimental and calculated secondary chemical shifts of residues 1-10 and 13-23 are also given in Table 3 for the NH and $C^{\alpha}H$ protons.

To examine the effects of secondary structure on chemical shifts, the observed ^{1}H Δδ values have been corrected for ring-current effects by subtracting the calculated chemical shifts attributable to aromatic groups in Xfin-31. The corrected Δδ shifts and the calculated average conformation-dependent shifts excluding ring-current effects are shown in Fig. 3. Comparison of the experimental and the calculated chemical shifts in Figs. 3A and B shows large ring-current effects that are predicted for protons in proximity to the aromatic rings in the folded structure, such as the amide and alpha protons of Lys^{13} and the alpha proton of Leu^{16}.

With the exception of NH protons, the chemical shifts of which are likely to be influenced by hydrogen-bonding both to carbonyl groups and solvent, the backbone nuclei show strong correlations between the secondary chemical shifts and the backbone dihedral angles, φ and ψ. The populated regions of the φ, ψ map for each residue calculated from 13 refined NMR structures are denoted α and β for helix and β-sheet, respectively in Fig. 3. Residues without α or β demarcations had φ and/or ψ angles with greater than 20% uncertainty and/or were located outside of the classically allowed regions of the φ, ψ map. Except for the N-terminal tyrosine, the residues denoted α all show upfield shifts of the $C^{\alpha}H$ resonance while residues with β symbol show downfield shifts corresponding to helical and β-sheet regions, respectively (Fig. 3B, hatched bars). The chemical

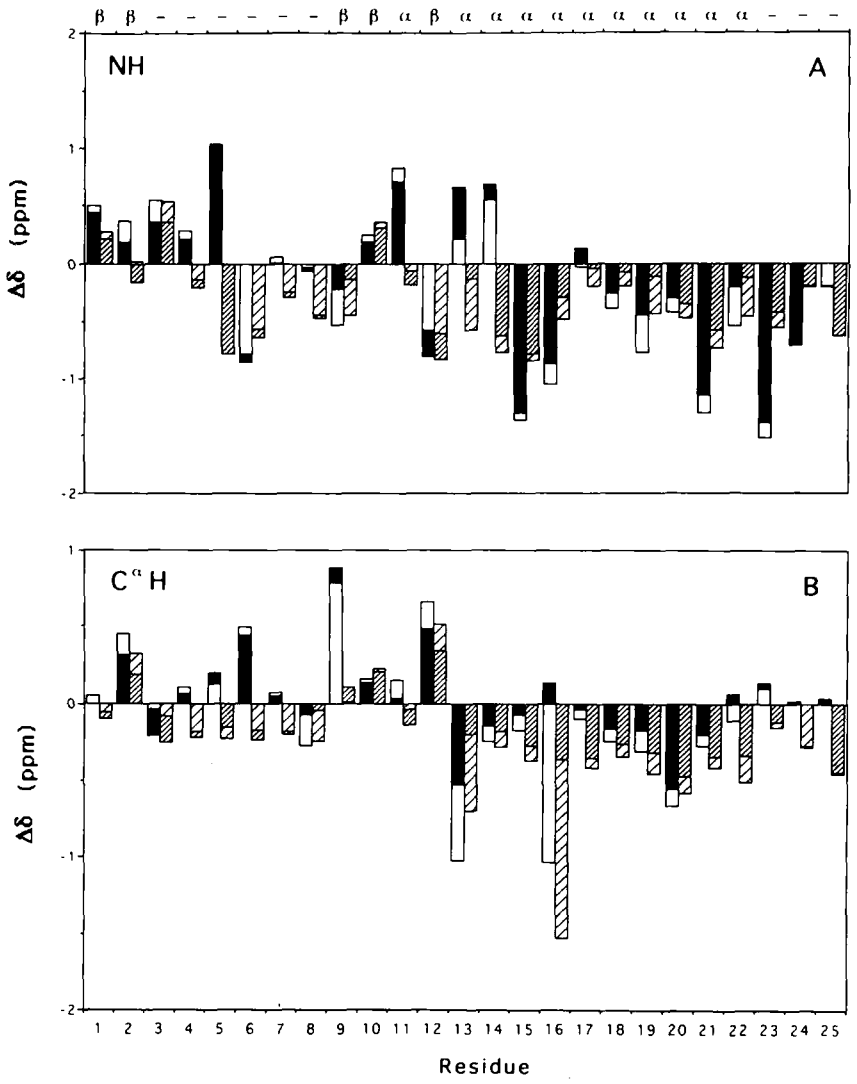

Fig. 3. Experimental chemical-shift differences Δδ (blank bars), chemical-shift differences calculated for experimental Δδ minus the calculated ring-current shifts (solid bars), total conformation-dependent chemical shifts calculated by including the effects of ring currents, peptide group anisotropy, and side-chain electrostatic interactions (see text) (single-hatched bars), and differences for the calculated contribution to chemical shift from peptide group anisotropy and side-chain electrostatic interactions but excluding ring-current effects (double-hatched bars) for (A) the NH protons and (B) the $C^{\alpha}H$ protons. Although there are differences in magnitude, similar patterns of chemical-shift differences characteristic of the elements of secondary structure in the zinc-complexed Xfin-31 are evident as for the differences relative to the random-coil peptides (Bundi and Wüthrich, 1979) and the averaged chemical shifts (Wishart et al., 1991). The calculated chemical-shift data are the means of the total secondary shifts (single-hatched bars) calculated for the 13 energy-refined structures of zinc-complexed Xfin-31. The standard deviations (not shown) range from < 5% in the helical region to > 100% in the N-terminal and loop regions for 4 backbone protons (the large uncertainty may well be due to the fact that the calculated total secondary shifts for these protons are approximately 0.05 ppm). The average standard deviation is ca. 10% excluding the 4 backbone protons. The symbols α and β represent residues with φ, ψ dihedral angles in the helical and in the β-sheet regions of the φ, ψ map, respectively. The dihedral angles are also derived from the calculated 13 energy-refined structures.

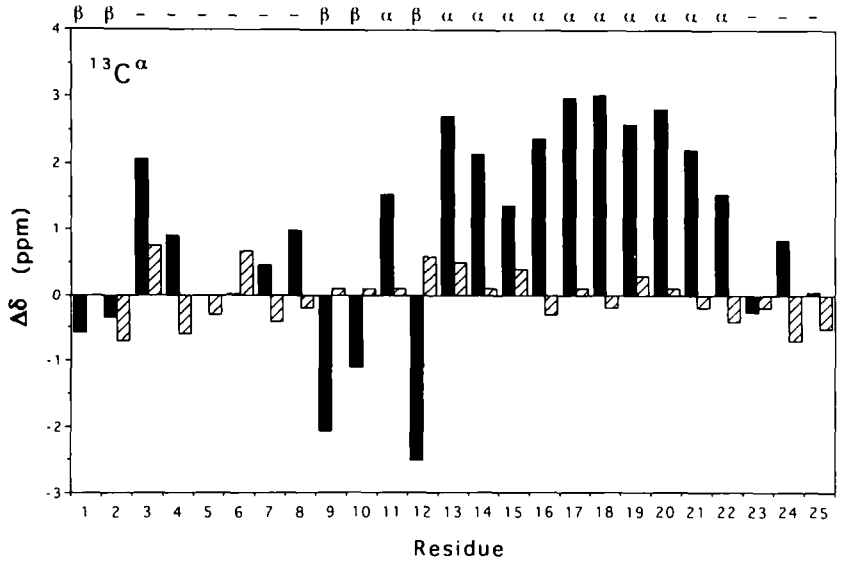

Fig. 4. Experimental chemical-shift differences Δδ for C^{α} carbons (solid bars). Chemical-shift differences Δδ of zinc-free Xfin-31 minus the random-coil values (Richarz and Wüthrich, 1978) are also shown (hatched bars); ideally, these differences should be zero for all residues but clearly this is not the case. This underscores the importance of chemical shifts due to the local amino acid sequence and the differences in the experimental conditions which should not be ignored when carrying out these types of analysis.

shifts of C^{α} carbons are somewhat more sensitive to the dihedral angles than are the proton shifts (Fig. 4). The experimental upfield and downfield shifts of C^{α} carbons (Fig. 4) mirror the α and β symbols in Fig. 3 (α's for residues 11, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22 show downfield shift; β's for residues 1, 2, 9, 10 and 12 show upfield shift) and clearly indicate the β-sheet and helical regions in Xfin-31.

In Xfin-31, the helical region extends from residues 12-24, as shown by the presence of medium-range NOEs (Lee et al., 1989a,b,1990). However, the dihedral angles place residue 12 in the β-sheet region of the φ, ψ map and accordingly, the alpha carbons and alpha protons show upfield and downfield shifts, respectively. The presence of medium-range NOEs indicative of helix starting from this residue may well be due to the presence of the turn leading from the β-sheet into the helix in this region of the peptide. Thus, in this case, the chemical shifts appear to be more sensitive to the φ and ψ dihedral angles of the residue than are the NOEs.

Although analysis of the chemical-shift dispersions of the Xfin-31 peptides demonstrates that the zinc-free peptide is less structured than the zinc-complexed peptide and is more similar to random-coil peptides, the chemical-shift evidence does not preclude the possibility that the free peptide dynamically samples an ensemble of ordered and disordered conformational states that differs from that sampled by a random-coil peptide. Small populations of partially-ordered conformers can be more sensitively detected by observation of medium-range NOE connectivities (Dyson et al., 1988; Wright et al., 1988; Wathlo et al., 1989). Observation of such NOEs for zinc-free Xfin-31 indicates the presence of minor populations of helical and turn-like conformations that are not detectable by analysis of the chemical shifts alone (Lee et al., 1991).

TABLE 3
AVERAGE SECONDARY CHEMICAL SHIFTS FOR Xfin-31

	NH	$C^{\alpha}H$	C^{α}
[Zinc-complexed] – [Zinc-free] Xfin-31			
Residues 1-10			
Δδ (ppm)	0.17 ± 0.53	0.19 ± 0.30	0.0 ± 1.2
rms (ppm)	0.58	0.28	
Residues 13-23			
Δδ (ppm)	−0.60 ± 0.68	−0.37 ± 0.37	2.1 ± 1.0
rms (ppm)	0.61	0.21	
[Zinc-complexed Xfin-31] – [random coil]			
Residues 1-10			
Δδ (ppm)	0.27 ± 0.48	0.10 ± 0.29	0.0 ± 1.3
Residues 13-23			
Δδ (ppm)	−0.63 ± 0.63	−0.53 ± 0.40	2.1 ± 1.1
[Zinc-complexed Xfin-31] – [averaged shifts]			
Residues 1-10			
Δδ (ppm)	0.27 ± 0.53	0.05 ± 0.33	−0.6 ± 1.6
Residues 13-23			
Δδ (ppm)	−0.50 ± 0.63	−0.45 ± 0.39	1.6 ± 1.1

Shown are the means and the sample deviations of the chemical-shift differences between zinc-complexed Xfin-31 and zinc-free Xfin-31, zinc-complexed Xfin-31 and random-coil shifts (Richarz and Wüthrich, 1978; Bundi and Wüthrich, 1979), and zinc-complexed Xfin-31 and averaged chemical shifts (Wishart et al., 1991), Δδ, for residues 1-10 and residues 13-23 which, respectively, encompass the β-sheet and the helix in zinc-complexed Xfin-31. Also reported are the rms differences between the observed and calculated secondary chemical shifts (Osapay and Case, 1991) for zinc-complexed Xfin-31.

The relationship between secondary and tertiary structure is exemplified further for other Cys_2/His_2 zinc fingers. The Cys_2/His_2 zinc fingers represent the largest group of structurally homologous proteins for which ^1H chemical-shift data are available. The amino acid sequences of 7 of these Cys_2/His_2 zinc fingers are shown in Table 4. The ^1H NMR chemical shifts for 7 of these fingers have been published (Xfin-31, Lee et al., 1989b; SWI-5, Neuhaus et al., 1990; EBP-1, Omichinski et al., 1990; ZFY-6T and ZFY-switch, Kochoyan et al., 1991a,b; Adr1a and T142I Adr1a mutant, Xu et al., 1991). Even under different experimental conditions, with few exceptions, the NH and $C^{\alpha}H$ chemical-shift differences between the above zinc-complexed peptide and the random coil chemical shifts (Bundi and Wüthrich, 1979) show remarkable similarities between the fingers (Fig. 5). In general, aside from the invariant ligands that bind zinc and the highly conserved hydrophobic residues, the zinc fingers display little sequence homology. The similarities in the pattern of the chemical shifts are thus not sequence but structure related.

Chemical shifts for zinc-complexed T5-xfin-31 (Xfin-31 with a 5-residue linker, TGERP, at the N-terminus) show a similar pattern to the zinc fingers described above (Fig. 5). The only notable chemical-shift differences observed between this peptide and Xfin-31 are for the ^1H resonances of Tyr^1 and the fingertip region (residues 10-13), against which the Tyr^1 side chain is packed (Lee et

TABLE 4
SEQUENCES OF Cys_2/His_2 TYPE OF ZINC FINGERS

	1	3	6													19		23			
Xfin-31		Y	K	C	..	C	E	R	S	F	V	E	K	S	A	L	S	R	H	...	H	K	N
ZFY-switch	..	Y	Q	C	..	C	E	Y	R	S	A	D	S	S	N	L	K	T	H	...	H	S	K	..
ZFY-6T	..	Y	Q	C	..	C	E	Y	R	S	A	D	S	S	N	L	K	T	H	....	H	S	K	..
Adr1a	..	Y	P	C	..	C	N	R	C	F	T	R	R	D	L	L	I	R	H	....	H	S	G	..
T142I(Adr1a)	..	Y	P	C	..	C	N	R	C	F	I	R	R	D	L	L	I	R	H	....	H	S	G	..
EBP-1	..	Y	H	C	..	C	N	F	S	F	K	T	K	G	N	L	T	K	H	.....	H	S	K	..
SWI-5	..	Y	S	C	....	C	D	K	A	F	V	R	N	H	D	L	I	R	H	...	H	N	E	..

Shown are 7 zinc finger peptides of the Cys_2/His_2 class for which ^1H NMR chemical shifts have been reported. Additional terminal and loop residues are indicated with a '.'; such residues have not been included in the analysis.

al., 1989a). The large difference in the backbone chemical shifts of Tyr^1 is a direct result of the presence and the absence of an additional peptide bond for T5-xfin-31 and Xfin-31, respectively. The absence of medium- and long-range NOEs and the values of chemical shifts indicate that the 5 linker residues are unstructured. Although these unstructured linker amino acids do not alter detectably the global fold of Xfin-31 (the NOEs that define the conformation of the peptide remain unchanged), small changes in backbone chemical shift occur for amino acids in the fingertip, more than 10 residues from the site of coupling of the linker but in close proximity within the folded structure. This emphasizes the extreme sensitivity of chemical shifts to subtle environmental effects. However, these perturbations are second-order effects that do not outweigh the primary contributions to chemical shifts from the secondary and tertiary structure of the zinc finger motif.

CONCLUSION

Essentially complete assignments of the ^1H and protonated ^{13}C NMR spectra of the single zinc finger peptide Xfin-31 in the presence and absence of zinc have been determined by 2D ^1H and natural abundance ^{13}C NMR spectroscopy. Comparison of the tertiary structure of zinc-complexed Xfin-31 (Lee et al., 1989a) with the chemical-shift differences between zinc-complexed and zinc-free Xfin-31 indicates that the location of the helix in the folded structure of the zinc-complexed Xfin-31 is strongly reflected in the secondary chemical-shifts of the backbone amide protons, alpha protons, and alpha carbons. A weaker correlation is observed between the secondary chemical shifts and the location of the β-sheet, perhaps due to perturbations introduced by the tight turn. Additional resonance shifts are observed for the alpha and the amide protons that are proximal to the aromatic residues in the folded structure. Although other potential contributions to the observed chemical-shift dispersions, such as electrostatic effects of the zinc atom, have not been evaluated, the calculated secondary chemical shifts of the protons are in good agreement with the observed secondary shifts.

The chemical-shift differences between zinc-complexed and zinc-free zinc finger peptides due to structural differences between the two species provide useful diagnostics of the conformational state of the peptides and are useful for investigations of dynamics, folding and stability in these

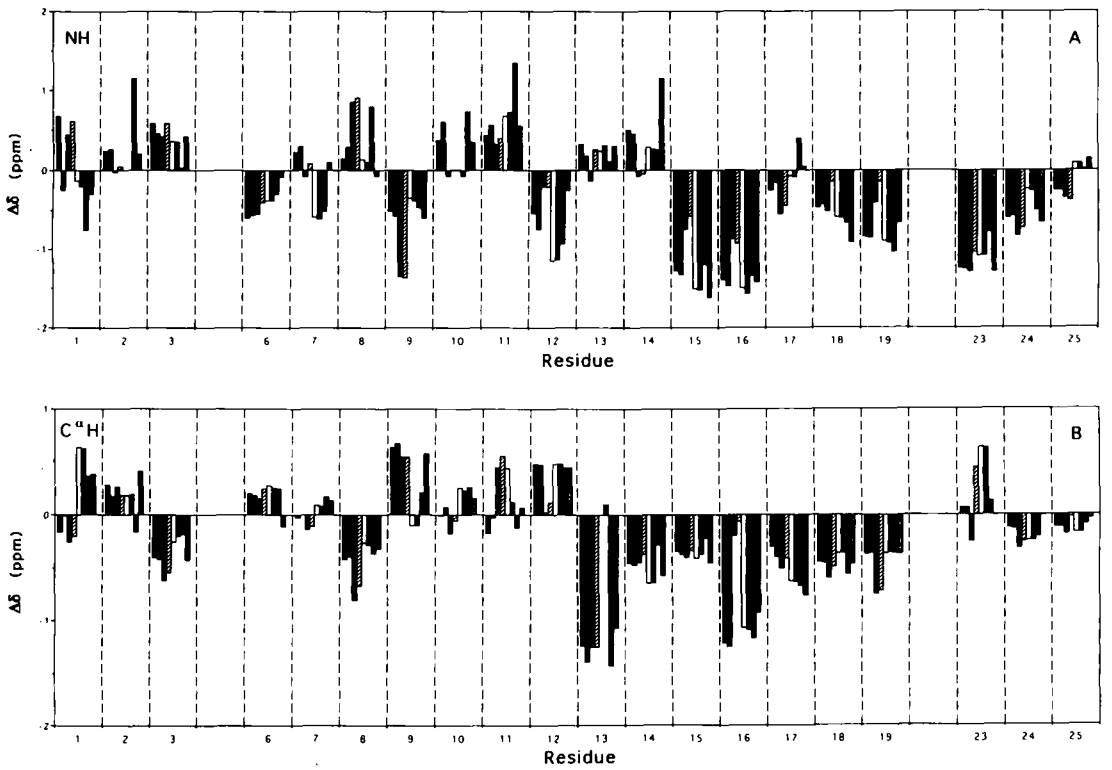

Fig. 5. ^{1}H NMR chemical-shift differences Δδ of zinc-complexed peptide minus the tabulated random-coil values of (A) the NH protons and (B) the C$^{\alpha}$H protons. The columns represent zinc finger peptides in the following order: Xfin-31, T5-xfin-31, ZFY-switch, ZFY-6T, Adr1a, T142I(Adr1a), EBP-1, and SWI-5 reported at 278 K and pH 5.5, 278 K and pH 5.5, 298 K and pH 6.0, 298 K and pH 6.5, 298 K and pH 5.45, 298 K and pH 5.45, 279 K and pH 5.8, and 283 K and pH 6.5, respectively.

peptides. In addition, the conservation of the chemical shifts for a large number of zinc finger peptides indicates a strong correlation with the common global fold for the Cys_2/His_2 type of zinc fingers.

ACKNOWLEDGEMENTS

We thank the National Institutes of Health for financial support (Grants GM-36643 and GM-38794). M. S. L. is the recipient of an American Cancer Society Postdoctoral Fellowship (Grant PF-3265). A. G. P. was supported by a National Science Foundation Postdoctoral Fellowship in Chemistry (CHE-8907510) awarded in 1989. We thank Dr. R.J. Mortishire-Smith for discussions and Drs. K. Osapay and D.A. Case for assistance in calculation of chemical shifts.

REFERENCES

Altaba, A.R., Perry-O'Keefe, H. and Melton, D.A. (1987) *EMBO J.*, **6**, 3065–3070.
Bax, A. and Subramanian, S. (1986) *J. Magn. Reson.*, **67**, 565–569.

Bax, A., Griffey, R.H. and Hawkins, B.L. (1983) *J. Magn. Reson.*, **55**, 301–315.
Bodenhausen, G., Vold, R.L. and Vold, R.R. (1980) *J. Magn. Reson.*, **37**, 93–106.
Brühweiler, D. and Wagner, G. (1986a) *J. Magn. Reson.*, **69**, 546–551.
Brühweiler, D. and Wagner, G. (1986b) *Biochemistry*, **25**, 5839–5843.
Bundi, A. and Wüthrich, K. (1979) *Biopolymers*, **18**, 285–298.
Carr, M.D., Pastore, A., Gausepohl, H., Rainer, F. and Roesch, P. (1990) *Eur. J. Biochem.*, **188**, 455–461.
Cavanagh, J. and Keeler, J. (1988) *J. Magn. Reson.*, **77**, 356–362.
Clore, G.M., Bax, A., Driscoll, P.C., Wingfield, P.T. and Gronenborn, A. (1990) *Biochemistry*, **29**, 8172–8184.
Clore, G.M., Omichinski, J.G. and Gronenborn, A.M. (1991) *J. Am. Chem. Soc.*, **113**, 4350–4351.
Dyson, H.J., Rance, M., Houghten, R.A., Wright, P.E. and Lerner, R.A. (1988) *J. Mol. Biol.*, **201**, 201–217.
Evans, R.M. (1988) *Science*, **240**, 889–895.
Frankel, A.D., Berg, J.M. and Pabo, C.O. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 4841–4845.
Havel, T.F. and Wüthrich, K. (1984) *Bull. Math. Biol.*, **46**, 673.
Ikura, M., Kay, L.E. and Bax, A. (1990) *Biochemistry*, **29**, 4659–4667.
Jung, G., Breitmaier, E., Voelter, W., Keller, T. and Tanzer, C. (1970) *Angew. Chem. Int. Ed. Engl.*, **9**, 894.
Kaptein, R. (1991) *Current Opin. Struct. Biol.*, **1**, 63–70.
Klevit, R.E., Herriott, J.R. and Horvath, S.J. (1990) *Proteins: Struct. Funct. Genet.*, **7**, 215–226.
Klug, A. and Rhodes, E. (1987) *Trends Biochem. Sci.*, **12**, 464–469.
Kochoyan, M., Havel, T.F., Nguyen, D.T., Dahl, C.E., Keutman, H.T. and Weiss, M.A. (1991a) *Biochemistry*, **30**, 3371–3386.
Kochoyan, M., Keutman, H.T. and Weiss, M.A. (1991b) *Biochemistry*, **30**, 9396–9402.
Krizek, B.A., Amann, B.T., Kilfoil, V.J., Merkle, D.L. and Berg, J.M. (1991) *J. Am. Chem. Soc.*, **113**, 4518–4523.
Lee, M.S., Gippert, G.P., Soman, K.V., Case, D.A. and Wright, P.E. (1989a) *Science*, **245**, 635–637.
Lee, M.S., Cavanagh, J. and Wright, P.E. (1989b) *FEBS Lett.*, **254**, 159–164.
Lee, M.S., Gippert, G.P., Soman, K.V., Case, D.A. and Wright, P.E. (1990) In *Structure and Methods, Vol. 2 (DNA Protein Complexes and Proteins)* (Eds, Sarma, R.H. and Sarma, M.H.) Adenine Press, Schenectady, pp. 83–91.
Lee, M.S., Gottesfeld, J. and Wright, P.E. (1991) *FEBS Lett.*, **279**, 289–294.
Lerner, L. and Bax, A. (1986) *J. Magn. Reson.*, **69**, 375–380.
Live, D.H., Davis, D.G., Agosta, W.C. and Cowburn, D. (1984) *J. Am. Chem. Soc.*, **106**, 1939–1941.
Marion, D. and Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.*, **113**; 967–974.
Miller, J., McLachlan, A.D. and Klug, A. (1985) *EMBO J.*, **4**, 1609–1614.
Muller, L. (1979) *J. Am. Chem. Soc.*, **101**, 4481–4484.
Neuhaus, D., Nakaseko, Y., Nagai, K. and Klug, A. (1990) *FEBS Lett.*, **262**, 179–184.
Nirmala, N.R. and Wagner, G. (1988) *J. Am. Chem. Soc.*, **110**, 7557–7558.
Omichinski, J.G., Clore, G.M., Appella, E., Sakaguchi, K. and Gronenborn, A.M. (1990) *Biochemistry*, **29**, 9324–9334.
Osapay, K. and Case, D.A. (1991) *J. Am. Chem. Soc.*, **113**, 9436–9444.
Otting, G. and Wüthrich, K. (1988) *J. Magn. Reson.*, **76**, 569–574.
Palmer, A.G., Rance, M. and Wright, P.E. (1991) *J. Am. Chem. Soc.*, **113**, 4371–4380.
Parraga, G., Horvath, S.J., Eisen, A., Taylor, W.E., Hood, L., Young, E.T. and Klevit, R.E. (1988) *Science*, **241**, 1489–1492.
Parraga, G., Horvath, S.J., Young, E.T. and Klevit, R.E. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 137–141.
Rance, M., Sorensen, O.W., Bodenhausen, G., Wagner, G., Ernst, R.R. and Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.*, **117**, 479–485.
Richarz, R. and Wüthrich, K. (1978) *Biopolymers*, **17**, 2133–2141.
Robertson, A.D., Rhyu, G.I., Westler, W.M. and Markley, J.L. (1990) *Biopolymers*, **29**, 461–467.
Saito, H. (1986) *Magn. Reson. Chem.*, **24**, 835–852.
Shaka, A.J., Keeler, J. and Freeman, R. (1983) *J. Magn. Reson.*, **53**, 569–574.
Spera, S. and Bax, A. (1991) *J. Am. Chem. Soc.*, **113**, 5490–5492.
Veitch, N.C., Concar, D.W., Williams, R.J.P. and Whitford, D. (1988) *FEBS Lett.*, **238**, 49–55.
Wang, J., Hinck, A.P., Loh, S.N. and Markley, J.L. (1990) *Biochemistry*, **29**, 102–113.
Wathlo, J.P., Feher, V.A., Lerner, R.A. and Wright, P.E. (1989) *FEBS Lett.*, **250**, 400–404.
Weiss, M.A. and Keutman, H.T. (1990) *Biochemistry*, **29**, 9808–9813.

Weiss, M.A., Dahl, C.E. and Keutman, H.T. (1990) *Biochemistry*, **29**, 5660–5664.
Williams, R.J.P. (1989) *Eur. J. Biochem.*, **183**, 479–497.
Williamson, M.P. (1990) *Biopolymers*, **29**, 1423–1431.
Wishart, D.S., Sykes, B.D. and Richards, F.M. (1991) *J. Mol. Biol.*, **222**, 311–333.
Wright, P.E., Dyson, H.J. and Lerner, R.A. (1988) *Biochemistry*, **27**, 7167–7175.
Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York.
Xu, R.X., Horvath, S.J. and Klevit, R.E. (1991) *Biochemistry*, **30**, 3365–3371.