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The zinc finger motif

Conservation of chemical shifts and correlation with structure

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Zinc fingers of the transcription factor IIIA (TFIIIA) type, in which zinc is co-ordinated by two cysteine and two histidine ligands (Cys_2/His_2), contain a length of helix packed against a β-hairpin. These zinc fingers comprise the widest range of structurally homologous proteins for which ^1H chemical shifts are available. A number of key resonances have chemical shifts that are highly sensitive to tertiary structure and are conserved between these peptides. The high conservation of these fingerprint chemical shifts is correlated with the common global fold of Cys_2/His_2 zinc fingers. These chemical shifts are largely independent of primary structure and should facilitate NMR assignments for future zinc finger proteins, as well as provide a diagnostic signature for the characteristic Cys_2/His_2 zinc finger fold.

Zinc finger; Secondary chemical shift; Conservation

1. INTRODUCTION

The zinc finger motif represents an important class of DNA/RNA binding proteins in eukaryotes [1–3]. A number of classes of zinc finger have been distinguished [3], the most extensively studied of which is the transcription factor IIIA (TFIIIA) type in which zinc is coordinated by two cysteine and two histidine ligands (Cys_2/His_2). The fold of these fingers comprises a length of helix packed against a β-hairpin with concomitant formation of a hydrophobic core [4–9]. ^1H NMR chemical shift data have been published for nine Cys_2/His_2 zinc fingers [5,7,8,10–13] and full assignments for a further two have been made in this laboratory (fingers 2 and 5 of the TFIIIA protein). The amino acid sequences of these zinc fingers are shown in Table I. The Cys_2/His_2-type zinc fingers comprise the widest range of structurally homologous proteins for which ^1H-chemical shift data are available. Here we identify a number of key chemical shifts which reflect the tertiary structure and are conserved between these peptides. These represent markers of successful folding and useful starting points for the sequential assignment process.

2. RESULTS AND DISCUSSION

The family of Cys_2/His_2 fingers for which NMR data are available can be subdivided on the basis of their intercysteine and interhistidine spacings ($Cx_2Cx_{12}Hx_3H$ subclass, Xfin-31; $Cx_2Cx_{12}Hx_4H$ subclass, Adr1a and ZFY-6T; $Cx_2Cx_{12}Hx_4H$ subclass, EBP-I; $Cx_4Cx_{12}Hx_3H$ subclass, SWI-5 and TFIIIA). Table II lists some of the conserved chemical shifts from differing subclasses of Cys_2/His_2-type zinc fingers. In general, except for the highly conserved hydrophobic residues at positions 1, 12, and 18 (Table I) and the invariant ligands that coordinate zinc, there is little sequence homology between the subclasses (for example, Xfin-31 and EBP-1 have only one residue in common, as do Xfin-31B and ZFY-6T). Thus, it is important to note that the high conservation of these chemical shifts is essentially independent of sequence. Consequently, these characteristic fingerprint markers are present in all the Cys_2/His_2 subclasses

Table I

Sequences of Cys_2/His_2 class of zinc fingers

	1 5 10 15 20 25	Ref.
	..|....|....|....|....|....|.....	
Xfin-31	YKC..GLCERSFVEKSALSRH..QRVHKN	[4,11,15]
Xfin-31B	YKC..GLCEFSGVEKSALSRH..QRVHKN	[13]
Xfin-31C	YKC..GLCERSLVEKSALSRH..QRVHKN	[13]
ZFY-switch	..YQC..QYCEYRSADSSNLKTH..IKTHSK..	[8]
ZFY-6T	..YQC..QYCEYRSADSSNLKTH.IKTKHSK..	[7]
Adr1a	..YPC..GLCNRCFTRRDLLIRH.AQKIHSG..	[6,10]
T142I (Adr1a)	..YPC..GLCNRCFIRRDLLIRH.AQKIHSG..	[10]
EBP-1	..YHC..SYCNFSFKTKGNLTKHMKSKAHSK..	[5]
SWI-5	..YSCDHPGCDKAFVRNHDLIRH..KKSHNE..	[12]
TFIIIA-2	..FPCKEEGCEKGFTSLHHLTRH..SLTHTG..	*
TFIIIA-5	YECPHEGCDKRFSLPSRLKRH..EKVHAG	*

*Lee, Mortishire-Smith and Wright, manuscript in preparation.

Abbreviations: TFIIIA, transcription factor IIIA; NMR, nuclear magnetic resonance; ppm, parts per million.

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and have values very much different from random coil [14].

The downfield shift of the 7-NH proton resonance is characteristic of all Cys_2/His_2 zinc fingers studied to date. In particular, for the Cx_2C subclass the 7-NH proton is the furthest downfield backbone NH proton (Table II). With the exception of Adr1a, T142I (Adr1a), and EBP-1, the $C^{\alpha}H$ proton of residue 11 is generally the furthest, or one of the furthest, downfield-shifted $C^{\alpha}H$ proton at ca. 4.95 ppm; this downfield shift is due to the residue at this position being part of the antiparallel β-sheet. A graph of $\Delta\delta$, (δ(experimental)–δ(random coil) [14]), for the 11 $C^{\alpha}H$ protons of eleven zinc finger peptides is shown in Fig. 1a. This graph clearly indicates the downfield-shifted $C^{\alpha}H$ proton for this residue at this particular position, for each of the zinc finger peptides, with the exception of Adr1a and the associated mutant T142I [10]. This residue is reported to be a part of the β-hairpin in Xfin-31 [4,11,13], ZFY [7,8], TFIIIA [Lee, Mortishire-Smith and Wright, manuscript in preparation], EBP-1 [5], and SWI-5 [12]; accordingly this proton is shifted downfield. However, this $C^{\alpha}H$ proton is shifted upfield in Adr1a and the mutant T142I.

Residue 15 lies in the loop between the hairpin and the helix; the 15 $C^{\alpha}H$ proton resonance is generally shifted upfield to ca. 3.2. ppm with the exception of T142I (Adr1a). This upfield shift is largely associated with a ring-current effect from Tyr-1, whose side-chain packs against the loop region [4,5,9]. The average conformation-dependent chemical shifts were calculated for Xfin-31 based on the three-dimensional solution structure in order to quantitate the chemical shifts associated with the ring-current effects from aromatic residues [15]. The conformation-dependent chemical shifts were calculated using a parameterized model that includes the effects of the anisotropy of the peptide group, side-chain electrostatic interactions, and aromatic ring currents [16]. The level of agreement between calculated and observed shifts is similar to that observed previously [16]. The root mean square (rms) deviation between the experimental and calculated conformation-dependent chemical shifts are 0.62 and 0.24 ppm for the NH and $C^{\alpha}H$ protons, respectively. These calculations of total conformation-dependent chemical shifts predict an upfield shift of 0.69 ppm for the 15 $C^{\alpha}H$ proton of Xfin-31; the dominant contribution to this upfield shift is from the aromatic residue, Tyr-1. This highly conserved aromatic residue is calculated to contribute approximately 0.49 ppm to the upfield shift of the 15 $C^{\alpha}H$ proton in Xfin-31; the calculated contribution to the upfield shift due to helical secondary structure is only 0.20 ppm.

The graph of $\Delta\delta$ for the 15 $C^{\alpha}H$ protons of eleven zinc

Table II

Key conserved ^1H chemical shifts for Cys_2/His_2 zinc fingers, reported at [a]278K and pH 5.5, [b]298K and pH 6.0, [c]298K and pH 6.5, [d]298K and pH 5.45 in H_2O and pH 7.45 in D_2O, [e]279K and pH 5.8, [f]283K and pH 6.5, [g]278K and pH 6.5.

	Ref.	Subclass	7NH	11$C^{\alpha}H$	15$C^{\alpha}H$	18NH	18$C^{\alpha}H$	27NH	27$C^{\alpha}H$	F12$^{\alpha}H$	H27$^{\alpha}H$
Xfin-31[a]	[15]	Cx_2C/Hx_3H	9.25	5.13	3.12	7.04	3.17	7.17	4.69	6.08	6.41
			(0.83)	(0.63)	(−1.24)	(−1.38)	(−1.21)	(−1.24)	(0.06)	(−1.26)	(−0.73)
Xfin-31B[a]	[13]	Cx_2C/Hx_3H	9.44	4.68	3.09	7.42	4.39	7.10	4.68	*	6.46
			(1.02)	(0.18)	(−1.27)	(−1.00)	(0.01)	(−1.31)	(0.05)	*	(−0.68)
Xfin-31C[a]	[13]	Cx_2C/Hx_3H	9.18	4.93	3.19	7.84	4.14	7.14	4.64	*	6.44
			(0.76)	(0.43)	(−1.17)	(−0.58)	(−0.24)	(−1.27)	(0.01)	*	(−0.70)
ZFY-switch[b]	[8]	Cx_2C/Hx_3H	9.66	4.92	3.25	7.55	4.18	7.12	4.38	*	6.55
			(1.48)	(0.54)	(−1.25)	(−0.87)	(−0.20)	(−1.29)	(−0.25)	*	(−0.59)
ZFY-6T[c]	[7]	Cx_2C/Hx_4H	9.65	4.92	3.25	7.49	4.32	7.37	5.07	*	6.59
			(1.47)	(0.54)	(−1.25)	(−0.93)	(−0.06)	(−1.04)	(0.44)	*	(−0.55)
Adr1a[d]	[10]	Cx_2C/Hx_4H	9.32	4.59	**	6.92	3.31	7.32	5.26	6.17	6.49
			(0.90)	(−0.35)		(−1.50)	(−1.07)	(−1.09)	(0.63)	(−1.17)	(−0.65)
T142I(Adr1a)[d]	[10]	Cx_2C/Hx_4H	9.32	4.59	4.48	6.85	3.30	7.33	5.25	6.14	6.47
			(0.90)	(−0.35)	(0.10)	(−1.57)	(−1.08)	(−1.08)	(0.62)	(−1.20)	(−0.67)
EBP-1[e]	[5]	Cx_2C/Hx_3H	9.83	4.71	2.93	7.08	3.21	7.63	4.76	6.66	6.49
			(1.65)	(0.21)	(−1.43)	(−1.34)	(−1.17)	(−0.78)	(0.13)	(−0.68)	(−0.65)
SWI-5[f]	[12]	Cx_4C/Hx_3H	8.98	4.93	3.68	7.00	3.46	7.17	4.74	6.53	6.40
			(0.59)	(0.58)	(−1.07)	(−1.42)	(−0.92)	(−1.29)	(−0.01)	(−0.81)	(−0.74)
TFIIIA-2[g]	+	Cx_4C/Hx_3H	9.12	4.43***	3.37	7.53	3.57	7.04	4.45	6.24	6.47
			(0.54)	(0.29)	(−1.01)	(−0.89)	(−0.80)	(−1.24)	(0.11)	(−1.10)	(−0.67)
TFIIIA-5[g]	+	Cx_4C/Hx_3H	9.07	4.94	3.55	6.99	3.32	7.12	4.62	6.41	6.31
			(0.49)	(0.56)	(−0.89)	(−1.43)	(−1.06)	(−1.37)	(0.18)	(−0.93)	(−0.83)

The values in parentheses represent chemical shift differences, $\Delta\delta$, δ(experimental)–δ(random coil) [14].
*Phe 12 is replaced in these fingers.
**The $C^{\alpha}H$ spin assignment for this residue is not reported.
***The residue at this position for TFIIIA-2 is Gly; chemical shift data for the most downfield proton are shown.
[+]Lee, Mortishire-Smith and Wright, manuscript in preparation.

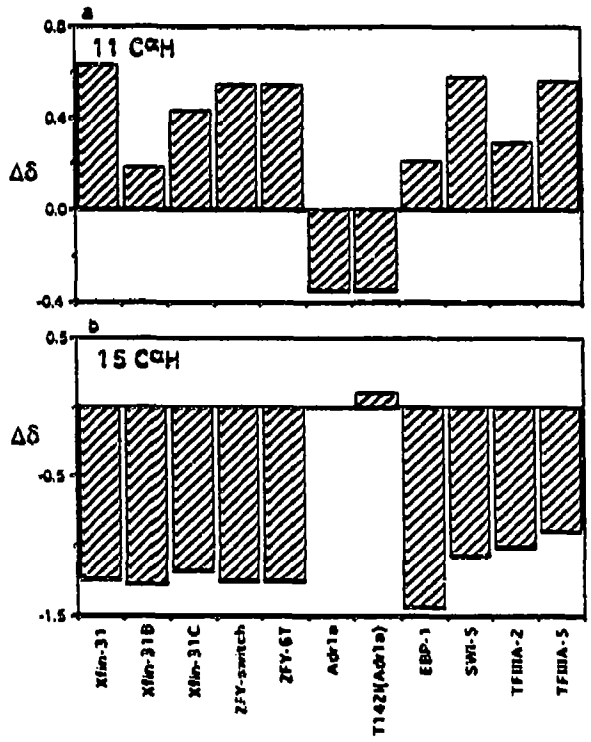

Fig. 1. Chemical shift differences, $\Delta\delta$,δ(experimental)-δ(random coil) [14] for (a) 11 $C^{\alpha}H$ protons and (b) 15 $C^{\alpha}H$ protons of eleven zinc finger peptides. The columns represent zinc finger peptides in the following order; Xfin-31, Xfin-31B, Xfin-31C, ZFY-switch, ZFY-6T, Adr1a, T142I(Adr1a), EBP-1, SWI-5, TFIIIA-2, and TFIIIA-5.

finger peptides are shown in Fig. 1b. Similar to Xfin-31, all zinc finger peptides, with the exception of the Adr1a mutant, T142I, exhibit an upfield shift for this $C^{\alpha}H$ proton (the chemical shift for this $C^{\alpha}H$ proton is not reported for Adr1a [10]). Given the close structural similarities between Adr1a [6] and the other zinc fingers [4,5,7,8] and the conservation of Tyr-1, the anomalous chemical shifts of the 11 $C^{\alpha}H$ and 15 $C^{\alpha}H$ resonances of Adr1a and associated mutant, T142I, may arise from incorrect assignments.

In those zinc fingers with Phe at position 12, the NH and $C^{\alpha}H$ protons of the highly conserved Leu at position 18 show large upfield shifts (ca. −1.1 and −1.0 ppm for the NH and $C^{\alpha}H$ protons, respectively, compared with the random coil [14] chemical shift value for Leu). These chemical shifts are reduced in Xfin-31B, ZFY-6T, and ZFY-switch (ca. −0.7 and 0 ppm for the NH and $C^{\alpha}H$ protons, respectively), which have Phe at position 10 instead of 12. Further, replacing the aromatic residue by an aliphatic residue (Xfin-31C) reduces the upfield shift of 18 NH to −0.4 ppm. Chemical shift calculations for Xfin-31 indicate upfield shifts by 0.48 and 1.52 ppm for the 18 NH and $C^{\alpha}H$ protons, respectively [15]. The upfield shifts solely due to contribution from secondary structure, excluding the ring-current effects, are 0.29 and 0.36 ppm for the NH and $C^{\alpha}H$ protons, respectively. Consequently, the aromatic residues contribute upfield shifts of ca. 0.19 and 1.16 ppm. An important distinction must be made for the Xfin-31C mutant which does not have an aromatic residue at position 12. Replacement of the conserved Phe-12 with leucine removes the ring-current contribution to the backbone chemical shifts of Leu-18, providing an excellent test of the quality of chemical shift calculation from a known three-dimensional structure. Thus, the calculated ring current contribution to δ(18 $C^{\alpha}H$) is −1.16 ppm, compared with an observed difference, δ(Xfin-31)-δ(Xfin-31C), of −0.95 ppm.

We note an interesting effect associated with the position of the conserved core aromatic residue in Cx_2C fingers. In those Cx_2C fingers with Phe at position 12, the 18 NH and $C^{\alpha}H$ protons exhibit chemical shifts of ca. 7.0 and 3.2 ppm, respectively (Xfin-31 and Adr1a). In contrast, when Phe appears at position 10, the 18 NH and $C^{\alpha}H$ protons exhibit shifts of ca. 7.5 and 4.3 ppm, respectively (Xfin-31B, ZFY-switch, and ZFY-6T). Thus, it appears that the position of the central conserved aromatic residue is reflected directly in the 18 NH and $C^{\alpha}H$ chemical shifts. We note that in EBP-1, which has Phe at both positions 10 and 12, the 18 $C^{\alpha}H$ and NH protons have shifts of 3.21 and 7.08 ppm, strongly indicating that the core hydrophobic packing in this finger involves Phe-12 rather than Phe-10. This conclusion is in agreement with the published solution structure of EBP-1 [5]. In addition, Hx_3H, Hx_4H, and Hx_5H subclasses can be distinguished on the basis of the NH and $C^{\alpha}H$ proton chemical shifts of the second His residue (His-27) that coordinates the zinc metal (Hx_3H subclass, ca. 7.1 and 4.6 ppm; Hx_4H subclass, ca. 7.3 and 5.2 ppm; Hx_5H subclass, 7.6 and 4.8 ppm, respectively). Although His-27 is one of the invariant residues which coordinate zinc, the differences in the NH and $C^{\alpha}H$ proton chemical shifts for each subclass exemplify an important distinction between the global fold and local differences in structure.

The conservation of chemical shifts extends not only to backbone protons but also to side-chain protons. The ζ proton of Phe-12 is invariably shifted upfield by its interaction with the His-21 side-chain [4,5]. The dispersion of histidine δ and ε proton resonances upon complex formation has already been noted [5–8,10–13]. However, in addition to dispersion, the His-27 aromatic protons display highly conserved chemical shifts (we list those for His-27δ in Table II). In contrast, the chemical shifts of His-21 aromatic protons are varied and are more dependent on the position of the core aromatic residue (position 10 or 12), in the same manner as the 18 NH and $C^{\alpha}H$ protons. When Val appears at position 26 (Xfin-31, Xfin-31B, Xfin-31C and TFIIIA-5), the resonance of one of the Val-26 $C^{\gamma}H_3$ groups is shifted upfield to ca. 0.4 ppm, and thus is a marker for folding of Hx_2VH fingers. These protons are 'sandwiched' between the two histidine ligands in the folded peptide and give rise to the furthest upfield resonance [4].

3. CONCLUSION

The conservation of chemical shifts described here is consistent with the common global fold of Cys_2/His_2 zinc fingers [4,5,7–9]. These extreme chemical shifts are largely independent of sequence and should facilitate NMR assignments for future zinc finger proteins. In addition, the observation that the chemical shift in this family of zinc fingers is correlated with structure rather than sequence underscores the importance of this parameter for NMR structural studies of homologous or mutant proteins.

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