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Zinc Finger Motif for Single-Stranded Nucleic Acids? Investigations by Nuclear Magnetic Resonance

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Abstract Nuclear magnetic resonance (NMR) methods have been used to address issues regarding the relevance and feasibility of zinc binding to "zinc finger-like" sequences of the type C-X_2-C-X_4-H-X_4-C [referred to as CCHC or retroviral-type (RT) zinc finger sequences]. One-dimensional (1D) NMR experiments with an 18-residue synthetic peptide containing the amino acid sequence of an HIV-1 RT-zinc finger domain (HIV1-F1) indicate that the sequences are capable of binding zinc tightly and stoichiometrically. ^{1}H-^{113}Cd spin echo difference NMR data confirm that the Cys and His amino acids are coordinated to metal in the ^{113}Cd adduct. The 3D structure of the zinc adduct [Zn(HIV1-F1)] was determined to high atomic resolution by a new NMR-based approach that utilizes 2D-NOESY back-calculations as a measure of the consistency between the structures and the experimental data. Several interesting structural features were observed, including (1) the presence of extensive internal hydrogen bonding, and (2) the similarity of the folding of the first six residues to the folding observed by X-ray crystallography for related residues in the iron domain of rubredoxin. Structural constraints associated with conservatively substituted glycines provide further rationale for the physiological relevance of the zinc adduct. Similar NMR and structural results have been obtained for the second HIV-1 RT-zinc finger peptide, Zn(HIV1-F2). NMR studies of the zinc adduct with the NCP isolated directly from HIV-1 particles provide solid evidence that zinc finger domains are formed that are conformationally similar (if not identical) to the peptide structures. The motif has been found in several other single-stranded nucleic acid binding proteins, including a human protein, and may represent a common motif analogous to the "classical" zinc finger motif widely distributed in duplex-DNA binding proteins.

Key words: CCHC, 18-residue synthetic peptide, retrovirus, 3D structure, zinc finger, NMR

In 1981, an amino acid sequence with conservatively spaced Cys and His amino acids was identified in retroviral nucleocapsid proteins (NCPs) [1]. Without exception, retroviral NCPs (and their *gag* precursor proteins) from all strains of all known retroviruses contain one or two copies of this conserved sequence [1–3]; see Figure 1 and Table I. In addition to the completely conserved Cys and His residues at positions 1, 4, 9, and 14 [the first Cys of the sequence is labeled Cys(1)], conservatively substituted glycines are found at positions 5 and 8, and aromatic or hydrophobic residues occur at positions 2 (or 3) and 10 (Fig. 1). A single copy of this sequence has also been observed in several plant viruses [4–9], and seven sequential copies of this sequence have been observed recently in a protein from humans that is apparently involved in sterol-mediated gene repression [10]. The latter protein, called a cellular nucleic acid binding protein (CNBP), binds with sequence specificity to single-stranded DNA *and* to single-stranded RNA [11].

Proteins that contain this conserved sequence appear to function physiologically by binding to single-stranded nucleic acids. In retroviruses, the array is contained in the *gag* polyprotein synthesized in infected cells. The *gag* protein functions by binding *with specificity* to retroviral RNA and anchoring the RNA to the cell wall for budding [12,13]. Extensive site-directed mutagenesis experiments involving the conserved and conservatively substituted residues have been performed [14–19]. In general, modifications of the Cys and His residues result in a significant decrease in (or loss of) RNA packaging, and modification of the conservatively substituted aromatic and hydrophobic residues al-

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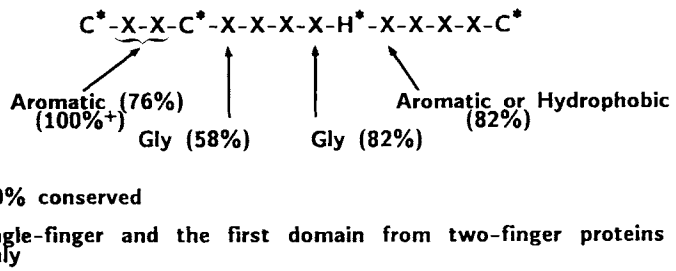

Fig. 1. CCHC amino acid sequence observed originally in retroviral NCPs. Residues that are conservatively substituted in NCPs are indicated. In the human CNBP, which contains seven copies of the CCHC sequence, the Gly residues are completely conserved.

ter the specificity of the viral RNA that is packaged. These results have led to speculation that the conserved array, as part of the *gag* polyprotein, participates directly in nucleic acid recognition and binding.

Subsequent to budding, the *gag* polyprotein is cleaved by the protease to give, among other products, the NCP which is bound non-specifically to RNA in mature particles. Further, several of the above-mentioned mutants that are capable of packaging RNA are also non-infective, suggesting that the conserved array (as part of the NCP) is involved in additional unknown functions.

WHAT IS THE ROLE OF THE CONSERVED CYS AND HIS RESIDUES?

A new field of bioinorganic chemistry emerged when, in 1985, Klug and co-workers suggested that repeated amino acid sequences of the type Cys-X_n-Cys-X_m-His-X_o-His observed in transcription factor IIIA (TFIIIA) from *Xenopus* oocytes bind zinc and form "DNA-binding fingers" [20]. Commonly referred to as "zinc fingers," these sequences have been observed in more than 100 different proteins (based largely on DNA sequence analyses), with some proteins containing 30 or more sequential zinc finger units [21,22]. In several cases, the requirement of zinc for sequence-specific DNA binding has been established, and a few synthetic zinc finger peptides have been structurally characterized by NMR methods [23,24]. In this paper, these sequences will be referred to as "classical- or CCHH-type" zinc fingers.

In 1986, Berg speculated [25] that the conserved arrays observed in retroviral nucleocapsid proteins might function physiologically by binding zinc in a manner similar to that proposed by Klug and co-workers for classical-type zinc fingers. For this reason, the motif observed originally in retroviral proteins has been called a "CCHC" or "zinc finger-like" or "retroviral-type" zinc finger motif. The mutagenesis data described above are consistent with a zinc binding function. On the other hand, early efforts to detect zinc in NCPs were unsuccessful, and it was reported that the NCP from avian myeloblastosis virus (AMV) binds zinc weakly and does not form zinc-binding fingers [19]. In addition, AMV particles apparently lack sufficient zinc to populate the NCP zinc fingers [19].

ARE THE CONSERVED SEQUENCES CAPABLE OF BINDING ZINC?

To determine if CCHC sequences are capable of binding zinc with significant affinity, metal binding and NMR experiments were carried out with a synthetic 18-residue peptide containing the amino acid sequence of the first CCHC sequence from the HIV-1 NCP (HIV1-F1) [26]. In the presence of one equivalent of Zn^{2+}, the peptide [Zn(HIV1-F1)] gives rise to sharp, well-resolved signals for the backbone amide protons whereas in the absence of zinc, broad, featureless signals are observed [26]; see Figure 2. The presence of sharp signals for the solution with zinc provides strong evidence that a stable, unique conformation is formed on zinc binding, whereas the broad signals observed in the absence of zinc are typical for a random coil-type conformation. Amide signals in spectra obtained at higher sample temperatures (50°C) and in the presence of solvent pre-saturation provide strong evidence that several amide protons are involved in hydrogen bonding [26].

To determine precisely which amino acids were coordinated to metal, the ^{113}Cd adduct [Cd(HIV1-F1)] was prepared, and a ^{1}H-^{113}Cd spin-echo-difference (SED) experiment was performed. In ^{1}H-^{113}Cd SED spectra, the only signals observed are those for protons that are scalar (i.e., through-bond) coupled to Cd. For Cd(HIV1-F1), SED signals were observed for the Cys-β and

TABLE I. CCHC Sequences From Retroviral Nucleocapsid, Plant Virus Capsid, and Human Cellular Nucleic Acid Binding Proteins*

Family type								
Retroviral family								
FeLV	Retrovirus	C	AY	C	KEKG	H	WVRD	C
R-MuLV	Retrovirus	C	AY	C	KEKG	H	WAKD	C
BaEV	Retrovirus	C	AY	C	KERG	H	WTKD	C
AKV MuLV	Retrovirus	C	AY	C	KEKG	H	WAKD	C
M-MuLV	Retrovirus	C	AY	C	KEKG	H	WAKD	C
M-MuSV	Retrovirus	C	TY	C	EEQG	H	WAKD	C
RSV	Retrovirus	C	YT	C	GSPG	H	YQAQ	C
		C	QL	C	NGMG	H	NAKQ	C
ASV	Retrovirus	C	YT	C	GSPG	H	YQAQ	C
		C	EL	C	NGMG	H	NAKQ	C
BLV	Retrovirus	C	YR	C	LKEG	H	WARD	C
		C	PI	C	KDPS	H	WKRD	C
HTLV-1	Retrovirus	C	FR	C	GKAG	H	WSRD	C
		C	PL	C	QDPT	H	WKRD	C
HTLV-2	Retrovirus	C	FR	C	GKVG	H	WSRD	C
		C	PL	C	QDPS	H	WKRD	C
HTLV-III	Retrovirus	C	FN	C	GKEG	H	TARN	C
		C	WK	C	GKEG	H	QMKD	C
HIV(LAV)	Retrovirus	C	FN	C	GKEG	H	IARN	C
		C	WK	C	GKEG	H	QMKD	C
HIV-2	Retrovirus	C	WN	C	GKEG	H	SARQ	C
		C	WK	C	GKPG	H	IMTN	C
ARV-2	Retrovirus	C	FN	C	GKEG	H	IAKN	C
		C	WR	C	GREG	H	QMKD	C
SRV-1	Retrovirus	C	FK	C	GRKG	H	FAKN	C
		C	PR	C	KRGK	H	WANE	C
SIV_{MAC}	Retrovirus	C	WN	C	GKEG	H	SARQ	C
		C	WK	C	GLMD	H	VMAK	C
SIV_{AGM}	Retrovirus	C	YN	C	GKFG	H	MQRQ	C
		C	LK	C	GKLG	H	LAKD	C
HERV	Retrovirus	C	YN	C	GQIG	H	LKKN	C
		C	PR	C	KKGK	H	WASQ	C
MPMV	Retrovirus	C	FK	C	GKKG	H	FAKN	C
		C	PR	C	KRGK	H	WANE	C
VISNA	Retrovirus	C	YN	C	GKPG	H	LARQ	C
		C	HH	C	GKRG	H	MQKD	C
EIAV	Retrovirus	C	YN	C	GKPG	H	LSSQ	C
		C	FK	C	KQPG	H	FSKQ	C
IAP	Retrovirus	C	FN	C	GRMG	H	LKKD	C
		C	YR	C	GKGY	H	RASE	C
FIV	Retrovirus	C	FN	C	KKPG	H	LARQ	C
		C	NK	C	GKPG	H	LAAK	C
Plant virus family								
COPIA	Plant virus	C	HH	C	GREG	H	IKKD	C
G	Plant virus	C	FR	C	QGFG	H	TQRY	C
F	Plant virus	C	TN	C	QEYG	H	TRSY	C
I	Plant virus	C	KK	C	LRFG	H	PTPI	C
CaMV	Plant virus	C	WI	C	NIEG	H	YANE	C
CERV	Plant virus	C	WV	C	WIEG	H	YANE	C
Others								
CNBP	Human	C	FK	C	GRSG	H	WARE	C
	Protein	C	YR	C	GESG	H	LAKD	C
		C	YN	C	GRGG	H	IAKD	C
		C	YN	C	GKPG	H	LARD	C
		C	YS	C	GEFG	H	IQKD	C
		C	YR	C	GETG	H	VAIN	C
		C	YR	C	GESG	H	LARE	C

*For references to these sequences, see refs. 21, 22, and 25.

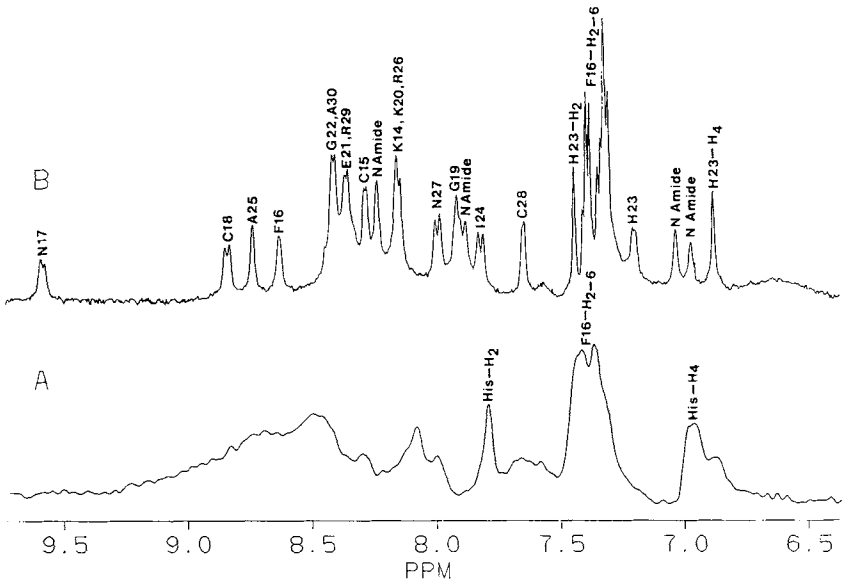

Fig. 2. NMR spectra of the aromatic and amide protons of HIV1-F1 (**A**) in the absence of Zn^{2+} and (**B**) in the presence of one equivalent of Zn^{2+}. Sharp signals obtained in the presence of zinc suggest formation of a unique solution conformation (reprinted from South et al. [26] with permission of the American Chemical Society).

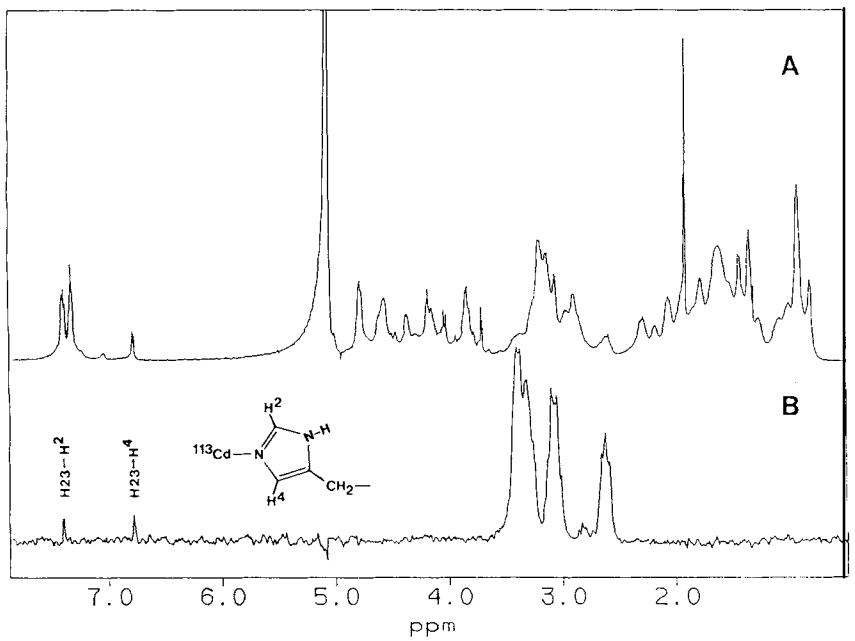

Fig. 3. One-dimensional ^1H (**A**) and ^1H-^{113}Cd spin-echo-difference (**B**) spectra obtained for ^{113}Cd(HIV1-F1). In spectrum B, only signals for protons that are scalar (though-bond) coupled to the ^{113}Cd nucleus (i.e., the His-H^2 and -H^4 and the Cys-Hβ protons) are observed, providing solid evidence that metal coordination occurs via the Cys-S and His-N^3 atoms (reprinted from South et al. [26] with permission of the American Chemical Society).

His-H^2 and -H^4 protons (Fig. 3), confirming that the metal was bound by the Cys sulfurs and the His-N^3 nitrogen [26].

We recently determined that a peptide with sequence of the second HIV-1 NCP CCHC sequence, HIV1-F2, also binds zinc, but with apparent reduced affinity [27]. On the other hand, in studies with the NCP isolated directly from HIV-1 particles, we have found that both CCHC domains bind zinc tightly and with high affinity,

and that local structures are formed that are conformationally similar (if not identical) to the structures exhibited by the individual synthetic peptides [28]; see below.

HOW TIGHTLY DO THE SEQUENCES BIND ZINC?

Green and Berg have used UV-vis spectrophotometric methods to quantitatively assess the affinity of CCHC finger peptides for Zn^{2+} and Co^{2+} [29,30]. The spectrum of the cobalt adduct with the murine leukemia virus CCHC sequence (MuLV-F) is consistent with a tetrahedral metal binding site, and charge-transfer bands at 314 and 350 nm are indicative of the presence of Co-S bonds. Titration experiments provided dissociation constants (Kd) for cobalt complexes with wild- and mutant-type sequences in the range ca. 1×10^{-5}–5×10^{-7}. Kd values for the zinc adduct, Zn(MuLV-F), and for the synthetic MuLV NCP of 10^{-10} M and 10^{-12} M, respectively, were reported, indicating that, under normal cellular conditions, these domains should be fully populated with zinc. Metal binding to the synthetic MuLV NCP has also been studied by NMR spectroscopy [31].

A ZN: CCHC PEPTIDE EXHIBITS A HIGHLY ORGANIZED STRUCTURE

High-resolution structural data have been published for the zinc adduct with HIV1-F1 [32]. Although attempts to obtain crystals of Zn(HIV1-F1) suitable for X-ray diffraction studies have thus far been unsuccessful, three-dimensional structural information for this peptide was obtained with a new NMR-based distance geometry (DG) approach that utilizes 2D nuclear Overhauser effect (NOESY) back-calculations as part of the refinement process [32]. Eight superpositioned DG structures that afforded back-calculated NOESY spectra consistent with the experimental spectra are shown in Figure 4.

Residues C(1) through K(6) fold in a manner nearly identical to the folding observed via X-ray crystallography for related residues in the iron-binding domain of rubredoxin [33]. Superposition of all backbone and Cys side chain atoms of residues C(1) through K(6) of Zn(HIV1-F1) (DG structure 1) onto the respective atoms of residues C(6) through Y(11) and residues C(39) through V(44) of rubredoxin gives RMSDs of 0.46 A and 0.35 A, respectively. The N(3) and C(4) backbone amide protons of Zn(HIV1-F1) are oriented in a manner consistent with hydrogen bonding to the C(1) sulfur in what has been described as a Type-I NH-S tight turn [33] (Fig. 5). The N-H(ϵ) side chain amide proton of N(3) appears to be hydrogen bonded to the C(14) sulfur, consistent with the low chemical exchange rate observed for this proton.

The C(4) α-carbon serves as a corner between the Type-I NH-S tight turn and a short, orthogonally directed Type-II NH-S tight turn, with hydrogen bonding between the amide of K(6) and the C(4) sulfur (Fig. 5). The carbonyl oxygen of K(6) is pointing in a direction consistent with hydrogen bonding to the amide hydrogen of C(1), and the amide hydrogen of G(5) exhibits hydrogen bonding to the C(1) carbonyl oxygen.

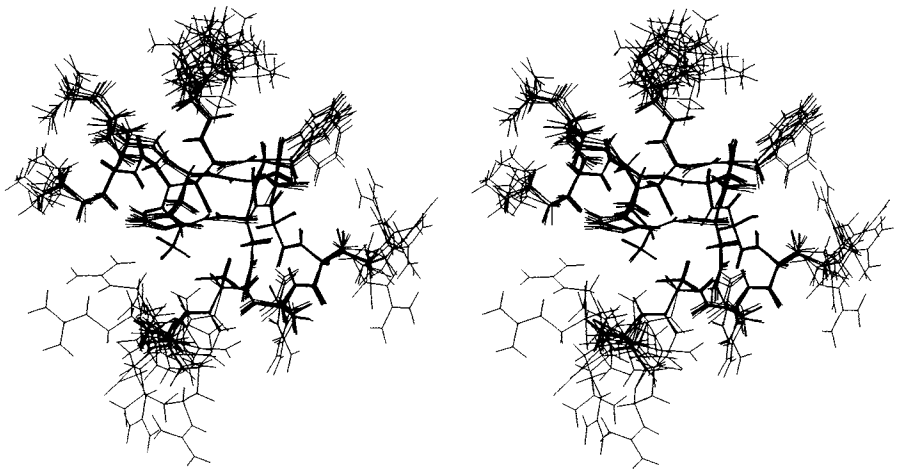

Fig. 4. Stereo view of eight superpositioned Zn(HIV1-F1) DG structures (reprinted from Summers et al. [34] with permission of the American Chemical Society).

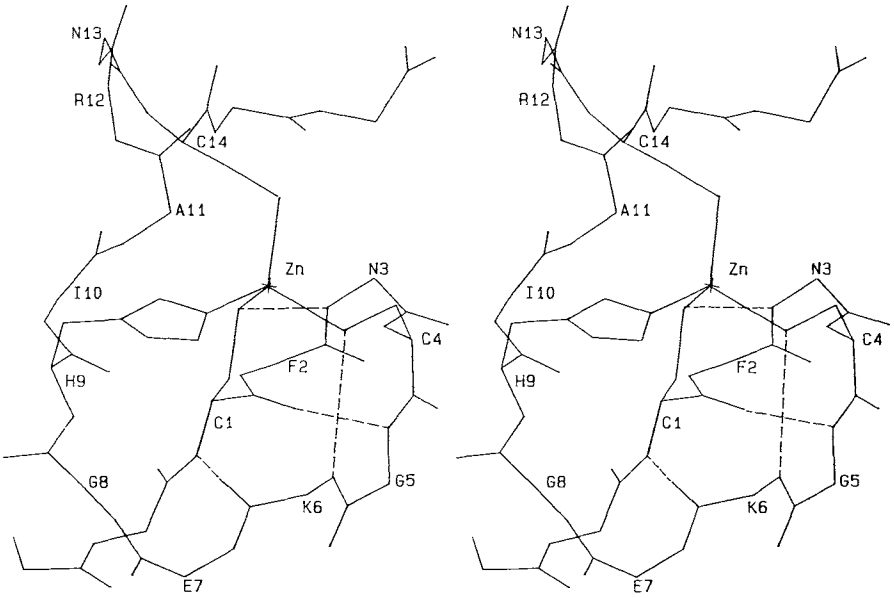

Fig. 5. Stereo view of a representative Zn(HIV1-F1) DG structure showing all backbone atoms and the side chain atoms (except protons) for residues C(1), C(4), H(9), and C(14). Dashed lines indicate hydrogen bonds (reprinted from Summers et al. [32] with permission of the American Chemical Society).

With this folding, the β-protons of K(6) are in the vicinity of the H(9) imidazole-H^2 proton, and the K(−1) α-proton is in the vicinity of the E(7) α-proton. The folding implicitly directs the hydrophobic phenyl group of F(2) away from the body of the peptide and into the solvent.

The G(8) α-carbon provides a corner for a 3_{10} turn, with the pro-R α-H in close proximity to the E(7) carbonyl oxygen. The backbone atoms including the carbonyl of G(8) through the amide of I(10) form a β-like stretch. None of the backbone atoms within this stretch appear to be involved in hydrogen bonding. The imidazole-N^3 nitrogen of H(9) is coordinated to Zn, and the hydrophobic side chain of I(10) is directed away from the body of the peptide and into the solvent. The methyl groups of I(10) are relatively close to the hydrophobic side chain of F(2).

The A(11) α-carbon serves as a corner that leads to a Type-I tight turn. This tight turn is comprised of residues A(11) through C(14) and is stabilized by apparent hydrogen bonding between the A(11) carbonyl oxygen and the C(14) amide proton, and by coordination of the C(14) sulfur to Zn. The methyl group of A(11) is located directly above the F(2) and N(3) backbone atoms, and the pseudo-plane made by the backbone atoms of the Type-I tight turn is nearly perpendicular to the pseudo-plane made by the backbone atoms of the Type-I NH-S tight turn (see Fig. 5). With this folding, the positively charged side chain of R(12) and the amide of N(13) are positioned above the hydrophobic side chains of residues F(2) and I(10).

IMPLICATIONS OF THE STRUCTURAL FINDINGS

It should be made clear that the structural studies described above were carried out in order to address issues regarding the physiological relevance of the zinc finger structures. As I will discuss further below, definitive proof for the physiological relevance of these structures is still lacking.

Two of the most striking structural features exhibited by Zn(HIV1-F1) are 1) the presence of at least seven internal hydrogen bonds within the 14-residue zinc binding domain, and 2) the similarity of the first six residues to related residues in the iron domain of rubredoxin. The extensive hydrogen bonding is consistent with the high thermal stability observed for Zn(HIV1-F1) [26]. Interestingly, Zn(HIV1-F1) and rubredoxin contain a Gly residue immediately following the C-X_2-C sequences. This Gly (position 5 in *gag* proteins) is conservatively substituted in retroviral *gag* proteins. The presence of a sterically non-demanding Gly at this position appears to stabilize Type-II NH-S folding [33]. Steric requirements associated with the 3_{10} turn

at position 8 may also explain the conservative substitution of Gly at this position in retroviral *gag* proteins. Thus, the structural constraints associated with the zinc-binding motif provide a rationale for the conservatively substituted Gly residues at positions 5 and 8, providing further evidence for the biological significance of a zinc adduct.

The backbone folding exhibited by Zn(HIV1-F1) implicitly positions the side chains of the conservatively substituted F(2), I(10), R(12), and N(13) residues in the same general spatial location. All of these side chains are directed away from the body of the peptide and into the solvent. As a consequence, the aromatic ring of F(2) and the methyl groups of I(10) form a hydrophobic surface patch. Adjacent to the hydrophobic patch is the positively charged, basic side chain of R(12) and the amide side chain of N(13). The orientation of these conservatively substituted side chains and preliminary nucleic acid binding experiments [34] are consistent with the proposal that these residues participate in gene recognition by binding to specific RNA structures [14,15]. Interestingly, residues within the C-X_2-C knuckles of steroid hormone receptor proteins dictate nucleic acid sequence specificity [35–37], and a model for the nucleic acid binding site of the glucocorticoid receptor protein includes contacts between residues in the zinc finger knuckle and specific base pairs [38].

STATUS OF THE FIELD

There is now overwhelming evidence from studies of synthetic peptides and proteins that the conserved CCHC sequences are capable of binding zinc stoichiometrically and with high affinity. It is also clear that the NCP isolated from HIV-1 particles is capable of binding zinc stoichiometrically and with high affinity, and that the structures formed on zinc binding are similar (or identical) to the structures of the synthetic peptides. The zinc finger peptides are capable of binding to nucleic acids via interactions involving the conservatively substituted hydrophobic residues implicated by mutagenesis experiments in genomic RNA recognition.

Despite the overwhelming evidence in favor of a physiologically relevant zinc adduct, one nagging question remains: Why has zinc not been detected in large quantities in retrovirus particles? Jentoft and co-workers were able to detect only very low quantities of zinc in AMV particles. In fact, zinc was detected at levels just sufficient to populate the two anticipated zinc sites of reverse transcriptase [19]. Note that there are typically ca. 100 ± 80 reverse transcriptase molecules per particle, compared to ca. 3,500 NEPs per particle. In preliminary experiments, Henderson and co-workers have determined that zinc co-elutes with HIV-1 particles [28], but quantitation of the zinc content has been difficult to achieve [39]. Interestingly, only about one in 1,000 retroviral particles generated from current cell lines is infectious [40], and the infectivity of harvested particles varies with time [41].

Unlike for classical-type zinc finger proteins which bind with specificity to duplex-DNA targets, detailed understanding of the nucleic acid targets for retroviral NCPs, including nucleic acid sequences and secondary (or tertiary) structure, is lacking. At the *gag* level in infected cells, CCHC sequences are apparently involved in genomic sequence recognition, and could involve interactions with linear or even knot-type RNA structures. At the NCP level in mature particles, CCHC sequences are involved in nonspecific interactions with RNA. In mature particles, proteins could interact with linear A-type RNA structures as observed by X-ray crystallography for bean-pod mottle virus [42]; of course, interactions with other RNA structures are also possible. Unfortunately, there is no known in vitro assay that can be used to test the physiological relevance of NCP-zinc adducts.

On the other hand, the human CNBP has been shown to bind with sequence specificity to single-stranded DNA, and has more recently been found to bind to single-stranded RNA [11]. Apparently, the specificity of CNBP for single-stranded DNA is not influenced by addition of modest amounts of EDTA. In this regard, we have found that the synthetic peptide, HIV1-F1, has a ca. 10-fold greater affinity for Zn^{2+} compared to EDTA, and it is not surprising to us that EDTA has little effect on CNBP-nucleic acid interactions. Since nucleic acid targets for CNBP are known, this protein may provide the best system for in vitro determination of the influence of zinc on CCHC protein-nucleic acid interactions.

REFERENCES

1. Henderson LE, Copeland TD, Sowder RC, Smythers GW, Oroszlan S: J Biol Chem 256:8400, 1981.
2. Copeland TD, Morgan MA, Oroszlan S: Virology 133: 137, 1984.

3. Karpel RL, Henderson LE, Oroszlan S: J Biol Chem 262:4961, 1987.
4. Mount SM, Rubin GM: Mol Cell Biol 5:1630, 1985.
5. DiNocera PP: Nucleic Acids Res 16:4041, 1988.
6. DiNocera PP, Casari G: Proc Natl Acad Sci USA 84: 5843, 1987.
7. Fawcett DG, Lister CK, Kellett E, Finnegan DJ: Cell 47:1007, 1986.
8. Franck A, Guilley H, Jonard G, Richards K, Hirth L: Cell 21:285, 1980.
9. Hull R, Sadler J, Lonfstaff M: EMBO J 5:3083, 1986.
10. Rajavashisth TB, Taylor AK, Andalibi A, Svenson KL, Lusis AJ: Science 245:640, 1989.
11. Lusis AJ: personal communication.
12. Dickson C, Eisenman R, Fan J, Hunter E, Teich N: In Weiss R, Teich N, Varmus H, Coffin J (eds): "RNA Tumor Viruses," 2nd ed., Vols 1 and 2. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1985.
13. Bolognesi DP, Montelaro RC, Frank H, Schafer W: Science 199:183, 1978.
14. Gorelick RJ, Henderson LE, Hanser JP, Rein A: Proc Natl Acad Sci USA 85:8420, 1988.
15. Meric C, Goff SP: J Virol 63:1558, 1989.
16. Gorelick RJ, Nigida SM, Bess JW, Arthur LO, Henderson LE, Rein A: J Virol 64:3207, 1990.
17. Aldovini A, Young RA: J Virol 64:1920–1926, 1990.
18. Dupraz P, Oertle S, Meric C, Damay P, Spahr P-F: J Virol 64:4978–4987, 1990.
19. Jentoft JE, Smith LM, Fu X, Johnson M, Leis J: Proc Natl Acad Sci USA 85:7094, 1988.
20. Miller J, McLachlan AD, Klug A: EMBO J 4:1609, 1985.
21. Berg JM: J Biol Chem 265:6513–6516, 1990.
22. South TL, Summers MF: Adv Inorg Biochem 8:199–248, 1990.
23. Parraga G, Horvath SJ, Eisen A, Taylor WE, Hood L, Young ET, Klevit RE: Science 241:1489, 1988.
24. Lee MS, Gippert GP, Soman KV, Case DA, Wright PE: Science 245:635, 1989.
25. Berg JM: Science 232:485–487, 1986.
26. South TL, Kim B, Summers MF: J Am Chem Soc 111:395–396, 1989.
27. South TL, Summers MF: unpublished results.
28. South TL, Blake PR, Sowder RC-III, Arthur LO, Henderson LE, Summers MF: Biochemistry 29:7786–7789, 1990.
29. Green LM, Berg JM: Proc Natl Acad Sci USA 86:4047–4051, 1989.
30. Green LM, Berg JM: Proc Natl Acad Sci USA 87:6403–6407, 1990.
31. Roberts WJ, Pan T, Elliott JI, Coleman JE, Williams KR: Biochemistry 28:10043–10047, 1989.
32. Summers MF, South TL, Kim B, Hare DR: Biochemistry 29:329–340, 1990.
33. Adman E, Watenpaugh KD, Jensen LH: Proc Natl Acad Sci USA 72:4854, 1975.
34. Summers MF, South TL, Kim B, Hare DR: Biochem Pharmacol 40:123–129, 1990.
35. Mader S, Kumar V, deVerneuil H, Chambon P: Nature 338:271, 1989.
36. Umesuno K, Evans RM: Cell 57:1139, 1989.
37. Danielsen M, Hinck L, Ringold GM: Cell 57:1131, 1989.
38. Hard T, Kellenbach E, Boelens R, Maler BA, Dahlman K, Freedman LP, Carlstedt-Duke J, Yamamoto KR, Gustafsson J-A, Kaptein R: Science 249:157–160, 1990.
39. Henderson LE: personal communication.
40. Rein A, Gerwin BI, Bassin RH, Schwarm L, Schidlovsky G: J Virol 25:146–156, 1978.
41. Smith RE: Virology 60:543–547, 1974.
42. Chen Z, Stauffacher C, Li Y, Schmidt T, Bomu W, Kamer G, Shanks M, Lomonossoff G, Johnson JE: Science 245:154–159, 1989.