p10 Single-Stranded Nucleic Acid Binding Protein from Murine Leukemia Virus Binds Metal Ions via the Peptide Sequence Cys^{26}-X_2-Cys^{29}-X_4-His^{34}-X_4-Cys^{39}†

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ABSTRACT: The RNA binding protein of 56 residues encoded by the extreme 3′ region of the gag gene of Rauscher murine leukemia virus (MuLV) has been chemically synthesized by a solid-phase synthesis approach. Since the peptide contains a Cys^{26}-X_2-Cys^{29}-X_4-His^{34}-X_2-Cys^{39} sequence that is shared by all retroviral gag polyproteins which has been proposed to be a metal binding region, it was of considerable interest to examine the metal binding properties of the complete p10 protein. As postulated, p10 binds the metal ions Cd(II), Co(II), and Zn(II). The Co(II) protein shows a set of d–d absorption bands typical of a tetrahedral Co(II) complex at 695 (ϵ = 565 M^{-1} cm^{-1}), 642 (ϵ = 655 M^{-1} cm^{-1}), and 615 nm (ϵ = 510 M^{-1} cm^{-1}) and two intense bands at 349 (ϵ = 2460 M^{-1} cm^{-1}) and 314 nm (ϵ = 4240 M^{-1} cm^{-1}) typical of Co(II) → $-S^-$ charge transfer. The ultraviolet absorption spectrum also indicates Cd(II) binding by the appearance of a Cd(II) → $-S^-$ charge-transfer band at 255 nm. The ^{113}Cd NMR spectrum of ^{113}Cd(II)–p10 reveals one signal at δ = 648 ppm. This chemical shift correlates well with that predicted for ligation of ^{113}Cd(II) to three $-S^-$ from the three Cys residues of p10. The chemical shift of ^{113}Cd(II)–p10 changes by only 4 ppm upon binding of $d(pA)_6$, indicating that the chelate complex is little changed by oligonucleotide binding. On the basis of the narrow line width of the p10 proton NMR spectrum, Cd(II)–p10 does not aggregate in solution even at concentrations of ~1 mM. Circular dichroism studies reveal that the apo-p10 protein has a secondary structure that contains only ~20% β-sheet; the rest assumes a conformation whose peptide bond ellipticity is equivalent to that of a "random coil". Zn(II) binding does not appear to induce significant additional secondary structure. Fluorescence titrations carried out with poly(rϵA) show that the affinity of p10 for this RNA, $K_a > 10^8$ M^{-1}, is not significantly altered by the presence of Cd(II), Co(II), or Zn(II). Although p10 clearly binds metal ions in vitro, metal ion coordination does not appear to result in any major change in p10 structure or in its nonspecific RNA binding properties. This finding may not, however, rule out a function of the metal ion in viral maturation.

The fourth gag gene product, p10 (nucleocapsid protein, NC), from Rauscher murine leukemia virus (MuLV) is a basic single-stranded nucleic acid binding protein encoded by the extreme 3′ region of the gag gene of MuLV type C. It contains the -Cys-X_2-Cys-X_4-His-X_4-Cys- sequence shared by all retroviral gag polyproteins. This region has been postulated to be a possible metal binding domain (Berg, 1986; Giedroc et al., 1986). The g32P protein of bacteriophage T4 contains a similar domain and has been shown to bind Zn(II) in a tetrahedral coordination (Giedroc et al., 1986). In the case of g32P, Zn(II) appears to organize a subdomain within the core of the protein that is essential along with the amino-terminal domain in allowing cooperative binding of g32P to oligonucleotides such as $d(pT)_{16}$ that are sufficiently long to permit protein–protein interactions along the DNA lattice. In addition, limited proteolysis, circular dichroism, and differential scanning microcalorimetry all indicate that Zn(II) makes a substantial contribution to maintaining the overall structure of g32P (Giedroc et al., 1986, 1987; Keating et al., 1988).

Optical spectroscopy has been applied to the Co(II) complex of an 18 amino acid fragment corresponding to the proposed metal binding sequence of p10 (Green & Berg, 1989). ^{113}Cd NMR has also been applied to a ^{113}Cd(II) complex of an 18 amino acid fragment containing the metal binding sequence found in the gag polyprotein of HIV-I (South et al., 1989). Both studies reveal that these fragments can form a tetrahedral metal complex utilizing the conserved Cys and His residues. The peptide results, however, cannot be directly used to infer biological function, since these fragments do not have properties of the native proteins such as high affinity for single-stranded nucleic acids. The complete p10 polypeptide was previously synthesized on the basis of the known primary structure of the protein by use of solid-phase synthesis techniques with blocking groups protecting the sulfhydryls (Roberts et al., 1988). Binding studies on this protected peptide revealed that it bound to RNA as well as did a native p10 isolated from HTG-2-infected cells. This result suggested that zinc binding if present was not required to achieve tight binding to RNA. In the present studies, the 56-residue polypeptide was synthesized without the blocking groups so that hydrofluoric acid cleavage of the peptide from the resin would yield three free sulfhydryl groups. The present studies have taken another step toward understanding the role of zinc in RNA binding and retroviral replication. We show that the p10 protein binds Cd(II), Co(II), and Zn(II) in a tetrahedral coordination. The metal ion does not significantly affect nonspecific binding of p10 to RNA. Possible other roles of metal ion binding in the function of the gag polyprotein are discussed.

Materials and Methods

Solid-Phase Synthesis of the p10 Peptide. Synthesis, cleavage, and purification of the 56-residue p10 peptide was performed as previously described (Roberts et al., 1988) except that during synthesis *N*-Boc-*S*-(4-methylbenzyl)-L-cysteine was used in all three cysteine positions instead of *N*-Boc-*S*-(acetamidomethyl (ACM))-L-cysteine. This strategy results in a

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peptide with free sulfhydryls following cleavage of the peptide from the resin by hydrofluoric acid. Purification was performed by preparative reverse-phase HPLC on two Vydac C_{18} columns (2.2 × 25 cm) connected in series. As with the ACM-derivatized p10, synthesis and purification proceeded extremely well with yields of 100 mg of lyophilized peptide which appear to be greater than 95% pure on the basis of reversed-phase HPLC and 97% pure on the basis of capillary electrophoresis.

Peptide Concentration. All peptide concentrations were determined by amino acid analysis carried out on a Beckman Model 7300 amino acid analyzer.

Visible Absorption Spectra. Visible absorption spectra were obtained on a Perkin-Elmer Lambda 6 UV/vis spectrophotometer that was computer controlled by employing Perkin-Elmer computerized spectroscopy software (PECSS). Aliquots of peptide were taken from freshly made 600 μM stocks in 10 mM Tris-HCl, pH 8.0, and 1 mM β-mercaptoethanol and were then diluted to 60 μM (1 mL) in 10 mM Tris-HCl, pH 8.0, and 0.1 mM β-mercaptoethanol. Cd(II), Co(II), or Zn(II) was added directly to the cuvette to a final concentration of 60 μM from a 10 mM solution made from a dilution in water of a 0.1 M stock at pH 1.5.

^{113}Cd NMR. Samples of p10 for ^{113}Cd NMR were prepared by dissolving lyophilized p10 in 10 mM phosphate, pH 7.8, to a final concentration of ~0.9 mM, followed immediately by addition of an equimolar amount of $^{113}CdCl_2$. Samples were stored under nitrogen to prevent oxidation of the free sulfhydryl groups. Formation of the Cd(II) protein complex can be confirmed by the appearance of Cd(II) → $-S^-$ charge-transfer bands at 255 and 246 nm. ^{113}Cd NMR was performed on a Bruker AM-500 spectrometer (110.93 MHz for ^{113}Cd) with a 10-mm broad-band probe. The spectra were acquired at 25 °C with a 45° pulse angle and a recycle time of 2 s. The spectral width was 15.2 kHz (136 ppm). The chemical shift is plotted relative to that of 0.1 M $^{113}Cd(ClO_4)_2$, δ = 0 ppm.

Fluorescence Measurements. The approximately 3-fold enhancement of the fluorescence of poly(riboethenoadenylic acid) [poly(rϵA)] (Pharmacia-LKB Biotechnology, Inc., ϵ = 3700 M^{-1} cm^{-1}) upon binding of p10 to this polynucleotide was monitored on a Model 8000 C SLM spectrofluorometer (SLM Instruments) using an excitation wavelength of 315 nm and an emission wavelength of 400 nm as previously described (Roberts et al., 1988). Peptide solutions were made from a 600 μM stock of the peptide in 10 mM Tris-HCl, pH 8.0, and 1 mM β-mercaptoethanol. Poly(rϵA), 1.38 μM in phosphate (2.0 mL) in 10 mM Tris-HCl, pH 8.0, and 0.1 mM β-mercaptoethanol, was titrated with a 20 μM solution of p10 peptide in 10 mM Tris-HCl and 0.1 mM β-mercaptoethanol. The metal ion titrations of the p10 peptide (20 μM) were made by the addition of $CdCl_2$, $CoSO_4$, or $ZnSO_4$ from 1 mM stock solutions of the metal salts in metal-free water. All stock buffer solutions were made metal free by passage through Chelex-100 (Bio-Rad) and were checked for metal content by atomic absorption. The binding constants were obtained by visually matching the data points to theoretical curves, in which varying K_a's were chosen, that were generated from eq 15 in McGhee and von Hippel (1974, 1976).

Atomic Absorption. Cd(II), Co(II), and Zn(II) concentrations were determined by flame atomic absorption on a Instrumentation Laboratories Model 157 atomic absorption spectrometer.

RESULTS

Cobalt–p10. Co(II) can substitute for Zn(II) in a number of metalloproteins and is useful as a spectroscopic probe of Zn(II) metal binding sites [for a review, see Vallee and Galdes (1984)]. The visible absorption spectrum of the protein–Co(II) complex not only indicates binding but also provides information on site symmetry as well. The Co(II)–p10 complex is cobalt blue in color with the following absorption bands (ϵ is in the units of M^{-1} cm^{-1}): 695 (ϵ = 565), 642 (ϵ = 655), 615 (ϵ = 510), 349 (ϵ = 2460), and 314 nm (ϵ = 4240) (Figure 1). The absorption bands between 600 and 700 nm arise from the d–d transition bands of Co(II), while the absorption bands between 310 and 350 nm are from $-S^-$ → Co(II) charge transfer. Experiments performed at greater than a 1:1 molar ratio of Co(II) to p10 showed no further increase in the extinction coefficient. Addition of 1 equiv of Zn(II) to Co(II)–p10 completely abolishes the Co(II) d–d transition bands at 600–700 nm and greatly reduces the charge-transfer bands at 310–350 nm (Figure 1). The remaining absorption between 310 and 350 nm arises from complexation of free Co(II) by β-mercaptoethanol in the buffer. Since the above extinction coefficients suggest that virtually all of the p10 protein is complexed with metal ion under the conditions tested, a lower limit for the Co(II)–p10 apparent association constant would be 6.3 × 10^6 M, assuming at least 95% binding.

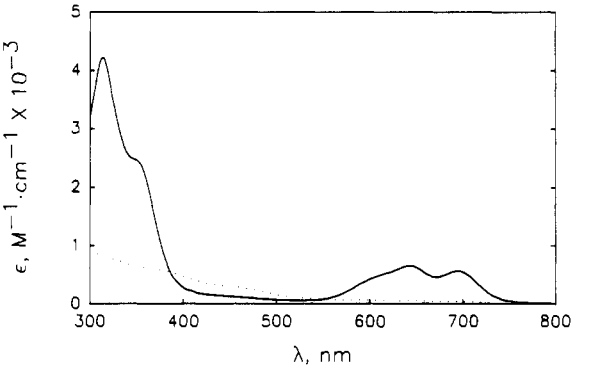

FIGURE 1: Visible absorption spectrum of Co(II)–p10. (—) Spectrum of the p10 peptide (60 μM) in the presence of (60 μM) Co(II); (···) same sample but after the addition of Zn(II) (60 μM). Buffer was 10 mM Tris-HCl, pH 8.0, and 0.1 mM β-mercaptoethanol. Both spectra were corrected for protein absorption by subtracting out the spectrum of apo-p10.

^{113}Cd NMR of ^{113}Cd(II)–p10. ^{113}Cd NMR can be used as a probe to determine the number of sulfurs involved in Cd(II) ligation in proteins [Giedroc et al., 1989; Pan & Coleman, 1989; for a review, see Armitage and Otvos (1982)]. When 1 equiv of ^{113}Cd(II) is added to apo-p10, a single resonance is observed at 648 ppm (Figure 2A). This is within the chemical shift range expected for ^{113}Cd(II) ligation to three $-S^-$. A similar ^{113}Cd(II) ligation in bacteriophage T4 g32P gives a chemical shift of 637 ppm (Giedroc et al., 1989). The signal/noise ratio and amplitude of the ^{113}Cd signal of p10 are considerably smaller than those of the signal from the similar site in g32P when compared on an equimolar basis (Figure 2C). Both proteins have S_3N donors to ^{113}Cd(II) and similar T_1 ~ 1 s. Cd(II)–p10 does not form aggregates at this concentration (0.9 mM) as judged by the line width of the signals in the ^{1}H NMR spectrum of the same sample (Figure 3A). Thus, aggregation or formation of Cd(II)-linked oligomers can be eliminated as a cause of this signal modulation. It is more likely that the loss of signal amplitude represents an exchange modulation caused by conformational flux in the p10 polypeptide. In support of this conclusion, the ^{113}Cd signal of ^{113}Cd(II)–p10 is sensitive to the Cl^- ion concentration such that the signal completely disappears in 20 mM Cl^- (data not shown). The original ^{113}Cd signal reappears on removal of the Cl^- by dialysis. This behavior is also in

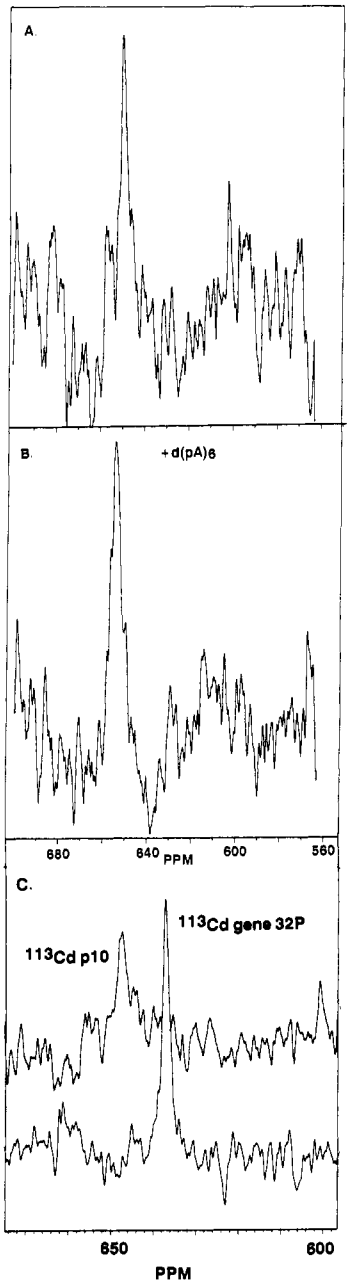

FIGURE 2: ^{113}Cd NMR of p10: (A) 0.9 mM protein in 10 mM phosphate, pH 7.8 (the number of transients was 10000 for this spectrum); (B) 0.9 mM p10–$d(pA)_6$ (1:1) complex (the number of transients was 29000). (C) ^{113}Cd NMR spectra of the ^{113}Cd(II) derivatives of p10 and g32P taken under the same conditions and concentrations (0.9 mM, pH 7.8 and 8.0 respectively).

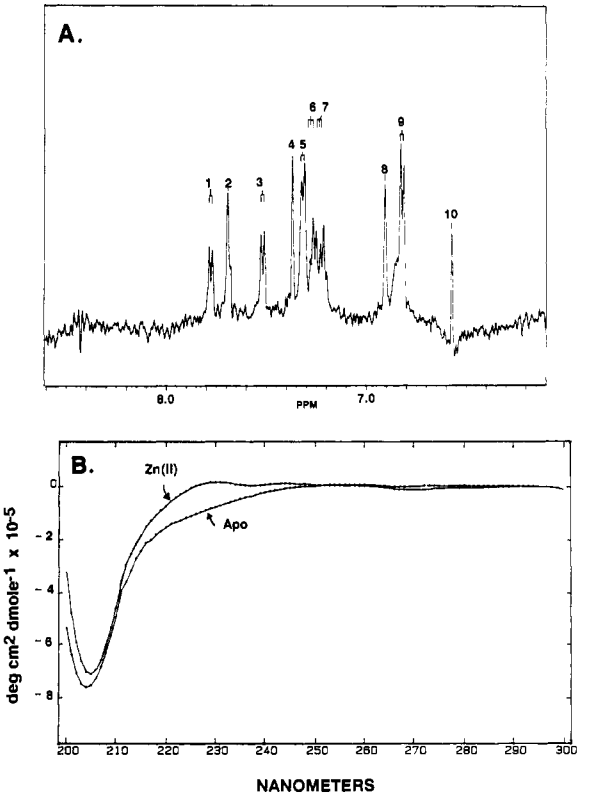

FIGURE 3: (A) Aromatic ^1H NMR spectrum of the ^{113}Cd(II)–p10 sample in D_2O. Assignments are 1, 3, 4, 6, and 7 to Trp, 5 and 9 to Tyr, 2 and 8 to His, and 10 (?) to α-proton. Singlet assignments are tentative. (B) Circular dichroism of apo-p10 and Zn(II)–p10: CD spectra of both Zn(II)–p10 (—) and apo-p10 (---) in 10 mM Tris/150 mM NaCl/1 mM β-mercaptoethanol/50 μM EDTA, pH 8.0, at ambient temperatures. Protein concentrations were 25 μM for both species.

contrast to that of the ^{113}Cd signal of ^{113}Cd(II)–g32P, which is insensitive to Cl^-. Although slightly broader than the ^{113}Cd NMR signal from ^{113}Cd(II)–g32P, the loss of signal appears to be due mainly to a fall in amplitude, not line broadening. Thus, the Cl^- modulation is probably not directly at the Cd(II) ion but involves anion binding to the protein. We have observed a ^{113}Cd NMR exchange modulation of this type previously in the case of ^{113}Cd(II) alkaline phosphatase (Gettins & Coleman, 1983) and have modeled a chemical exchange process for ^{113}Cd NMR which accounts for the loss of signal amplitude (Coleman et al., 1979). The modulation of the type shown by p10 is best explained by the interchange between at least three separate conformations, A = B = C, in which the exchange between species A and B is slow on the NMR time scale, $<10^2$ s^{-1}, and that between species B and C is intermediate on the NMR time scale, 10^2–10^4 s^{-1}. Both rates, however, are fast on the time scale of ^1H NMR, and therefore, a single highly resolved proton spectrum is observed (Figure 3A).

Addition of equimolar $d(pA_6)$ shifts the ^{113}Cd signal 4 ppm downfield (Figure 2B), comparable to the shift observed in g32P upon complexation with $d(pA)_6$ (Giedroc et al., 1989). The signal is less intense than that of free protein, indicating some additional sort of chemical exchange broadening in the ^{113}Cd(II)–p10–$d(pA_6)$ complex (see Discussion).

Circular Dichroism of p10. As shown previously, ACM-blocked p10 shows a CD spectrum indicating a high percentage of structure which gives a peptide bond ellipticity similar to that of a "random coil" (Roberts et al., 1988). Deblocked apo-p10 shows a similar CD spectrum (Figure 3B). Zn(II) binding does not induce significant additional secondary structure of the type, α or β, which would give rise to more negative ellipticity in the far-UV. The CD spectrum of Zn(II)–p10 does show more positive molar ellipticity between 220 and 240 nm. Similarly, ellipticity of g32P at 240 nm becomes more positive upon Zn(II) binding accompanied at the same time by more negative changes at 280 nm (Giedroc et al., 1989). This phenomenon can be explained by a change in the environment of the single Tyr or Trp residue of p10, both of which are located in the near vicinity of the Cys ligands. A reasonable graphical fit of the CD of Zn(II)–p10 is obtained by a combination of 0% α-helix, 20% β-sheet, and 80% random coil (Greenfield & Fasman, 1969).

Binding of the Metallo Derivatives of p10 to Poly(ethenoadenylic acid). The binding of a protein to RNA can be monitored with the fluorescent RNA poly(riboethenoadenylic acid) [poly(rϵA)]. During these experiments the fluorescence

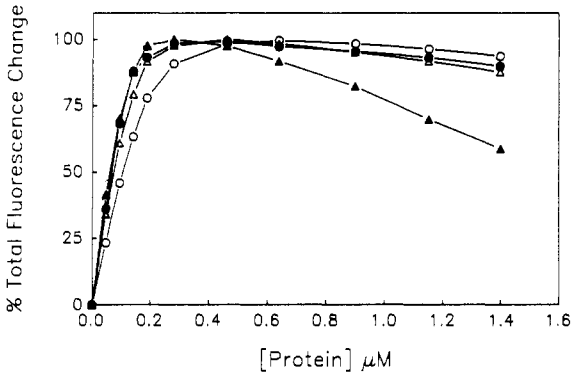

FIGURE 4: Titration of poly(riboethenoadenylic acid) [poly(rεA)] with p10 peptide in the presence or absence of Zn(II), Cd(II), or Co(II). Poly(rεA) (1.38 μM) was titrated with p10 in the presence or absence of metal ions in 10 mM Tris-HCl, pH 8.0, and 0.1 mM β-mercaptoethanol. The increase in fluorescence of the RNA was normalized to a scale of 0–100, where 100 represents the maximal fluorescence enhancement. (○) apo-p10; (●) p10 plus Zn(II); (△) p10 plus Cd(II); (▲) p10 plus Co(II). The decrease in fluorescence at high concentrations of Co(II)–p10 is due to quenching of the fluorescence by Co(II).

of the RNA increases due to an unstacking of the nucleic acid bases upon protein binding. The titrations of poly(rεA) with p10 in the presence or absence of Cd(II), Co(II), or Zn(II) indicate that under these conditions of low ionic strength the binding curves are not significantly different for all four species (Figure 4). Stoichiometric binding is achieved in each case where the phosphate/p10 ratio taken at the break point of each curve gives a site size of approximately six nucleotide residues. The K_a of each curve is greater than 10^8 M^{-1} and represents a minimum estimate determined by the fitting of theoretical curves to the data (see methods). Titrations similar to those shown in Figure 4 were carried out at a NaCl concentration of 80 mM, where binding is no longer stoichiometric. There was still no apparent effect of metal ion binding on the affinity of p10 of poly(rεA) (data not shown).

Discussion

The single-stranded nucleic acid binding protein formed by the proteolytic release of the C-terminal domain from the polyprotein product of the gag gene of murine leukemia virus, known as p10, forms a 1:1 complex with Zn(II), Co(II), or Cd(II). A comparison of the Co(II)–p10 absorption spectrum with that of the Co(II)-substituted ssDNA binding protein, g32P from phage T4, normally a Zn(II) protein, shows remarkable similarity between the two Co(II) spectra (Giedroc et al., 1986). As also observed for g32P, the ^{113}Cd NMR signal from the ^{113}Cd(II)-substituted p10 protein suggests a binding site containing three $-S^-$ ligands (Giedroc et al., 1989).

A -Cys-X_2-Cys-X_4-His-X_4-Cys- sequence occurs in the C-terminal domains of the gag gene polyproteins from all retroviruses, often as a tandem repeat. This amino acid sequence has been predicted to form a metal binding site (Berg, 1986; Giedroc et al., 1986). There are only three Cys in p10, and they occur in the sequence -Cys^{26}-X_2-Cys^{29}-X_4-His^{34}-X_4-Cys^{39}-. Since the Co(II) complex is tetrahedral, the likely candidate for a fourth ligand completing the tetrahedral site is the imidazole nitrogen of the single His of the molecule, which occurs in the midst of this Cys-containing sequence.

A peptide of 18 residues corresponding to one of the two copies of the above sequence (residues 13–30) found in the protein p7, the product of the gag gene of HIV-I, has been synthesized and binds ^{113}Cd(II) with the appearance of a ^{113}Cd NMR signal at 653 ppm, suggesting coordination to its three Cys residues (South et al., 1989). Likewise, an 18-residue peptide corresponding to the sequence found in the predicted metal binding domain of p10 has been shown to bind Co(II) with the appearance of an absorption spectrum expected from a tetrahedral sulfur containing complex of Co(II) (Green & Berg, 1989). More recent data on the 18-residue peptide from HIV-I include a 3D solution structure determined by 2D NMR methods, which shows the metal ion coordination to be to the three Cys and one His in the sequence (Summers et al., 1989).

While the peptide–metal ion complexes demonstrate that the above amino acid sequence can fold to form a reasonably stable tetrahedral metal complex utilizing the three $-S^-$ and imidazole nitrogen as ligands, the possible structural or functional role of a metal ion in the native gag proteins remains unclear. Thus far, model peptide–Zn(II) complexes synthesized to mimic Zn(II)-containing DNA binding proteins have not shown high affinity for DNA (Frankel et al., 1987). With this in mind we undertook to study the metal binding properties of the complete p10 molecule synthesized by solid-phase peptide synthesis. In earlier studies we had examined both the metal binding and oligonucleotide binding properties of p10 isolated from virus infected cells kindly supplied by Louis Henderson (J. Casas-Finet, K. Williams, and J. Coleman, unpublished data). The native protein bound tightly to single-stranded polynucleotides such as poly(dT) ($K_{app} \sim 10^7$ M^{-1} in 1 mM phosphate, pH 7.0, 0.1 mM DTT, and 20 μM EDTA) but never contained more than 0.5 mol of zinc/mol. Difficulties in obtaining sufficient quantities of purified protein prevented significant progress with the protein from virus-infected cells. The initial synthesis of p10 utilizing the *N*-Boc-*S*-(acetamidoethyl) derivative of cysteine to prevent oxidation of the sulfhydryls showed that we generated a protein with the same nonspecific RNA binding properties as the p10 isolated from virus-infected cells (Roberts et al., 1988). This result already led us to doubt the essential role of a metal ion in the binding of p10 to a nonspecific RNA. The binding of the apo, Zn(II), Co(II), and Cd(II) of derivatives p10 to poly(rεA) amply confirms the fact that metal ion binding to p10 does not significantly affect the interaction of p10 with a nonspecific RNA (Figure 4).

What then is the function of Zn(II) in p10? The affinity of p10 for Zn(II) ($K_d < 10^{-6}$ M) suggests that this site would be filled in the presence of the Zn(II) concentrations found in most mammalian cells. Zn(II) binding can be predicted to stabilize the protein. On the other hand, CD shows very little folding to be induced by Zn(II) binding (Figure 4). The CD spectrum of apo-p10 is suggestive of a high percentage of random coil like structure. In fact, p10 is very Gly (6 out of 56) and Pro (6 out of 56) rich, and thus, the protein may contain a large percentage of turns of various kinds. The ^{113}Cd NMR shows the metal ion binding site to be subject to significant exchange modulation (Figure 2). This most likely represents conformational changes in the protein that take place on a 0.1–1-ms time scale (Coleman et al., 1979). This suggests that p10 is considerably more flexible in the vicinity of the metal binding domain than is the case for the similar metal binding domain in g32P. It could be that by stabilizing a particular p10 conformation Zn(II) might modulate the interactions of p10 with other MuLV-encoded proteins that are crucial for packaging of the retroviral RNA genome (Gorelick et al., 1988). While it does not prove the function of Zn(II), it has been shown by mutations of the three Cys of the putative metal binding domain of p10, one at a time, that all three are essential for viral particle maturation. Viral

particles are produced by the mutants, but they do not specifically recognize and package the viral RNA genome. Instead, the viral particles contain host cell RNA sequences. Incorporation of a stabilizing Zn(II) ion could also affect the processing of the gag polyprotein by the retroviral protease. Although the Gorelick et al. studies demonstrate that mutagenesis of the cysteine residues in p10 has no apparent effect on the processing of the p30 protein from its p65 precursor, it is possible that incorporation of a stabilizing Zn(II) ion could affect the processing of the p10 protein. Detailed protein chemistry studies would be required to rule out this latter possibility, which is prompted by the finding that apo-g32P has many bonds sensitive to trypsin while the binding of Zn(II), Cd(II), or Co(II) renders a core domain completely resistant to proteolysis (Giedroc et al., 1987).

Although our RNA binding studies would appear to rule out a role of Zn(II) in terms of p10 binding to nonspecific RNA, it is possible that p10 and the analogous proteins from other retroviruses interact at some point in virus assembly with a specific region of the viral RNA. The metal ion could have a more pronounced effect on a specific interaction as opposed to the nonspecific RNA binding monitored here. There is evidence that freshly prepared p10 does specifically interact with its viral RNA, an interaction that can be destroyed by denaturing the RNA (Nissen-Meyer & Abrahem, 1980).

Finally, it is the p65 gag precursor and not the p10 protein that initially interacts with the viral RNA that is destined for encapsidation. It is quite possible that Zn(II) might have a more substantial effect on the binding properties of the p65 protein, which binds more tightly than p10 does to poly(rεA) (Karpel et al., 1987), than it does on p10. Similarly, it is also possible that the binding constant for Zn(II) of this Cys-containing amino acid sequence located in the gag polyprotein is considerably larger than that of the same sequence in the released p10. This might relate to the reports that suggest that Rous sarcoma virus particles or Moloney MuLV do not contain enough zinc to populate each of the nucleotide binding core proteins with a Zn(II) ion (Jentoft et al., 1988; Gorelick et al., 1988).

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