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Nucleic Acid Interactive Properties of a Peptide Corresponding to the N-Terminal Zinc Finger Domain of HIV-1 Nucleocapsid Protein†

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ABSTRACT: An 18-residue peptide (NC-F1) with an amino acid sequence corresponding to the N-terminal zinc finger of human immunodeficiency virus-1 nucleocapsid protein has been shown to bind to nucleic acids by fluorescence and NMR methods. Previously, this peptide has been shown to fold into a defined structure when bound to zinc (Summers et al., 1990). We have used a fluorescent polynucleotide, poly(ethenoadenylic acid), to monitor binding of this peptide to nucleic acids. In the presence of zinc, the peptide had a smaller site size (1.75 nucleotide residues/peptide) than in the absence of the metal ion (2.75). The salt sensitivity of the interaction indicated that two ion pairs are involved in the association of Zn^{2+}(NC-F1) with polynucleotide, whereas one ion pair is found in the metal-free peptide–nucleic acid complex. Competition experiments with single-stranded DNA (ss DNA) in either the presence or absence of Zn^{2+} showed that the peptide bound to ss DNA. Using NMR methods, we monitored the binding of a synthetic oligonucleotide, d(TTTGGTTT), to Zn(NC-F1). The hydrophobic residues F_2 and I_{10}, which are on the surface of the peptide and have been implicated in viral RNA recognition, were shown to interact with the oligomer. In accord with this observation, analysis of the salt dependence of the polynucleotide–peptide interaction indicates a nonelectrostatic component of about −6 kcal/mol, a value consistent with theoretical estimates of stacking energies of phenylalanine with nucleic acid bases.

The nucleocapsid (NC) proteins of retroviruses have been shown to bind to nucleic acids, but the full extent of their participation in the life cycle of the virus remains to be explicated. NC proteins are associated with the RNA genome in the core of the viral particle (Dickson et al., 1984; Karpel et al., 1987). They have also been shown in vitro to promote the annealing of primer tRNA to viral RNA (Barat et al., 1989) and viral RNA dimerization (Prats et al., 1988). Single-stranded (ss) nucleic acids bind nonspecifically to these proteins, with a 15–30-fold greater affinity than double-stranded (ds) nucleic acids (Davis et al., 1976).

Retroviral NC proteins have one or two copies of a conserved sequence that is similar to the zinc binding domain of the eukaryotic transcription factor TFIIIA (Berg, 1986). Classical zinc finger proteins, such as TFIIIA, have a Cys-Cys-His-His motif and form a stable structure when a tetrahedrally coordinated zinc is bound (Miller et al., 1985; Lee et al., 1989). The conserved sequence in retroviral NC proteins is of the Cys-Cys-His-Cys type [C-$(X)_2$-C-$(X)_4$-(H)-$(X)_4$-C]

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(CysHis motif), which was proposed to form a finger structure when bound to zinc (Berg, 1986). NC proteins bind Zn^{2+}, as indicated by the results of a zinc blotting technique applied to full-length protein (Schiff et al., 1988) and spectroscopic methods used with a finger peptide (South et al., 1989; Green & Berg, 1989). Upon interaction with the metal ion, the retroviral finger peptides fold into stable three-dimensional structures. This folding is only seen when zinc is bound (South et al., 1989; Summers et al., 1990). However, the metal appeared to have little or no effect on the ultraviolet circular dichroism or nucleic acid–interactive properties of avian retroviral-derived or synthetic murine NC proteins (Jentoft et al., 1988; Roberts et al., 1989). Atomic absorption studies suggested that there is not enough zinc in the mature virion to bind stoichiometrically to NC (Jentoft et al., 1988). These results must be contrasted with the clear requirement for this conserved motif in the assembly of infectious particles (Gorelick et al., 1988, 1990; Mêric & Goff, 1989; Dupraz et al., 1990). Moreover, recent NMR results on the HIV-1 NC, p7, indicate that this protein, which contains two fingers, binds 2 equiv of the metal stoichiometrically, which brings about formation of folded domains conformationally very similar to those seen for the finger peptides (South et al., 1990a). In addition, tightly-bound zinc has been found in several retroviruses in quantities sufficient to populate the NC CCHC arrays (Bess et al., 1992), and EXAFS studies indicate that zinc is coordinated to the NC zinc fingers in mature particles (Summers et al., 1992; Chance et al., 1992).

The classical finger proteins bind in a sequence-specific manner to ds DNA (Vrana et al., 1988; Kadonaga et al., 1987, 1988; Nagai et al., 1988). Zinc is required for sequence-specific binding (Hanas et al., 1983; Johnston, 1987; Kadonaga et al., 1988). In one case, it was found that sequences adjacent to, rather than within, the zinc finger contribute to the sequence-specific binding (Corton & Johnston, 1989). With respect to the effect of (and requirement for) bound metal on nucleic acid interaction, varying (qualitative) results have been obtained. In one case, there was no obvious effect of Zn^{2+} on DNA binding (Frankel et al., 1987), whereas other data suggest an enhancement (Parraga et al., 1988) or a requirement for the metal ion (Lee et al., 1991). It should be emphasized that these classical proteins contain in general a relatively large number of zinc fingers, unlike the retroviral NC proteins which possess one or two metal binding domains.

Despite the evidence that the conserved Cys-His motif of NC is important for the selection of viral RNA for packaging, NC has not yet been demonstrated to bind nucleic acid with any sequence specificity. However, other interesting nucleic acid–interactive properties have been demonstrated in vitro: NC plays a role in annealing the primer tRNA to the viral RNA (Barat et al., 1989; Prats et al., 1990) and in viral RNA dimerization (Prats et al., 1988). The involvement of Zn^{2+} in these processes has yet to be explicated.

A high-resolution structure of the first zinc finger of HIV-1 has been determined by NMR methods (South et al., 1989; Summers et al., 1990). This 18-residue peptide is about one-third the length of the full protein (55 residues) and has the sequence (numbered from the first Cys):

$$V_{-2}K_{-1}C_1F_2N_3C_4G_5K_6E_7G_8H_9I_{10}A_{11}R_{12}N_{13}C_{14}R_{+1}A_{+2}$$

We have examined this peptide, nucleocapsid peptide finger 1 (NC-F1), with a view toward determining the extent of interaction of the peptide for nucleic acids, the effect of the zinc-induced structure on this interaction, and the specificity, if any, of the peptide for single- vs double-stranded nucleic acids. Using fluorescence methods, we have demonstrated that the peptide will interact with both single- and double-stranded nucleic acids nonspecifically. The presence of bound Zn^{2+} had a significant effect on the nucleic acid occluded site size and binding affinity. Preliminary 1D NMR studies of a complex between Zn(NC-F1) and a single-stranded oligodeoxynucleotide, d(TTTGGTTT), indicated that conserved hydrophobic residues are involved in an intercalative type of binding (South et al., 1989). The present analysis of 2D NMR data collected for a complex of the peptide with d(TTTGGTTT) is consistent with intercalative binding involving the heterocyclic base and the hydrophobic residues of the peptide. The combined results of NMR experiments and salt dependence of NC-F1–polynucleotide binding provide a consistent picture of the electrostatic and nonelectrostatic contributions in NC–nucleic acid interactions.

Materials and Methods

Materials. The first finger sequence of the HIV-1 nucleocapsid protein (NC-F1) was purchased from Peptide Technologies Inc. (Washington, DC). Peptide purity was established first at Peptide Technologies Inc. by detection of a single peak for the purified sample on reverse-phase HPLC and by amino acid analysis. A 1D ^1H NMR spectrum of this material showed that, except for very small signals due to residual solvent, only a single set of signals was observed. The sample purity exceeds 98%, with 0% detected peptide impurities, at the signal-to-noise ratio of the collected data. The sequence of the peptide at pH 7 in the presence of Zn^{2+} was determined unambiguously by 2D NMR methods (Summers et al., 1990). The peptide was again submitted to HPLC reverse-phase analysis and found to contain only a single peak, and amino acid analysis confirmed the composition of the peptide.

Two nucleic acid–interactive peptides lacking a zinc binding domain, obtained from J. Elder (Scripps Institute, La Jolla, CA) and referred to as peptide 6127 and peptide 81, were used as controls. Peptide 6127 (GGGQYFAKPRNQGGC) is a 15-residue peptide corresponding to residues 291–304 of the rat heterogeneous ribonucleoprotein A1. Peptide 81 (SLKRGNTPRNQGPC) is an immunologically related 14 amino acid peptide from the viral gp70 protein of recombinant murine leukemia virus (MuLV). Poly(ethenoadenylic acid) [poly(ϵA)] was purchased from Pharmacia. Polynucleotide concentration was determined spectrophotometrically using the following extinction coefficients (per mole of phosphate): $\epsilon(257) = 3.7 \times 10^3$ M^{-1} cm^{-1} for poly(ϵA) (Karpel et al., 1987), and $\epsilon(260) = 6.5 \times 10^3$ M^{-1} cm^{-1} for calf thymus DNA (Jensen & von Hippel, 1976). Heat-denatured calf thymus DNA was prepared by boiling the solution in a water bath for 5 min and rapidly cooling on ice. Zinc chloride (ACS reagent grade) was purchased from Sigma. All other reagents were of reagent grade. All solutions were prepared in doubly distilled water containing less than 12 ppb zinc (185 nM), as determined by atomic absorption spectrometry.

Fluorescence Methods. The enhancement of fluorescence emission upon peptide binding to poly(ϵA) was measured using a Jasco FP-4 (samples at 23 °C) or SLM 48000S (at 25 °C) spectrofluorometer. The excitation wavelength used was 305 nm (with 1–4-nm bandwidth) and the emission wavelength was 400 nm.

Titrations were performed using a solution of poly(ϵA) with additions of small aliquots of peptide. Lyophilized peptide was dissolved in doubly distilled water, zinc chloride was added to the solution (or an equivalent volume of water), and then an aliquot of concentrated buffer was added, so that the peptide concentrations of both apopeptide and zinc-bound

peptide were the same in identical volumes. The assay cuvette contained 1–3 μM (p) poly(εA) and sodium phosphate buffer (1.00 mM, pH 7.3) in a total volume of 1000 μL. Competing nucleic acid, when present, was in 10-fold molar excess (residues) over poly(εA). Fluorescence enhancement was measured after addition of aliquots of a solution of peptide to which a 3–6% molar excess of zinc chloride had been added. All readings were corrected for volume changes. Control experiments were performed in the absence of $ZnCl_2$ or peptide. The effect of ionic strength on the fluorescence enhancement was determined by adding aliquots of a 1 M sodium chloride solution to the peptide–poly(εA) mixture.

Binding isotherms were computer analyzed using the extended Scatchard equation for noncooperative binding, eq 10 of McGhee and von Hippel (1974):

$$\nu/L = K(1 - n\nu)[(1 - n\nu)/(1 - (n - 1)\nu)]^{n-1}$$

where ν is the binding density of the ligand (peptide) on the lattice (nucleic acid) in moles of ligand per mole of nucleotide residue, L is free ligand concentration (in moles per liter), n is the binding site size, and K is the intrinsic binding constant. ν is related to the fractional saturation of the lattice, θ, which is equal to νn. The binding model takes into account the effects of ligand crowding at high levels of saturation. For noncooperative binding, this leads to an inability to achieve complete saturation under most conditions, even at high [ligand]/[lattice] (Kowalczykowski et al., 1986). Accordingly, we calculated K and n from the experimental binding isotherms by the following procedure: Values for θ at the highest observed [ligand]/[lattice] (θ_{max}) were estimated from a comparison of our isotherms and the theoretical curves of Kowalcykowski et al. (1986). For each value of θ_{max}, θ was calculated for all of the data points in the titration. Then, for a given value of n, the corresponding sets of ν, L, and ν/L were calculated, and the best value of K was determined by a computerized nonlinear least squares program (NFIT, Island Products, Galveston, TX) based on the Marquardt–Levenberg algorithm (Bevington, 1969). In this curve fitting, data points of low [peptide]/[nucleic acid] were excluded, since binding under these conditions is near stoichiometric. The initial estimates of θ_{max} and n were systematically varied, and the calculation was repeated. The values reported below for K and n represent the highest R^2 obtained for the full range of the θ_{max}, n sets. There was no evidence for any cooperativity in the interaction, and use of the extended form of the Scatchard equation for this type of interaction [eq 15 of McGhee and von Hippel (1974, 1976)] yielded poorer fits.

The effect of salt on complex formation was monitored by the decrease in poly(εA) fluorescence enhancement brought about by the addition of NaCl to solutions of the polynucleotide in the presence of high levels of peptide. The concentrations of the peptide–nucleic acid complex, free peptide, and free poly(εA), and therefore ν and ν/L, were calculated from the (remaining) fluorescence enhancement, total peptide concentration, and total poly(εA) concentration. K's were calculated from eq 10 of McGhee and von Hippel (as above), with the assumption that n was constant through the range of [NaCl] used. Utilizing eq 14a of Record et al. (1976), log K vs log [NaCl] plots were interpreted in terms of the number of electrostatic interactions between peptide and nucleic acid, assuming an average number of Na^+ bound per poly(εA) nucleotide of 0.77 (Record et al., 1976). An estimate for the affinity of the peptide for single- or double-stranded DNA was obtained by performing binding experiments with poly(εA) in the presence of these substrates. Under these conditions, assuming invariant site size, the association constant of peptide for the competitor DNA is

$$K_{DNA} = (K/K_{App} - 1)/[DNA]_p$$

where K is the association constant for poly(εA) (for Zn^{2+}-bound or -free peptide) in the absence of competitor, K_{App} is the apparent affinity of peptide for poly(εA) in the presence of competitor (calculated as if no competitor were present), and $[DNA]_p$ is the residue concentration of single- and double-stranded DNA.

NMR Methods. NMR data were collected with a GE GN-500 (500 MHz, ^1H) spectrometer. Sample conditions were as follows: 5 mM Zn(NC-F1) plus 5 mM d(TTTGGTTT) in D_2O (99.9%, MSD Isotopes, Inc.); T = 30 °C. NOESY data were collected and processed with the following parameters: 300-ms mixing period; 64 scans per t_1 increment; 2 × 256 × 1024 data matrix size, zero-filled to a final matrix size of 2048 × 2048 points; 6-Hz Gaussian and 90° shifted-square sine bell filtering in the t_2 and t_1 dimensions, respectively. Signal assignments for the Zn^{2+}(NC-F1)–nucleic acid complex were made with procedures described in detail by Wuthrich (1986).

Results

The Presence of Bound Zn^{2+} Affects the Site Size and Affinity of Finger Peptide–Poly(εA) Binding. Addition of NC-F1 prepared with and without Zn^{2+} to solutions of poly(εA) produced an increase in the fluorescence emission of the polynucleotide. At low ionic strength, plots of fluorescence vs [NC-F1]/$[poly(\epsilon A)]_p$ were hyperbolic, with a maximal fluorescence increase of around 3-fold in both the presence and absence of the metal ion. The apparent occluded site size was initially estimated with the assumption that maximal binding saturation was achieved at high [peptide]/[nucleic acid]. As Kowalczykowski et al. (1986) note, under conditions of high affinity, the binding isotherm of an experiment where ligand (protein or peptide) is added to lattice (nucleic acid) is approximated by two straight lines that intersect at a $[ligand]/[lattice]_p$ of $1/n$, corresponding to full saturation of the nucleic acid. For protein–nucleic acid interactions, conditions of high affinity can be met, or at least approached, by reducing the salt concentration. In low ionic strength (1 mM sodium phosphate) titrations, the apparent site size obtained for NC-F1 prepared with Zn^{2+} was consistently smaller, by about one-third, than that derived in the absence of metal ion. This was clearly evident in parallel experiments run in the presence and absence of the metal ion, where the site size was determined to be about 2 for Zn(NC-F1) and 3 for the apopeptide. As we show below, the same procedure run with the control peptides did not show any significant difference in n. If anything, the apparent site size in these cases is slightly *smaller* in the absence of metal. Thus, even if this reflects an effect of metal on the assay, which is unlikely (see below), it does not account for the difference in site size. Titrations with NC-F1 performed on different days produce some variation in site size, which is estimated to be ±25%, with a ratio of n for apopeptide to n for Zn(NC-F1) of 1.6 ± 0.2. The uncertainties, therefore, are a limitation on the *absolute* value of the site size but do not negate the aforementioned reproducible effect of the metal. This reduction in the occluded site size probably reflects the metal-induced formation of a compact structure in the peptide.

Although the above method was useful for approximating site sizes, the assumption that full saturation was achieved in the titrations was not completely correct. Plots of 1/(fluorescence change) vs 1/[free ligand] (double reciprocal) became nonlinear at high levels of binding saturation, par-

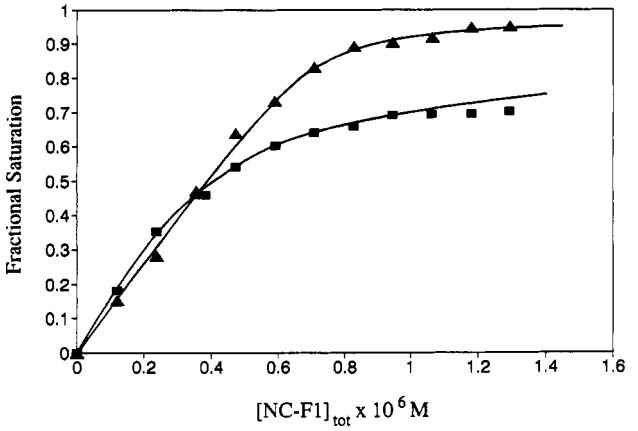

FIGURE 1: Forward titration of poly(ϵA) and HIV-1 NC-F1. [poly(ϵA)] = 1.34 × 10^{-6} M (p) in 0.001 M sodium phosphate, pH 7.3. The lines represent the best fit for the values of K and n given in the text. Symbols: (■) NC-FI; (▲) Zn(NC-F1) ([NC-F1]:[$ZnCl_2$] = 1:1.06).

Table I: Calculated K and n for NC-F1 Binding to Poly(ethenoadenylic acid)

θ_{max} (%)	K (M^{-1})	n	R^2
	(A) Zn(NC-F1)		
100	2.4 × 10^8	1.8	0.9868
95	9.6 × 10^7	1.75	0.9946
90	4.0 × 10^7	1.75	0.9880
	(B) Zn-Free NC-F1		
75	5.9 × 10^6	2.6	0.9991
70	3.8 × 10^6	2.75	0.9992
65	2.33 × 10^6	3.0	0.9989

ticularly in the case of the Zn^{2+}-free ligand. This is a consequence of the overlapping of binding sites that occurs when a ligand covers more than one residue on the lattice [and is accounted for by the extended Scatchard relationship (eq 10) of McGhee and von Hippel (1974)]. In order to obtain precise values of n and K, the calculational method described under Materials and Methods was utilized. The results are shown in Figures 1 and 2. As we noted, in this method no prior assumptions were made as to the values of θ_{max} and n, although our initial estimates of n were used for this curve fitting procedure and, as it turned out, proved to be close to the final calculated values. Our calculations showed that optimal fits were obtained with θ_{max} values of 0.95 and 0.70 for peptide with bound and unbound zinc, respectively (Figure 1). The ν/L vs ν plots gave optimal fits for Zn^{2+}-bound peptide with K = 9.6 × 10^7 and n = 1.75, and for the metal-free peptide, K = 3.8 × 10^6 and n = 2.75 (Figure 2). The statistics of these fits, as well as for the fits using θ_{max} 5% above and below the optimal values, are listed in Table I. As can be seen in the table, the calculated values of K are strongly dependent on the magnitude of θ_{max}, which even at suboptimal levels yields excellent R^2 values. Nevertheless, it is quite clear that binding of zinc to the peptide increases its affinity for nucleic acid by 1–2 orders of magnitude and reduces the site size by about one-third.

Control experiments indicated that the effect of Zn^{2+} on peptide–poly(ϵA) binding was clearly due to its binding to peptide, rather than to any effect of the metal on polynucleotide fluorescence. In the absence of peptide, Zn^{2+} concentrations up to 0.5 μM had no effect on the polynucleotide's fluorescence emission, and only a moderate increase (28%) was seen at 0.7 μM. Since there was less than 0.2 μM free Zn^{2+} in the titrations (6% molar excess of zinc over peptide), no effect on poly(ϵA) fluorescence would be

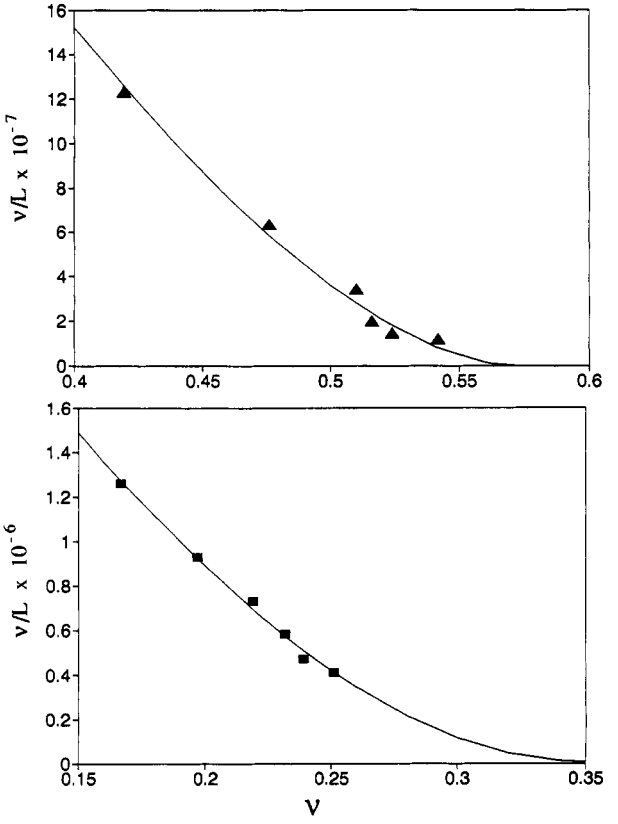

FIGURE 2: Scatchard plots of the data in Figure 1: (top panel) Zn(NC-F1); (bottom panel) NC-F1.

expected. Even had any amount of metal dissociated from the peptide during the course of the titration, its effect on the polynucleotide fluorescence would have been very minor compared to the enhancement brought about by peptide binding. Note that the NMR data showed no evidence of displacement of zinc from the peptide upon binding nucleic acid (see below).

A preliminary titration experiment performed in the presence of excess EDTA ([EDTA]:[Zn^{2+}] = 3.8:1) showed no apparent effect of Zn^{2+} on the affinity of NC-F1 for poly(ϵA). This indicates that the metal ion, though tightly bound in the peptide, is accessible to the chelating agent. Note that a similar finger peptide has been shown to have a K_d(Zn) = 6 × 10^{-10} M (Green & Berg, 1990); the K_d of EDTA for Zn^{2+} is about 10^{-16} (Sillén & Martell, 1964).

The NC-F1–Poly(ϵA) Interaction Is Salt-Dependent. Addition of concentrated NaCl to solutions of poly(ϵA) fully bound by NC-F1 brought about complete reversal of the polynucleotide fluorescence enhancement, indicating that the formation of the complex depends, at least in part, on ionic interactions. A higher salt concentration was required to dissociate the complex in the presence of zinc chloride. From the salt reversal plots, the NaCl concentrations at 50% binding saturation were 8 mM NaCl for NC-F1 and 40 mM for Zn(NC-F1) (Figure 3). log K vs log [Na^+] plots showed slopes of −1.51 and −0.84, respectively, for the Zn^{2+}-bound peptide and apopeptide (Figure 4). These plots could be interpreted in terms of the number of charge interactions using eq 14a of Record et al. (1976):

$$-\log K/\log [\mathrm{NaCl}] = k + m'\Psi$$

where k is the number of anions displaced from the peptide by the interaction, m' is the number of ion pairs formed during the interaction, and Ψ is the number of counterions bound per phosphate of nucleic acid [0.77 for poly(rA), assumed to be

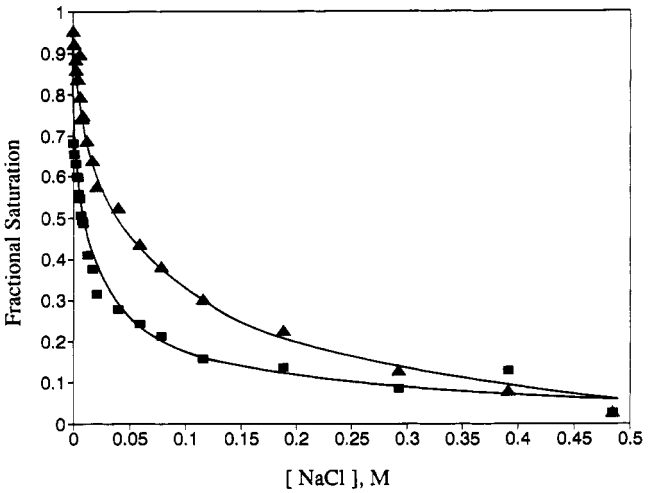

FIGURE 3: Reversal of NC-F1–polynucleotide binding by NaCl. Buffer conditions are as in Figure 1, with NaCl as indicated. [NC-F1] = 1.29×10^{-6} M (p); [poly(ϵA)] = 1.32×10^{-6} M (p) prior to addition of NaCl. Symbols: (■) NC-FI; (▲) Zn(NC-F1) ([NC-F1]:[$ZnCl_2$] = 1:1.06).

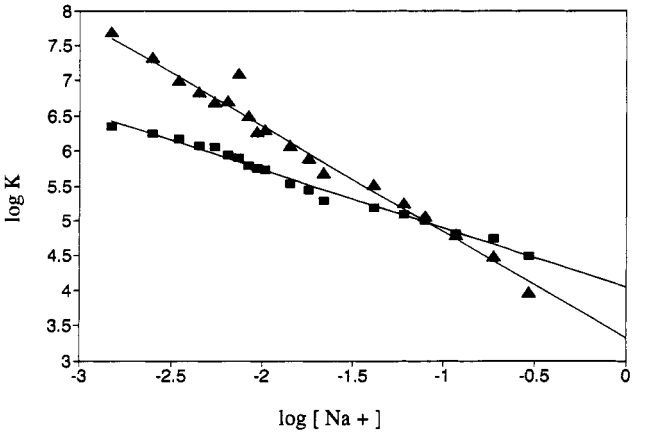

FIGURE 4: log k_{app} vs log [NaCl] plot. Data are from Figure 3 plotted according to Record et al. (1976).

the same for poly(ϵA)]. Although k is not known, no more than 2.0 (with zinc) or 1.1 ion pairs (without metal) could be formed with the four positively charged residues in the peptide (K_{-1}, K_6, R_{12}, and R_{+1}). The higher slope for the Zn-bound peptide could reflect an additional ionic interaction occurring within the metal-induced compact structure of the peptide (see Discussion). Alternatively, the number of peptide–nucleic acid ionic interactions may be the same in both cases, but an additional counterion is displaced in the case of the Zn^{2+} peptide.

The nonelectrostatic component of the binding affinity can be estimated by extrapolation to 1 M NaCl, where no electrostatic contribution to binding occurs (Record et al., 1976). The estimated values for log K at 1 M NaCl are similar for the Zn-bound and Zn-free peptide, 3.3 and 4.0, respectively. This corresponds to about −5 kcal/mol for the nonelectrostatic component of peptide binding to nucleic acid, consistent with our NMR results and theoretical estimates (see below).

The Retroviral Zn Finger Peptide Interacts with Nonfluorescent Single- and Double-Stranded Nucleic Acids. The affinity of the peptide for nonfluorescent, natural nucleic acids can be assessed by their ability to compete with poly(ϵA) in fluorometric titrations carried out in the presence of limiting NC-F1. Over the full range of the titration, the fluorescence enhancement of poly(ϵA) by metal-bound or -free peptide was decreased when calf thymus DNA was present at a concentration 10-fold that of poly(ϵA). This effect was seen with

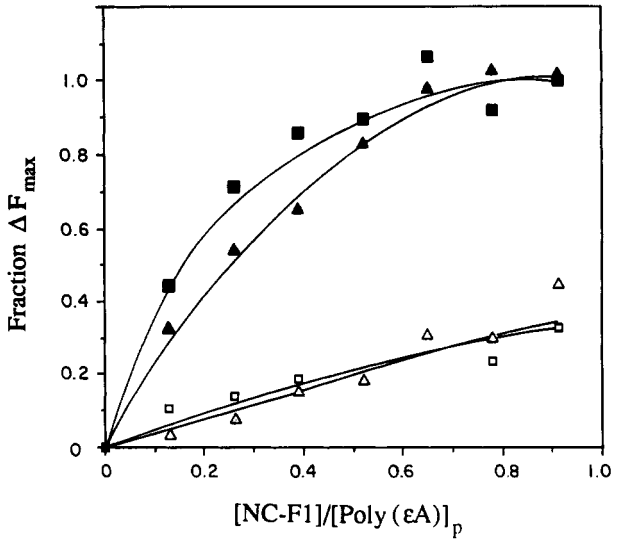

FIGURE 5: Competition of single-stranded calf thymus DNA and poly(ϵA) for NC-F1. [poly(ϵA)] = 2.68×10^{-6} M (p); [ss CTDNA] = 2.69×10^{-5} M (p); buffer conditions are as in Figure 1. Symbols: (■) NC-F1 + poly(ϵA); (□) NC-F1 + poly(ϵA) + ss CTDNA; (▲) Zn(NC-F1) + poly(ϵA); (△) Zn(NC-F1) + poly(ϵA) + ss CTDNA ([NC-F1]:[$ZnCl_2$] = 1:1.03).

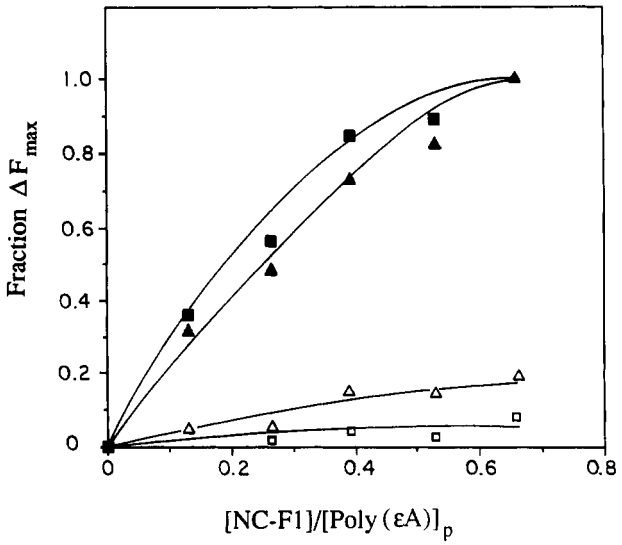

FIGURE 6: Competition of double-stranded calf thymus DNA and poly(ϵA) for NC-F1. [poly(ϵA)] = 2.61×10^{-6} M (p); [ds CTDNA] = 2.62×10^{-5} M (p); buffer conditions are as in Figure 1. Symbols (■) NC-F1 + poly(ϵA); (□) = NC-F1 + poly(ϵA) + ds CTDNA; (▲) Zn(NC-F1) + poly(ϵA); (△) Zn(NC-F1) + poly(ϵA) + ds CTDNA ([NC-F1]:[$ZnCl_2$] = 1:1.01).

both ss DNA (Figure 5) and ds DNA (Figure 6). Within the experimental error of the data, and assuming the same site size on DNA as on poly(ϵA), the metal-bound peptide interacted with both ss and ds DNA with affinities comparable to its association constant for the fluorescent polynucleotide and therefore did not display any obvious specificity for the conformational state of the DNA. The apopeptide displayed an affinity for ss DNA about one-quarter that for poly(ϵA) ($\approx 1 \times 10^6$ M^{-1}) and an affinity for ds DNA of about twice the value for the polyribonucleotide ($\approx 6 \times 10^6$ M^{-1}). Qualitatively, we can state that NC-F1 can bind to ds DNA, ss DNA or poly(ϵA) and, at least in the case of its Zn^{2+}-bound form, displays no obvious specificity for single vs double strands. Conceivably, the peptide might bring about the denaturation of the ds DNA upon binding.

Effect of Zn^{2+} on Nucleic Acid Interaction of Peptides That Do Not Bind This Metal Ion. Two peptides, 6127 and 81,

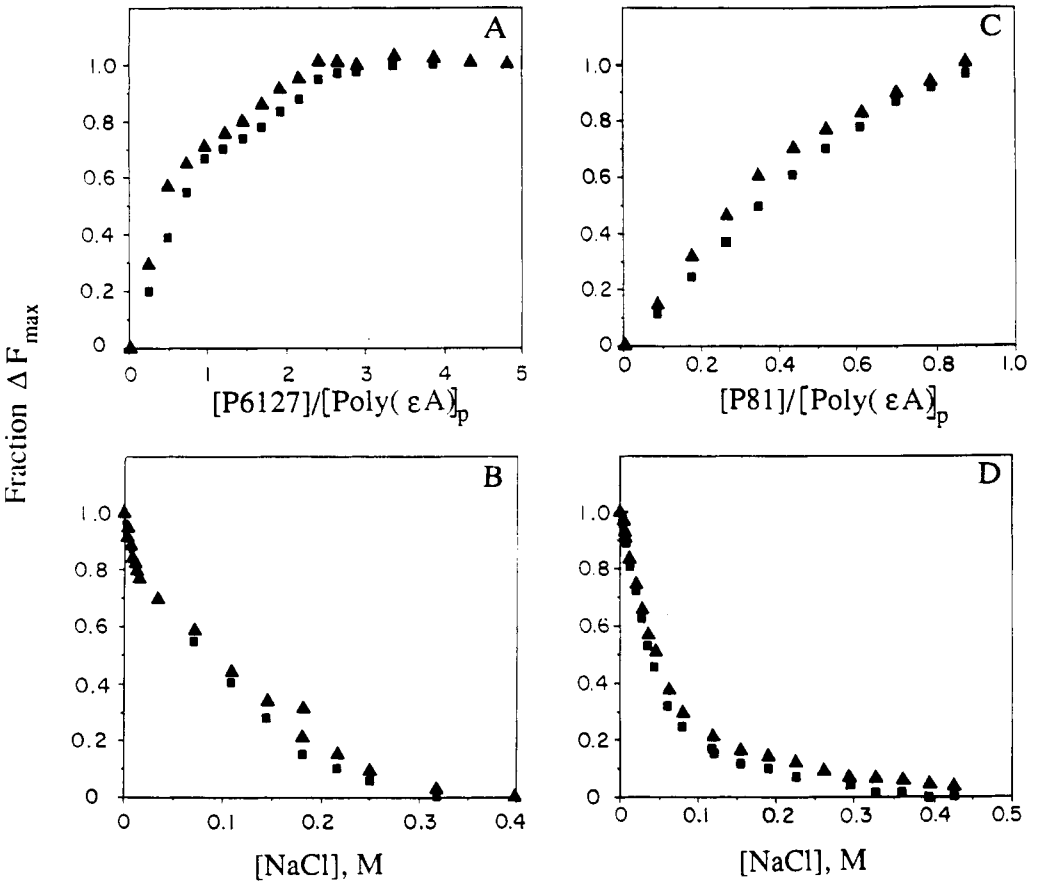

FIGURE 7: Control peptide binding to poly(ϵA) and salt reversal in 0.001 M sodium phosphate, pH 7.3. (A) Peptide 6127 + poly(ϵA); [poly(ϵA)] = 1.44×10^{-6} M (p). (B) Reversal of peptide 6127 and poly(ϵA) binding with NaCl; [poly(ϵA)] = 1.41×10^{-6} M (p). (C) Peptide 81 and poly(ϵA); [poly(ϵA)] = 1.44×10^{-6} M (p). (D) Reversal of peptide 81 and poly(ϵA) with NaCl; [poly(ϵA)] = 1.43×10^{-6} M (p). Symbols: (▲) peptide; (■) Zn(peptide) ([peptide]:[$ZnCl_2$] = 1:1.01).

were selected as controls because they have previously been shown to bind to poly(ϵA) at low ionic strength and have neither a conserved zinc binding sequence nor any likelihood of strongly coordinating this metal ion (Trauger et al., 1990). The presence of zinc had little effect on the binding to or salt reversal of either control peptide. Apparent site size as well as the salt reversal profile was unaffected by the presence of Zn^{2+} (Figure 7). A slightly greater enhancement of poly(ϵA) fluorescence was noted for the control peptides in the presence of zinc chloride, which can be accounted for by the effect of (unbound) Zn^{2+} on the fluorescence emission of poly(ϵA). In contrast, for NC-F1, where the zinc is bound to peptide and not free in solution, the fluorescence increase was *lower* in the presence of metal ion.

NMR Studies: Binding of NC-F1 to Oligonucleotides. In order to further elucidate the binding of Zn(NC-F1) with single-stranded nucleic acids, its interaction with a synthetic oligomer, d(TTTGGTTT), was studied using NMR methods. The cross-peak pattern in the 2D NOESY spectrum was essentially identical to that observed previously for Zn(NC-F1) in the absence of nucleic acid (Summers et al., 1990). Thus, the overall three-dimensional structure of the peptide, and therefore its binding of zinc, is unperturbed when bound to the oligomer. No intermolecular NOE cross peaks were detected.

The chemical shift differences observed for nucleic acid-bound vs free peptide are summarized in Figure 8. The results are consistent with the binding of the deoxyoligonucleotide at a hydrophobic surface patch containing F_2 and I_{10} (see Discussion). First, signals for residues V_{-2}–C_1 were relatively insensitive to the presence of nucleic acid, with shift changes generally less than 0.2 Å. Extremely large shift changes were observed for several of the signals associated with the F_2 residue; specifically, the F_2 amide proton exhibits a downfield shift of 0.6 ppm, whereas the signal due to the aromatic protons shows an upfield shift of 0.6 ppm. One of the F_2 β protons also exhibits a large upfield shift (0.4 Å). For N_3, the backbone amide proton signal shifts downfield by 0.5 ppm on binding to nucleic acid, whereas the remaining N_3 protons are not significantly influenced. Small shifts were generally observed for protons on residues C_4–H_9. In fact, chemical shifts associated with the H_9 protons were all 0.1 ppm or less.

I_{10} displayed large shifts upon binding the oligonucleotide. The α-proton signal shifted upfield by 0.2 ppm, whereas the β, γ-CH_2, γ-CH_3, and δ-CH_3 signals exhibited larger upfield shifts of 0.45, 0.3, 0.9, and 0.35 ppm, respectively. The A_{11} backbone amide and methyl signals shifted downfield by 0.37 and 0.30 ppm, whereas the α-proton signal moved upfield by 0.25 ppm. The side-chain amide proton signals for N_{13} shifted downfield by 0.2–0.5 ppm on complexation, but remaining N_{13} proton signals were only slightly influenced (≤0.1 Å). Signals for the β protons of C_{14} moved upfield by 0.27–0.44 ppm, but the remaining C_{14} signals were not affected significantly. For A_{+2}, the backbone amide proton signal shifted downfield by 0.55 ppm whereas the remaining signals were only moderately affected (≤0.2 ppm).

Discussion

The peptide NC-F1, a fragment of the HIV NC protein containing the first zinc finger, binds to nucleic acids under low salt conditions. Full-length NC proteins from M-MuLV and AMV (avian myeloblastosis virus) interact with nucleic

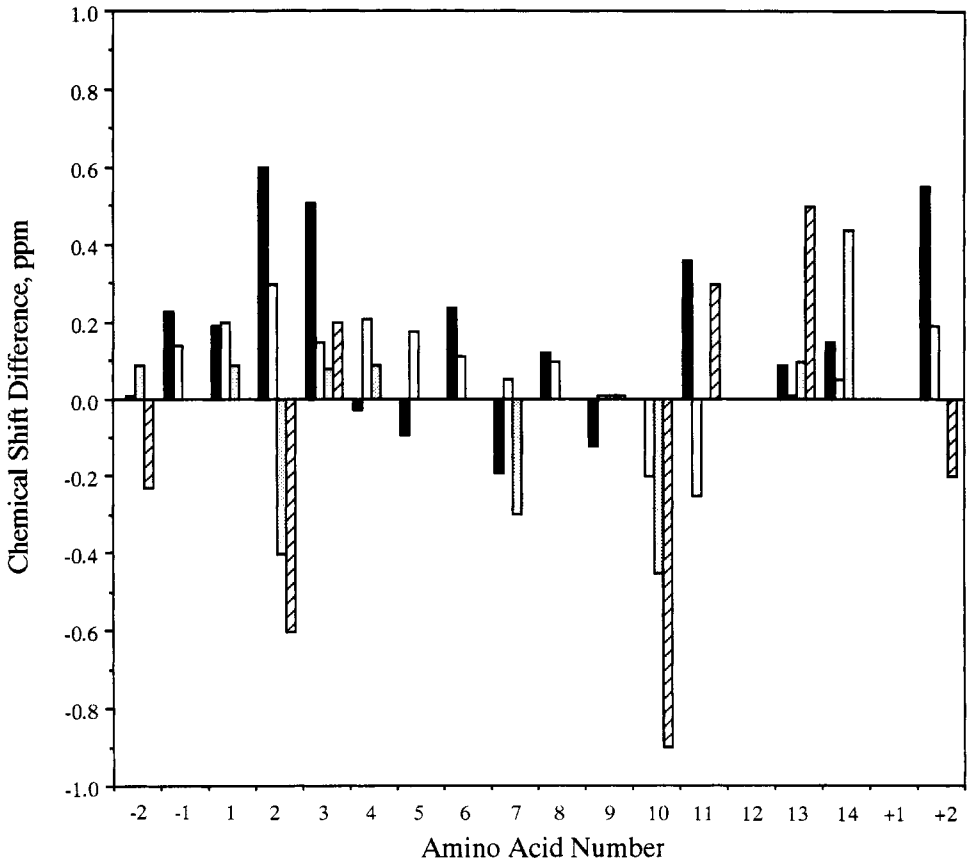

FIGURE 8: Chemical shift differences in ppm of the amide (black shaded), α (open), and β (light shaded) protons obtained for Zn(NC-F1) residues upon binding d(TTTGGTTT). Others (hatched) refer to V_{-2} methyl, F_2 aromatic, N_3 side-chain amide, I_{10} γ-methyl, A_{11} methyl, N_{13} side-chain amide, and A_{+2} methyl protons. For geminal protons (e.g., Gly α or Phe β protons) the larger of the chemical shift differences was used.

acids (Karpel et al., 1987; Jentoft et al., 1988; Roberts et al., 1989), and this binding can be observed under higher salt conditions, indicating that the complete NC protein has a greater affinity for nucleic acid than does the finger peptide. This is consistent with at least some of the results from non-retroviral systems, where single finger domains from classical zinc finger proteins have been shown to bind to nucleic acids with lower affinity than the full-length proteins (Parraga et al., 1988; Kadonaga et al., 1988).

The effect of Zn^{2+} on the nucleic acid binding parameters of NC-F1 is clearly a consequence of the metal ion on the structure of the peptide. Zn^{2+} had no effect on the nucleic acid binding properties of control peptides that lack zinc finger sequences. The number of nucleic acid residues covered by the apopeptide was 2.75, and 1.75 for Zn(NC-F1). As we have noted, although there was some variation in these numbers, the ratio of site sizes [for apoprotein/Zn(NC-F1)] obtained from experiments run in parallel was constant, 1.6 ± 0.2. This is consistent with the NMR-derived compact structure of the Zn-containing peptide. In the absence of the metal ion, NC-F1 does not fold into a compact structure but rather is a set of random structures which present no clear NMR spectrum (Summers et al., 1990). The larger site size obtained for the apopeptide is consistent with the more extended structure(s) of this form of the peptide. Note that full-length NC proteins, such as M-MuLV and AMV, typically show larger site sizes (n = 5–6; Karpel et al., 1987) than those reported in this study, as would be expected for polypeptide chains 3–4 times the size of NC-F1.

In 1 mM sodium phosphate, the presence of bound zinc in the peptide brings about a 25-fold increase in affinity for nucleic acid ($K = 9.6 \times 10^7$ M^{-1} compared to 3.8×10^6 M^{-1}). These differences in affinity and site size between the apopeptide and zinc peptide are not unusually large; however, even small differences in nucleic acid binding affinity can be physiologically relevant. For example, the cooperative binding of bacteriophage T4 gene 32 protein to its own message, which is responsible for the translational autoregulation of this protein, can be accounted for by relatively small differences in its binding affinities for ss DNA, 32 mRNA, and other RNAs (von Hippel et al., 1982). The special affinity of 32 protein for its own message appears to be related to the existence of a pseudoknot structure in the mRNA (McPheeters et al., 1988), and it has been proposed (Karpel, 1990) and recently demonstrated (Shamoo et al., 1991) that this specificity is dependent on the presence of zinc in 32 protein. Thus, the differences in binding parameters seen in vitro for the apo- and zinc-bound peptide might reflect biologically significant differences in specific interaction(s) in vivo. Furthermore, within the contexts of the whole NC protein or the gag polyprotein precursor, the conformational constraints imposed on the structure by zinc binding could affect the site size, affinity, or other binding parameters (such as cooperativity) to a greater extent than that seen with the NC-F1 peptide. Analogous to the selective recognition of 32 protein of its own mRNA, such factors could be essential for the selective recognition and packaging of viral RNA by the precursor. The initial steps in this process might be precursor binding at retroviral RNA sequences identified to be essential for packaging (Lever et al., 1990; Mann et al., 1983), which, analogous to the autoregulatory region of T4 32 mRNA, are generally located 5′ to the translational start codon.

A comparison of the nucleic acid binding affinities of NC-F1 with data for a full-length NC protein, murine leukemia viral

(MuLV) p10, is instructive. Unlike HIV-1 p7, MuLV p10 has only one Zn finger. Data are available for the interaction of the full-length protein in the absence of the metal (Karpel et al., 1987). At 60 mM NaCl, p10 binds poly(ϵA) with an affinity of 2×10^6 M^{-1} (Karpel et al., 1987). Under these conditions, the affinities of NC-F1 for poly(ϵA) is about 1.2×10^5 M^{-1} in the absence of zinc. If the Zn finger of MuLV p10 had an affinity for poly(ϵA) comparable to that for HIV-1 NC-F1, then the full-length protein would show a $\Delta G°$ of nucleic acid binding about 2 kcal/mol more negative than the peptide. This difference is likely the result of additional electrostatic interactions present in full-length NC protein–nucleic acid binding. For MuLV p10, the slope of the log K vs log [NaCl] plot is −2 (Karpel et al., 1987), about twice that seen with the apopeptide. Thus, the number of electrostatic interactions increases with polypeptide length as well as with the presence of bound metal ion. Given the effect of metal on the salt dependence of peptide–nucleic acid binding, the Zn-bound form of the full-length protein would presumably display a greater dependence of affinity on [salt] than the apoprotein, with a corresponding increase in ion-pairing interactions. The nonelectrostatic portion of the binding free energy is about the same for apopeptide and apoprotein, $\Delta G°$ $\sim$ −6 kcal/mol, based on a predicted log K of 4 at 1 M NaCl.

Single- and double-stranded calf thymus DNA bound with comparable affinity to NC-F1 as judged by competition experiments with poly(ϵA) for the peptide, in the presence or absence of zinc. In contrast, full-length NC proteins (in the absence of metal) were found to selectively bind single-stranded nucleic acids (Davis et al., 1976). Preferential binding to single-stranded nucleic acids therefore likely relies on a larger portion of the NC protein than the 18 amino acids of NC-F1.

Further insight into the nature of the peptide–nucleic acid interaction can be gained by consideration of the NMR data presented in this study. Significant perturbations in the chemical shifts of residues F_2 and I_{10} of Zn(NC-F1) were seen upon interaction with the oligodeoxynucleotide TTTGGTTT. Inspection of the three-dimensional structure of the peptide (Summers et al., 1990) indicates that these side chains are in close proximity to each other on the surface of the peptide, exposed to solvent, and form a hydrophobic surface patch. There is an obvious potential for intercalation of the phenylalanine ring with the nucleic acid bases, most likely the internal guanines of the oligonucleotide. [Upon interaction with TTTGGTTT, there was a considerable broadening and downfield shift of the G-H_8 signals but only a small effect on the T-H_6 and -CH_3 signals (South et al., 1990b).] The nonelectrostatic component of the binding free energy, $\sim$−6 kcal/mol, its consistent with theoretical estimates for the stacking energies of phenylalanine with a nucleotide base (−6.3 to −9.1 kcal/mol; Kumar & Govil, 1984). Interactions of other nonpolar residues, such as I_{10}, may also contribute to the nonelectrostatic interactions. These results are likely relevant for the biological function of NC protein, since mutational analysis of analogous retroviruses indicated that nonpolar residues at positions 2 or 3 and 10 play critical roles in viral replication and the selective packaging of viral RNA. In MuLV, $Y_3 \rightarrow$ S or G and $W_{10} \rightarrow$ G led to a loss of infectivity and inhibition of MuLV genomic RNA packaging (Méric & Goff, 1989). Dupraz et al. (1990) showed that a $Y_2 \rightarrow$ S change in the nucleocapsid sequence of Rous sarcoma virus reduced viral RNA packaging efficiency and viral infectivity.

With respect to electrostatic interactions, the positioning of R_{12} on the same face of the Zn^{2+} peptide as the hydrophobic surface patch makes it a likely candidate for ion-pairing interactions with single-stranded nucleic acid. With our estimate of two ion pairs formed between peptide and nucleic acid, an additional basic residue would be needed (assuming that counterion release is not a significant factor). The ϵ-NH_3^+ group of K_{-1} is a potential participant in the interaction. In a model-building exercise with a dinucleotide in the A-conformation (the length was chosen to concur with the observed occluded site size), we found that with the help of the flexible side chains of K_{-1}, I_{10}, and R_{12} and the allowable rotation of the F_2 ring we could make van der Waals contacts between the two nonpolar side chains and the bases, while bringing the positive charges to within close proximity ($\leq$3 Å) of nonesterified phosphate oxygens. Additional degrees of freedom would be obtained were the dinucleotide allowed to distort from the A-conformation, which, given the perturbation in poly(ϵA) fluorescence, likely does occur to some extent. Note that basic residues are also capable of interactions with the nucleic acid bases. For example, a recent crystallographic study of the complex of the DNA binding domain of the Zif268 protein (which contains three Zn fingers) with a consensus double-stranded DNA binding site shows a number of arginine–guanine contacts, which could be important for sequence recognition (Pavletich & Pabo, 1991).

Specific recognition of viral RNA by NC has not as yet been demonstrated, although, as we have noted, there is genetic evidence that the CysHis motif of NC is required for selective packaging of viral RNA (Gorelick et al., 1988, 1990; Méric & Goff, 1989, Dupraz et al., 1990). The CysHis motif may be involved in the specific interaction of NC with viral RNA, whereas other parts of the protein are required for high-affinity binding. This separation of function within nucleic acid binding proteins is known for other zinc fingers, such as Sp1, where the zinc fingers have been shown to be required for binding specificity, while another portion of the protein was responsible for high-affinity binding (Kadonaga et al., 1988). If NC is similar to Sp1 in this respect, then the zinc fingers of NC could have a role in specific sequence recognition of viral RNA.

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Registry No. NC-F1, 123963-60-0; d(TTTGGTTT), 129809-10-5; Zn, 7440-66-6.

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