DNA binding properties of the zinc-bound and zinc-free HIV nucleocapsid protein: supercoiled DNA unwinding and DNA–protein cleavable complex formation

Esther Priel[a,*], Esther Aflalo[a], Iftach Seri[a], Louis E. Henderson[b], Larry O. Arthur[b], Mordechai Aboud[a], Shraga Segal[a], Donald G. Blair[c]

[a]*Departement of Immunology and Microbiology, Cancer Research Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

[b]*AIDS Vaccine Program, Program Resources Inc/Dyn Corp., NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702–1201, USA*

[c]*Microbiology Section, Laboratory of Molecular Oncology, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702–1201, USA*

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Abstract The HIV nucleocapsid (NC) protein contains, as those of other retroviruses, two Cys-His arrays which function as zinc finger binding domains. The nucleic acid binding properties of retroviral NC have been previously demonstrated. In this study, we characterized the DNA binding ability of the zinc-bound and zinc-free forms of HIV NC. We found that in addition to binding single-stranded DNA, both forms bind and unwind supercoiled plasmid DNA. The binding ability of the zinc-bound form was higher than the zinc-free form. In addition we showed the formation of NC protein–DNA cleavable complex which is the result of a presumably covalent bond formed between the protein and the phosphate moiety of the DNA backbone. The NC unwinding activity and the protein–DNA cleavable complex formation resembles the first step of the relaxing mechanism of DNA topoisomerase. Our results shed light on the possibility of a novel physiological function for the HIV NC protein in the viral life cycle.

Key words: HIV nucleocapsid protein; Supercoiled DNA; DNA–protein complex

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immune deficiency syndrome (AIDS) [1–3]. Its replication is controlled by a complex system of viral and cellular regulatory proteins, some of which act at the transcriptional and others at the post transcriptional level [4]. Retroviruses establish persistent infection by integrating proviral DNA into the host cell genome [5]. It is possible that both provirus integration and expression require topological changes [6]. DNA topoisomerases are essential nuclear enzymes which control and modify the topological state of DNA. We have demonstrated a topoisomerase I (topo I) activity in purified virions of HIV-1, equine infectious anemia virus (EIAV) [7], Moloney murine leukemia virus [8] and human T cell leukemia virus (unpublished data). We have recently purified the topo I activity from EIAV particles and found that this activity is associated with the p11 nucleocapsid protein (manuscript in preparation).

As in other replication competent retroviruses, the HIV genome contains the structural genes *gag*, *pol* and *env* as well as protease and regulatory genes. The *gag* gene encodes a 55 kDa core precursor polypeptide, $pr55^{gag}$ which is cleaved by the viral protease to the matrix antigen, the capsid antigen, the nucleocapsid (NC) and three other proteins designed p1, p2 and p6 [9]. The NC is a 7 kDa protein (p7) containing two stretches of Cys-X_2-Cys-X_4-His-X_4-Cys which are known as Cysteine–Histidine arrays [9]. Such arrays are also present in the NC of most other retroviruses and are presumably involved in the viral RNA packaging by $pr55^{gag}$ during virus assembly [10–15]. They are also required for the subsequent functions of NC within the mature virions, such as the dimerization of the genomic viral RNA [16] and the binding of the primer tRNA to the primer binding sequence in this genomic RNA [17]. Nucleocapsid proteins from several retroviruses bind single-stranded DNA and RNA [18–20] and are capable of unwinding folded RNA structures by binding selectively to single-stranded RNA [21]. Moreover, the NC of HIV-1 and HTLV-I have recently been shown to be zinc finger proteins capable of binding two Zn^{2+} cations for each NC molecule [21]. It was of interest to analyze the ability of the HIV p7 NC protein to bind and unwind supercoiled DNA and to elucidate its possible relationship to the viral topoisomerase activity. We also compared the DNA binding ability of the zinc-bound to the zinc-free p7 proteins. Our data show that both forms bind and unwind double-stranded supercoiled DNA, although the zinc-bound form was more efficient in binding single-stranded DNA.

In addition, our results present the possibility that the unwinding of supercoiled DNA by this protein is the result of its ability to form a DNA–protein cleavable complex.

2. Materials and methods

2.1. HIV p7 NC

The HIV NC protein used in this work was from a p7 stock prepared by Henderson L.E from the MN strain of HIV [9,21]. We examined the purity of this protein preparation using SDS-polyacrylamide gel (PAG) electrophoresis (SDS-PAGE). Increasing amounts of NC protein (1–10 μg) were loaded on 10% SDS-PAG or 15% SDS-PAG containing 4 M urea. The zinc-bound and zinc-free forms were obtained as described by Summers et al. [21].

*Corresponding author. Fax: (972) (7) 277453.

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2.2. DNA binding and DNA unwinding assay

Increasing amounts of the zinc-bound and zinc-free p7 protein were added to 25 μl DNA binding buffer containing 20 mM Tris-HCl, pH 8.1, 1 mM dithiothreitol (DTT), 20 mM KCl and 0.25 μg pUC-19 supercoiled plasmid DNA. The mixture was incubated at 37°C for 30 min. Where indicated, proteinase K was added followed by incubation at 50°C for 1 h. The reaction was terminated by adding 5 μl of stopping buffer (final concentration: 1% SDS, 15% glycerol, 0.5% Bromophenol blue, 50 mM EDTA, pH 8). The reaction products were analyzed by electrophoresis in 1% agarose using the TBE buffer (0.089 M Tris-HCl, 0.089 M boric acid, 0.062 M EDTA) at 1 V/cm. The gel was stained with ethidium bromide (1 μg/ml) and photographed using a short-wavelength ultraviolet lamp.

2.3. Agarose gel shift assay

DNA labeling and purification. ^{32}P-labeled pUC-19 fragments were prepared using the Random Primer DNA labeling system (BRL). To separate the labeled DNA from the unincorporated labeled nucleotides, we purified the DNA by chromatography through Sephadex G-50 column (nick column, Pharmacia). The obtained labeled DNA was further purified from the enzymes used for DNA labeling by phenol/chloroform extraction and was precipitated by ethanol. The labeled pUC-19 DNA was then dissolved in TE (10 mM Tris-HCl, pH 8, 1 mM EDTA).The DNA was boiled for 10 min, and immediately cooled prior to adding to the reaction mixture.

Gel shift assay. 0.3 μg of HIV p7 protein and 10 ng (6×10^5 cpm) of labeled single-stranded pUC-19 fragments were added to the DNA binding buffer. Where indicated, increasing amount of unlabeled double-stranded pUC-19 DNA fragments (*Aat*II–*Eco*RI) were added.

The binding assay was performed as described above and the reaction products were analyzed by electrophoresis on 1% agarose gel (4 V/cm) in a TBE buffer. The gel was exposed to autoradiography as described by Sambrook et al. for unfixed wet polyacrylamide gel [22]. We found that it was also possible to use this method for agarose gel. The DNA fraction bound to the p7 was estimated by densitometric analysis of the autoradiogram.

2.4. DNA–protein cleavable complex assay

HIV p7 protein (0.6 μg) was added to the DNA binding buffer containing 10 ng (6×10^5 cpm) labeled single-stranded pUC-19 fragments which were purified from the free dCTP nucleotide and the enzymes which were used for DNA labeling as described above. Where indicated free [^{32}P]dCTP was added instead of labeled DNA, or increasing amounts of cold pUC-19 DNA plasmid were added together with the labeled DNA. The reaction mixture was incubated for 1 h at 37°C and boiled for 2 min at 100°C. The P1 nuclease digestion was performed as described previously [23,24]. 0.1 M (final concentration) sodium acetate pH 5.6 and 30 units of P1 nuclease (BRL) were added for 2 h, at 37°C. Where indicated, 400 μg/ml of proteinase K were added and the reaction was further incubated for 1 h at 50°C. The reaction was stopped by 5 μl of protein sample buffer (0.5 M Tris-HCl, pH 6.8, 4% SDS, 0.1% Bromophenol blue, 20% glycerol, 4% V/V, β-mercaptoethanol). The reaction products were boiled for 10 min and analyzed on 4–20% SDS-polyacrylamide gradient gel and the protein was transferred to nitrocellulose membrane as described [25]. Autoradiography was performed using X-ray film. The membrane was then further analyzed by Western blot using HIV p7 antibodies (1:2,000 dilution) and developed by ECL Western blotting detection reagents (Amersham).

2.5. V8 proteinase digestion

The HIV p7 protein was labeled with ^{32}P as described above using the P1 nuclease assay. The pH of the reaction mixture was adjusted to pH 7.5 with 2.5 μl of 1 M Tris pH 10.5. Four units of V8 proteinase (Sigma) were added and the reaction was incubated for 1 h, at 37°C. The reaction was stopped by 5 μl of protein sample buffer and loaded on 10–18% gradient SDS-PAGE containing 7 M urea for separation of low molecular mass polypeptides.

The proteins were then transferred to nitrocellulose membrane followed by autoradiography and Western blot detection using HIV p7 antibodies.

3. Results

3.1. Purity of the p7 NC protein preparation

The p7 NC protein used in this study was from the same batch of purified p7 preparation used in experiments reported elsewhere [9]. To verify the purity of this preparation, increasing amounts of this protein (1–10 μg) were analyzed on SDS-polyacrylamide gel electrophoresis following silver staining. Fig. 1A shows a major band of 7 kDa and a ladder of higher molecular weight bands. These additional bands represent a ladder of multimers of 7 kDa and could be dissociated into 7 kDa monomers by treatment with 4 M urea (Fig. 1B), it should be notice that this method was previously used for separation of histones and non-histone nuclear proteins. No other bands were observed, even at high protein concentrations, indicating that the p7 protein is highly purified. Interestingly, this tendency of p7 protein to form multimers at high concentrations resemble other nuclear non-histone protein.

3.2. p7 NC binds and unwinds supercoiled DNA

The DNA binding capacity of the zinc-free and zinc-bound p7 was examined with the plasmid pUC-19. The plasmid prep-

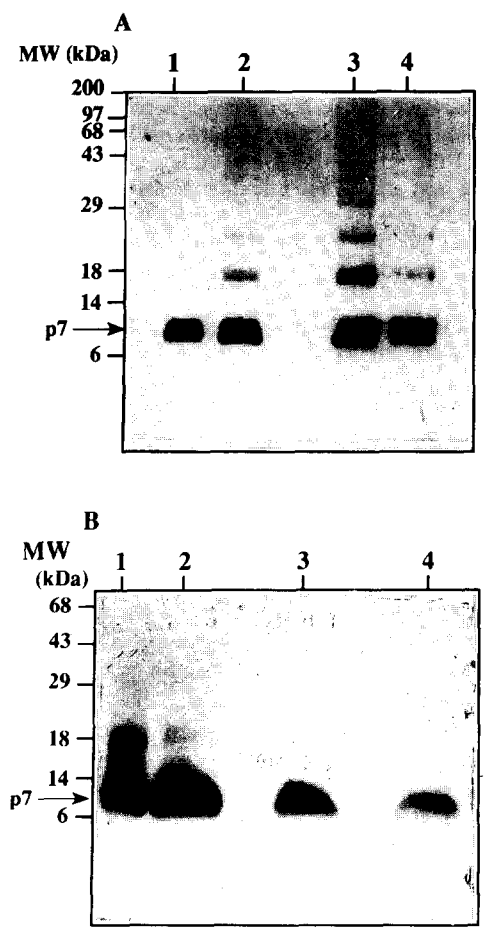

Fig. 1. Examination of the purity of the p7 NC preparation . Increasing amounts of the p7 protein were loaded on 10% SDS polyacrylamide gel (A) or on 15% SDS polyacrylamide gel containing 4 M urea (B). 1 μg protein (lanes 1A,4B), 5 μg protein (lanes 2A,3B), 7.5 μg protein (lanes 3A,2B), 10 μg protein (lanes 4A,1B). The gel was stained using the silver staining reagent (BRL).

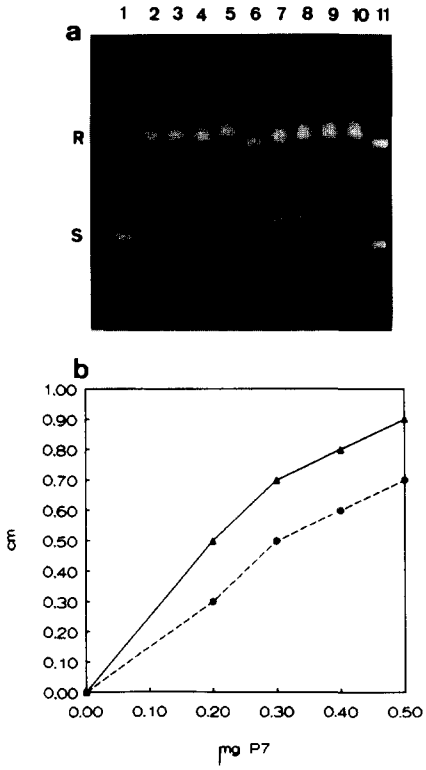

Fig. 2. p7 binds and unwinds supercoiled DNA. Increasing amounts of the zinc-free form of p7 (lanes 2–6) or the zinc-bound form (lanes 7–11) were added to a DNA binding buffer and contained 0.25 μg supercoiled pUC-19 DNA. The reaction products were analyzed by agarose gel electrophoresis (a): 0.2 μg p7 (lanes 2,7), 0.3 μg (lanes 3,8), 0.4 μg (lanes 4,9), 0.5 μg (lanes 5–6 and 10–11). Lane 1, supercoiled pUC-19 DNA; lanes 6 and 11 demonstrated the results obtained after proteinase K treatment. (b) The relative supercoiled DNA migration rate was calculated from the results demonstrated in part (a). ▲, zinc-bound form; ●, zinc-free form. Each data point represents a substraction between the migration distance of the control supercoiled DNA and the migration distance observed in the presence of p7.

aration used in this study contained both supercoiled and relaxed molecules.

Fig. 2a, shows that the migration of both DNA forms was retarded in the presence of either the zinc-bound or zinc-free p7, although the retardation of the supercoiled DNA was more prominent than that of the relaxed DNA. Both proteins imposed this retardation in a dose-dependent manner (see lanes 2–5 for zinc-free p7 and lanes 7–10 for the zinc-bound p7). Proteinase K digestion of the reaction products, formed by the highest amount of either the zinc free (lane 6) or zinc-bound (lane 11) p7, completely abolished their retardation. It is therefore evident that this retardation resulted from the binding of these proteins, rather than from permanent conformational changes of the DNA. Interestingly, the relative amounts of the supercoiled DNA molecules decreased by increased doses of the proteins, whereas that of the unwound molecules correspondingly increased. This quantitative transition remained unchanged following treatment with proteinase K (lane 6 and 11, for the zinc-free and bound p7 respectively), indicating that the binding of p7 eventually leads to a complete unwinding of some of the supercoiled DNA molecules. Higher levels of p7 protein formed protein–DNA complexes which did not enter into the agarose gel; while treatment of these complexes with proteinase K increased the amount of the relaxed form of the DNA (data not shown). It is notable that at equal concentrations of protein, the retardation imposed by the zinc-bound p7 was greater than that imposed by the zinc-free p7 (Fig. 2b). This could

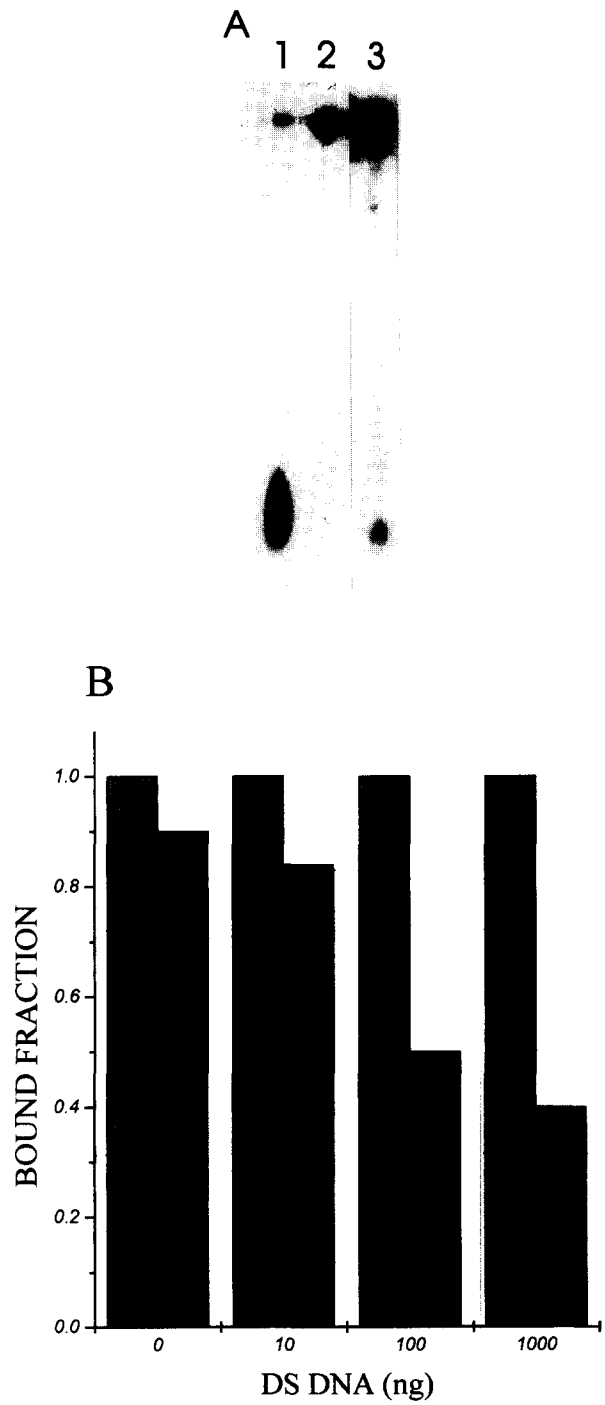

Fig. 3. Binding of p7 to single-stranded versus double-stranded pUC-19 DNA fragments. 0.3 μg of zinc-bound (lane 2, shading) or zinc-free (lane 3, filled) p7 protein were added to DNA binding buffer containing ^{32}P-labeled single-stranded DNA fragments. The reaction products were analyzed on 1% agarose gel followed by autoradiography (A). The binding assay was also performed in the presence of increasing doses of unlabeled double-stranded DNA fragments and densitometric analysis was performed on the autoradiogram and the bound single-stranded DNA fraction was calculated (B). Lane 1A represent ^{32}P-labeled single-stranded DNA.

either reflect differences in the DNA binding capacity of these two forms of the protein or result from different conformation of their DNA protein complexes.

3.3. Binding of p7 to single-stranded DNA

The binding capacity of p7 to supercoiled and relaxed double-stranded DNA and its unwinding activity, poses two major questions. (i) Since it is known that NC proteins from retroviruses bind single-stranded DNA [18–20], does p7 protein bind single-stranded region present in the supercoiled DNA or may it also bind the double-stranded regions? (ii) How does p7 protein unwind the covalently closed circular supercoiled DNA?

To address the first question, we examined the binding capacity of its zinc-free and -bound forms to ^{32}P-labeled single-stranded DNA fragments derived from the pUC-19 plasmid. We developed an agarose gel shift assay which enabled us to examine the DNA binding capacity of this protein to single-stranded fragments of DNA. Fig. 3A demonstrates a significant DNA retardation with both of the p7 forms, suggesting that both forms of the protein efficiently bind single-stranded DNA fragments.

3.4. Comparison of p7 affinity to double- versus single-stranded DNA

To compare the affinity of p7 to double- versus single-stranded DNA, the two forms of p7 protein were incubated with labeled single-stranded DNA in the presence of increasing amounts (10 ng, 100 ng, 1,000 ng) of unlabeled double-stranded DNA fragments derived from pUC-19 plasmid (*Aat*II–*Eco*RI).

The reaction products were analyzed on agarose gel electrophoresis followed by autoradiography. Densitometric analysis on the autoradiogram was performed and the single-stranded DNA-bound fraction was calculated. The results demonstrated in Fig. 3B indicated that the zinc-bound form of the NC protein had a greater affinity to single-stranded DNA than to double-stranded DNA. Incubation of the protein in the presence of increasing amounts of double-stranded DNA fragments did not influence its potential to bind single-stranded DNA. However,

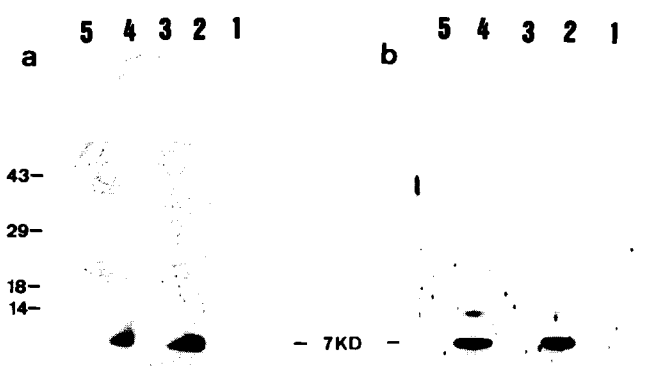

Fig. 4. p7-DNA cleavable complex formation. 0.6 μg of zinc-free (lanes 4a,5a and 4b,5b) or zinc-bound (lanes 2a,3a and 2b,3b) of p7 protein were added to the DNA binding buffer containing ^{32}P-labeled single-stranded DNA. P1 nuclease assay was performed and the reaction product was analyzed on 4–20% polyacrylamide gradient gel followed by Western blot analysis and autoradiography (a) or detection by p7 antibodies (b). Lanes 3a, 5a and 3b,5b represent the results obtained after proteinase K treatment. The P1 nuclease treatment of the ^{32}P-labeled DNA is shown in lane 1.

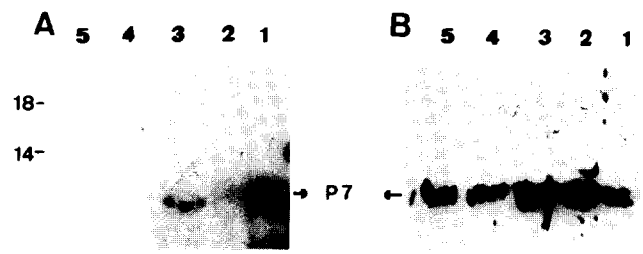

Fig. 5. Inhibition of ^{32}P transfer from labeled DNA to HIV-NC protein by unlabeled pUC-19 DNA. 0.6 mg of zinc-bound p7 protein was added to the DNA binding buffer containing ^{32}P labeled single-stranded DNA (lanes 1A,1B) or dCTP (^{32}P) nucleotides (lanes 5A,5B). Increasing amounts of unlabeled pUC-19 DNA were added to the reaction: 10 ng (lanes 2A,2B), 100 ng (lanes 3A,3B), 1,000 ng (lanes 4A,4B). P1 nuclease assay was performed and the reaction products were analyzed on 10% polyacrylamide gel, transfered to nitrocellulose membrane followed by autoradiography (A) or detection by p7 antibodies (B).

increasing amounts of double-stranded DNA fragments abrogated the potential of the zinc-free form of p7 to bind single-stranded DNA fragments (i.e. 40% of the labeled single-stranded DNA were bound by the protein in the presence of 1 μg of double-stranded DNA). The zinc-bound form of p7 specifically bound single-stranded DNA whereas double-stranded DNA even a concentration of 100-fold, did not prevent the binding of this protein. On the other hand, the zinc-free form of p7 bound the two DNA structures. These data suggest that the two forms of the p7 differ in their DNA binding characteristics.

3.5. Formation of p7 protein–DNA cleavable complex

The HIV p7 protein, as other retroviral NC proteins contains regions which are positively charged and contribute to electrostatic interactions between p7 protein and the negatively charged phosphate backbone of the DNA. However, these electrostatic interactions cannot account for the p7 unwinding activity because to unwind a closed circular supercoiled DNA plasmid in an irreversible manner, a single-stranded DNA break in the phosphodiester backbone of the DNA should be formed. It has been proven that for DNA relaxing enzymes, such as DNA topoisomerases, the relaxation activity involves a formation of an enzyme–DNA cleavable complex as an intermediate product, in which a covalent bond is formed between the active site of the enzyme and the phosphate moiety from the DNA backbone [23,26–27]. Thus, topoisomerases unwind supercoiled DNA by their nicking closing activity [26–27]. Therefore, we investigated whether p7 protein unwinds supercoiled DNA by the same mechanism and therefore we used the protein–DNA cleavable complex assay. If such a complex of protein–DNA is formed by covalent bonds between the protein and the phosphate residues of the DNA , these residues should remain bound to the protein after P1 nuclease digestion. Thus, the ^{32}P-labeled single-stranded DNA fragments were interacted with either of the two p7 forms, followed by digestion with P1 nuclease. After boiling in the presence of SDS, the reaction products were subjected to SDS polyacrylamide gel electrophoresis and then transferred to a nitrocellulose filter by Western blotting. The blot was first exposed to X-ray film for autoradiography and then analyzed with p7 specific antibodies and the ECL detection reagent. As can be seen in Fig. 4a,b, a 7 kDa

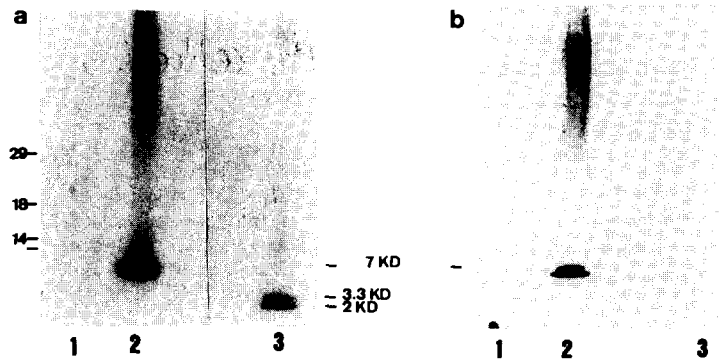

Fig. 6. V8 proteinase digestion of ^{32}P-labeled p7 obtained by P1 nuclease assay. The labeled p7 (lane 2) was treated with 4 units of V8 proteinase (lane 3) and the reaction products were analyzed on 10–18% polyacrylamide gradient gel containing 7 M urea. The proteins were transferred to nitrocellulose and autoradiography was performed (a) and the presence of the protein was also detected using HIV p7 antibodies (b).

band was detected in both procedures (lanes 2 and 4), suggesting that the phosphate residues did indeed remain linked to the p7 protein. This was further substantiated by the finding that this band was eliminated by treating the P1 digestion product with proteinase K prior to being loaded onto the gel (Fig. 4a,b, lanes 3 and 5). No significant differences were detected between the two p7 forms in this analysis. These results suggest that the p7 protein was labeled by transfer of ^{32}P from the DNA to the protein. It is possible that the p7 was labeled by free labeled dCTP which might have remained with the DNA preparation after its purification. Therefore, a control assay was performed in which the template DNA was omitted from the random primer reaction. The reaction mixture was purified through a Sephadex G-50 column (nick column) and an equivalent volume to that used with the labeled DNA reaction was added to the reaction mixture containing both forms of p7 protein. The results depicted in Fig. 5 are for the zinc-bound p7, similar results were obtained for the free zinc p7 (data not shown). Fig. 5 lanes 5A and 5B, show that the p7 protein was not labeled by free dCTP. The labeling of p7 was observed only in the presence of labeled DNA (lanes 1A,1B). Moreover, increasing doses of unlabeled pUC-19 supercoiled plasmid added together with ^{32}P-labeled DNA completely prevented the ^{32}P labeling of the p7 protein (Fig. 5A,B, lanes 2–4). These results strongly indicated that the radio-labeling of p7 protein was due to the ^{32}P transfer from the labeled DNA. Since boiling of the reaction products twice (once prior to the P1 digestion and once in the presence of SDS) did not affect the labeling of p7, it could be assumed that a very stable bond (probably a covalent bond) was formed between the labeled phosphate moiety in the DNA backbone and the p7 protein.

To determine whether p7 binds the DNA through one or more bonds, the P1 nuclease digested product was treated with V8 proteinase which cleaves p7 to several peptides. Fig. 6a shows two ^{32}P labeled peptides, one of 3.3 kDa and the second of 2 kDa (lane 3). This may suggest that phosphate residues of the DNA were bound to at least two sites in the p7 protein. We cannot rule out the possibility that these two bands reflected incomplete digestion of the p7 protein by V8 protease or that additional labeled peptides in the V8 digest existed that could not be detected because of their minute size. The p7 antibodies recognized the labeled p7 protein (Fig. 6b, lane 2), but not the p7 fragments (Fig. 6b, lane 3).

4. Discussion

The ability of retroviral NC protein to bind nucleic acids has been previously demonstrated for several retroviruses [18–20]. It has recently been shown that HIV p7 NC binds tightly to DNA fragments, including supercoiled plasmid containing proviral DNA sequences, and forms a high molecular weight DNA–protein complexes [28]. Since HIV p7 NC contains two zinc finger binding domains [16,21] we examined the DNA binding properties and characteristics of the zinc-bound and zinc-free forms of the HIV NC. Our data demonstrate that both forms (in addition to their ability to bind single-stranded DNA) are capable of binding double-stranded supercoiled DNA plasmid and the zinc-bound form was more effective in this respect. Our data also suggest that the affinity of the zinc form to single-stranded DNA fragments is significantly higher than for double-stranded DNA fragments, while that of the zinc-free form binds both DNAs. These data may suggest that zinc influences the DNA binding properties of the NC. In addition, we found that when incubated with ^{32}P-labeled single-stranded DNA followed by P1 nuclease digestion, the p7 protein was labeled. We demonstrated that the labeling of p7 can be observed only with labeled DNA and not by free labeled nucleotide. Moreover, we found that the labeling of p7 protein significantly decreased, when unlabeled DNA was present in the reaction mixture. These data strongly indicate that the p7 protein is labeled by transfer of the ^{32}P from the DNA backbone to the protein. Since the labeling of the p7 protein by this procedure was stable in the presence of SDS and boiling, it is clear that the interaction between the phosphate moiety from the DNA and the protein is not due to electrostatic charge or hydrophobic interaction. Thus, we may assume that the p7 protein forms a covalent bond with the phosphate moiety of the DNA backbone. Indeed it was shown that using the same P1 nuclease method DNA topoisomerase I protein is labeled and this labeling is a consequence of a covalent bond formed between the enzyme and the phosphate moiety of the DNA backbone [23,24,29]. It has been previously shown that NC proteins are capable of unwinding a folded RNA structure by binding selectively to single-stranded RNA [21]. We demonstrated that the HIV NC protein unwinds supercoiled DNA plasmid. Since NC protein binds single-stranded DNA more efficiently than double-stranded DNA, we suggest that the HIV p7 binds sin-

gle-stranded regions present in the supercoiled double-stranded DNA. We showed that the products of the unwinding activity are permanently fully relaxed DNA plasmid molecules. In order to obtain this DNA form from a covalently closed circular supercoiled DNA, a nick had to be formed in the phosphodiester bond of the supercoiled DNA. It is possible that this nick is formed by the p7 protein due to its ability to bind, presumably, covalently, the phosphate moiety of the DNA backbone. This bond formation is preceded by cleavage of the DNA strand and will lead to the relaxation of the supercoiled DNA.

This relaxation mechanism resembles the first step of DNA topoisomerase I activity and is consistent with our previously reported results of the association of the HIV topoisomerase I activity and the viral NC protein [7]. However the purified p7 protein did not form the typical ladders of partially relaxed molecules which characterized the topo I activity. It is interesting to mention that we found that V8 digestion of the ^{32}P-labeled p7 protein (which was labeled through ^{32}P transfer from DNA to the protein) yielded two ^{32}P-labeled fragments, (3 kDa and 2.3 kDa). This observation suggest that at least one, and possibly two, bonds were formed between the p7 and the DNA. In accordance with the published sequence of the p7 [9], digestion of V8 proteinase produces 2.8 kDa, 2.84 kDa, 1.2 kDa and 0.5 kDa fragments. The 2.8 kDa and the 2.84 kDa, each contain one zinc finger domain. Since we found that two labeled fragments were observed after the V8 proteinase digestion and their size, according to our electrophoresis gel conditions, were 3 kDa and 2.3 kDa, we may assume that the two zinc finger domains of the p7 are involved in the bond formation between the phosphate moiety in the DNA backbone and the p7 protein. This is a possible explanation since it was shown that zinc coordination in *E. coli* DNA topoisomerase I is required for the transition of the noncovalent complex with DNA to the cleavable state [29].

The ability of p7 protein to unwind supercoiled DNA suggests an additional physiological role for this protein. It is possible that it is involved in the integration process of proviral DNA into the cellular genome influences the topological state of the viral or cellular DNA and thus facilitates the recombinational process between the two DNAs. Moreover, during the reverse transcriptional process of the viral RNA, especially during the synthesis of the second proviral DNA strand, topologically restricted structures may be formed. These structures should be removed in order to permit efficient DNA synthesis. Thus, p7 NC unwinding activity may be involved in this process, and indeed, it has been postulated that the NC protein play a role in assisting the replication process carried out by the reverse transcriptase [30].

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References

[1] Barre Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Charmaret, S., Gruest, J., Dawguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. and Montagnier, L. (1983) Science 220, 868–871.
[2] Levy, J.A., Hoffman, A.D., Kramer S.M., Landis J.A., Shimabukuro, J.M. and Oshiro, L.S. (1984) Science 225, 840–842.
[3] Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. (1984) Science 224, 497–500.
[4] Rosen C.A. (1991) TIG J. 7, 9–14.
[5] Varmus, H.E. and Swanstrom, R. (1985) in: RNA Tumor Viruses (R. Weiss, N. Teich, H. Varmus and J. Coffin, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[6] Fujiwara, T. and Mizucchi, K. (1988) Cell 54, 497–504.
[7] Priel, E., Showalter, S.D., Roberts, M., Oroszlan, S., Segal, S., Aboud, M. and Blair, D.G. (1990) EMBO J. 9, 4167–4172.
[8] Priel, E., Aflalo, E., Chechelnitsky, G., Benharroch, D., Aboud, M. and Segal, S. (1993) J. Virology, 67, 3624–3629.
[9] Henderson, L.E., Bowers, M.A., Sowder II, R.C., Serabyn, S.A., Johnson, D.G., Bess Jr., J.W., Arthur, L.O., Bryant, D.K. and Fenselan, C. (1992) J. Virol. 66, 1856–1865.
[10] Aldovini A. and Young, R.A. (1990) J Virol. 64, 1920–1926.
[11] Gorelick, R.J., Henderson, L.E., Hamser, J.P. and Rein, A. (1988) Proc. Natl. Acad. Sci. USA 85, 8420–8424.
[12] Gorelick, R.J., Nigida Jr., S.M., Bess Jr., J.W., Arthur, L.O., Henderson, L.E. and Rein, A. (1990) J. Virol. 64, 3207–3211.
[13] Meric, C. and Spahr, R.-F. (1986) J. Virol. 60, 450–459.
[14] Meric, C., Gouilloud, E. and Spahr, R.-F. (1988) J. Virol. 62, 3328–3333.
[15] Meric, C. and Goff, S.P. (1988) J. Virol. 63, 1558–1568.
[16] Bess Jr., J.W., Powell, P.J., Issaq H.J., Schumack, L.J., Grimes, M.K., Henderson, L.E. and Arthur, L.O. (1992) J. Virol. 66, 840–847.
[17] Pratts, A.C., Sarih, L., Gabus, C., Litvak, S., Keith, G. and Darlix, J.L. (1988) EMBO J. 7,1777–1783.
[18] Karpel R.L., Henderson, L.E. and Oroszlan, S. (1987) J. Biol. Chem. 262, 4961–4967.
[19] Copeland, T.D., Morgan, M.A. and Oroszlan, S. (1984) Virology 133, 137–145.
[20] Schulein M., Burnette, W.N. and August, J.T. (1978) J. Virol. 26, 54–60.
[21] Summers, M.F., Henderson, L.E., Chance, M.R., Bess, J.W., South T.L., Blake, P.R., Sagi, I., Perez-Alvarado, G., Sowder III, R.C., Hare, D.R. and Arthur, L.O. (1992) Protein Sci. 1, 563–574.
[22] Sambrook, J., Fritsch E.F., Maniatis, T. (1989) in: Molecular Cloning: a Laboratory Manual, 2nd edition., pp. 6.45 Cold Spring Harbor Laboratory Press.
[23] Chin Chow, K., Johnson, T.L. and Pearson, G.D. (1985) Biotechniques 3, 290–297.
[24] Shuman, S. and Moss, B. (1987) Proc. Natl. Acad. Sci. USA 84, 7478–7482.
[25] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[26] Wang, J.C. (1985) Annu. Rev. Biochem. 54, 665–697.
[27] Wang, J.C. (1987) Biochem. Biophys. Acta 909, 1–9.
[28] Lapadat-Tapolsky, M., De Rocquigny, H., Van-Gent, D., Roques, B., Plasterk, R. and Darlix, J.C. (1993) Nucleic Acids Res. 21, 831–839.
[29] Tse-Dinh, Y.-C. (1991) J. Biol. Chem. 266, 14317–14320.
[30] Darlix, J.C., Vincent, A., Gabus, C., De Rocquigny, H., Roques, B. (1993) CR Acad. Sci. Paris/Life Sci. 316, 763–771.