

DNA Strand Exchange and Selective DNA Annealing Promoted by the Human Immunodeficiency Virus Type 1 Nucleocapsid Protein

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Nucleocapsid protein (NC) of human immunodeficiency virus type 1 (HIV-1) was expressed in *Escherichia coli* and purified. The protein displayed a variety of activities on DNA structure, all reflecting an ability to promote transition between double-helical and single-stranded conformations. We found that, in addition to its previously described ability to accelerate renaturation of complementary DNA strands, the HIV-1 NC protein could substantially lower the melting temperature of duplex DNA and could promote strand exchange between double-stranded and single-stranded DNA molecules. Moreover, in the presence of HIV-1 NC, annealing of a single-stranded DNA molecule to a complementary DNA strand that would yield a more stable double-stranded product was favored over annealing to alternative complementary DNA strands that would form less stable duplex products (selective annealing). NC thus appears to lower the kinetic barrier so that double-strand ↔ single-strand equilibrium is rapidly reached to favor the lowest free-energy nucleic acid conformation. This activity of NC may be important for correct folding of viral genomic RNA and may have practical applications.

Nucleocapsid (NC) proteins are small structural proteins present in the cores of all retroviruses (51). In a virion, the dimeric viral RNA genome is thought to be covered with NC proteins. NC proteins are basic, typically include one or two zinc finger-like motifs, and can bind to either DNA or RNA, with a preference for single strands (13, 24, 25, 28, 29, 31, 37, 46, 48, 52). NC protein is synthesized as a part of the Gag or Gag-Pol precursor polyprotein. These polyproteins assemble into virus particles and package the viral RNA. The polyproteins are subsequently cleaved by the viral protease to form mature proteins, including NC protein (51). Viruses with mutations in the region encoding NC show disrupted packaging of viral RNA, without other apparent defects in viral particle formation (4, 14, 16–20, 32, 33, 38). In the case of avian leukosis virus, it is not yet clear whether mutations in Cys-His motifs directly block packaging of its genome or reduce the stability of packaged RNA (5). These results have led to the proposal that the NC region of the Gag polyprotein is responsible for recognition of the packaging signals on viral RNAs. In support of this notion, purified Gag polyprotein as well as the NC protein of human immunodeficiency virus type 1 (HIV-1) bound HIV RNA with some specificity (6, 30).

In a test tube, NC protein has been shown to promote annealing of primer tRNA and DNA oligonucleotides to viral RNA and to promote dimerization of model viral genomic RNAs containing a dimer linkage sequence (7, 12, 42, 43). Recently, a DNA renaturation activity of HIV-1 NC was described, and its kinetic properties were characterized (13). NC has also been shown to stimulate both formation of strong-stop cDNA and strand transfer by reverse transcriptase (28).

In this study, we tested the possibility that HIV-1 NC could promote transfer of DNA strands between alternative DNA

duplexes by virtue of its DNA-annealing and DNA-melting activities. We found that NC could overcome the kinetic barrier to equilibrium between alternative pairs of complementary strands. This activity might play important roles in the viral life cycle.

MATERIALS AND METHODS

DNA oligonucleotides. DNA oligonucleotides were obtained from the PAN facility (Stanford University) and Operon (Alameda, Calif.). The sequences of these oligonucleotides are as follows: 24A–, ACT GCT AGA GAT TTT CCA CAA GTC; 24A+, GAC TTG TGG AAA ATC TCT AGC AGT; 28X–, TCG AAC TGC TAG AGA TTT TCC ACA GAC T; 21A–, ACT GCT AGA GAT TTT CCA CAT; 21A+, ATG TGG AAA ATC TCT AGC AGT; 28A–, ATG CAC TGC TAG AGA TTT TCC ACA AGT C; 28A+, GAC TTG TGG AAA ATC TCT AGC AGT GCA T; and 32A–, ACT GCT AGA GAT TTT CCA CAT AGT ATC GAA TT.

Preparation of HIV-1 NC protein. The gene encoding the 71-amino-acid form of HIV-1 NC was amplified by PCR (19) from pNL4-3 plasmid DNA (1). At the same time, codons encoding the sequence Met-(His)₆ were added to the N-terminal end of the native NC gene for the purpose of one-step purification using Ni-nitrilotriacetic acid resin (Qiagen, Chatsworth, Calif.). This hybrid gene was cloned into the T7-7 vector (49) to make plasmid pHIVp7his1. This plasmid was introduced into *Escherichia coli* BL21(DE3), which has an isopropyl-1-thio-β-D-galactopyranoside (IPTG)-inducible T7 RNA polymerase gene (47). Transformed cells were grown in 2 liters of LB medium with 70 μg of carbenicillin per ml at 37°C until they reached an optical density at 595 nm of 0.5. IPTG was then added at 1 mM, and incubation was continued for 2 h before harvest. Harvested cells were suspended in 10 ml of 50 mM Tris-Cl (pH 7.5)–10% sucrose (TS) and frozen in liquid nitrogen. Frozen cells were thawed, and 4 ml of TS, 1.5 ml of 50 mM Tris-Cl (pH 7.5)–(0.3 M spermidine–10% sucrose–0.1 M NaCl, 14 μl of β-mercaptoethanol–2 M Tris base, 20 μl of

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0.25 M phenylmethylsulfonyl fluoride (PMSF), 0.35 ml of 10-mg/ml lysozyme, and 0.2 ml of 10% Triton X-100 were added. After successive incubations at 0°C for 50 min and at 37°C for 3 min, the cell suspension was centrifuged in a Sorvall SS34 rotor at 14,000 rpm for 30 min. NC protein was extracted from the pellet with 10 ml of extraction buffer (50 mM Tris-Cl [pH 7.5], 100 mM NaCl, 15% glycerol, 0.1% Triton X-100, 10 mM β -mercaptoethanol, 0.25 mM PMSF, 6 M guanidium-HCl), and insoluble material was removed by centrifugation (Beckman SW41 rotor, 35,000 rpm for 20 min). The supernatant was loaded directly onto a 1-ml column of Ni-nitrilotriacetic acid agarose resin in a 3-ml syringe. The resin was then washed with 40 ml of extraction buffer with 1 mM imidazole and 30 ml of buffer A (50 mM Tris-Cl [pH 7.5], 100 mM NaCl, 15% glycerol, 0.1% Triton X-100, 10 mM β -mercaptoethanol, 0.25 mM PMSF) plus 20 mM imidazole. Recombinant NC protein was then eluted with 25 ml of buffer A plus 100 mM imidazole followed by 25 ml of buffer A plus 200 mM imidazole. Approximately 15 mg of NC was recovered, and the peak fractions used in this work were judged to be >95% pure by Coomassie brilliant blue staining of sodium dodecyl sulfate (SDS)-polyacrylamide gel. Protein concentration was determined by using a Bradford protein determination kit (Bio-Rad) with bovine serum albumin as a standard.

Labeling of DNA substrates. The 93-mer DNA fragment was derived from pT7-5 DNA (49) digested with *Hind*III and *Pvu*II. The 5' ends of this DNA fragment were labeled with [γ -³²P]ATP (ICN) and T4 polynucleotide kinase (New England Biolabs) (44), and the labeled DNA fragment was purified by electrophoresis through a 10% polyacrylamide-7 M urea gel in Tris-borate-EDTA (TBE) buffer (44). Specific activity of the radiolabeled DNA was estimated by thin-layer chromatography. 5' labeling of oligonucleotides was done with [γ -³²P]ATP (ICN) and T4 polynucleotide kinase, and labeled fragments were purified through a spin column of Sephadex G-15 (Pharmacia) (44).

DNA annealing assay. The standard reaction mixture (10 μ l) contained 50 mM Tris-Cl (pH 7.9), 1 mM EDTA, 1 mM dithiothreitol, 0.1% Triton X-100, and 3 mM MgCl₂ (standard buffer for annealing assay). ³²P-labeled DNA substrates and HIV-1 NC were added as described for each assay. Double-stranded DNA (dsDNA) substrates were initially heat denatured by incubation at 94°C for 5 min in TE (10 mM Tris-Cl [pH 7.9], 1 mM EDTA) and then rapidly chilled on ice before addition to the reaction mixture. Complementary oligonucleotide DNAs were added separately at the start of the reaction. After the reaction, 5 μ l of stopping solution (0.25% bromophenol blue, 20% glycerol, 20 mM EDTA, 0.2% SDS, 0.4 mg of yeast tRNA [Sigma] per ml) was added, and the samples were electrophoresed on a 10 or 15% polyacrylamide gel in TBE at 4°C. The gel was then dried, and an autoradiogram was exposed at -40°C. Quantitation of the radioactivity was done with a Molecular Dynamics PhosphorImager.

RESULTS

Stimulation of DNA annealing by HIV NC. Figure 1A shows the time course for annealing of 93-mer complementary DNAs with and without purified recombinant His₆-tagged HIV-1 NC protein. In the absence of NC protein, only 10% of the DNA had renatured after 30 min, while in the presence of the recombinant His₆-tagged NC protein preparation, renaturation was almost complete at the first time point (10 s). Thus, this NC protein preparation accelerated annealing by at least 2,000-fold under these conditions. Overexpression in bacterial cells with a histidine tag, and the guanidium chloride treatment

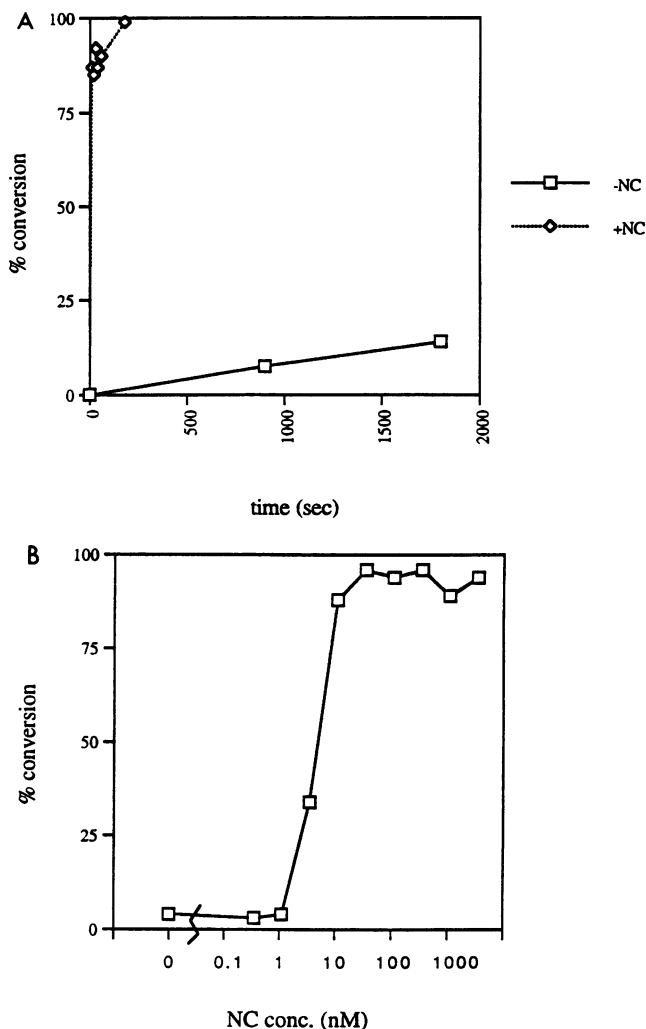


FIG. 1. (A) Time course of annealing of 93-mer complementary DNAs. The 12.5- μ l reaction mixture contained 50 mM Tris-Cl (pH 7.9), 1 mM EDTA, and 0.1% Triton X-100, with 1 nM heat-denatured ³²P-labeled 93-mer DNA with or without 1 μ M HIV-1 NC protein. After incubation at 37°C for the indicated length of time, reaction mixtures were treated as described in Materials and Methods, and ssDNA and renatured dsDNA were resolved by 10% polyacrylamide-TBE gel electrophoresis. (B) Titration of HIV-NC in an annealing reaction. The indicated amount of NC protein was added to 12.5 μ l of standard reaction mixture with 1 nM ³²P-labeled 93-mer DNA. After incubation at 37°C for 2 min, samples were resolved by gel electrophoresis and the amounts of ssDNA and dsDNA were determined.

and refolding protocol used for purification, therefore did not appear to compromise the protein's activity. MgCl₂ stimulated this reaction, with an optimal concentration of between 2 and 4 mM. No effect of ATP was observed (data not shown). For 1 nM (as double-stranded molecules) of 93-mer DNA, full activity required 11 nM NC under these conditions (Fig. 1B), or one NC molecule per 17 nucleotides.

Stimulation of annealing by NC was also observed with short DNA oligonucleotides (Fig. 2A), but at high concentrations, NC prevented annealing of these short DNAs (data not shown). A possible explanation for this inhibition was that by binding more strongly to single-stranded DNA (ssDNA) than dsDNA, NC could shift the equilibrium toward the single-

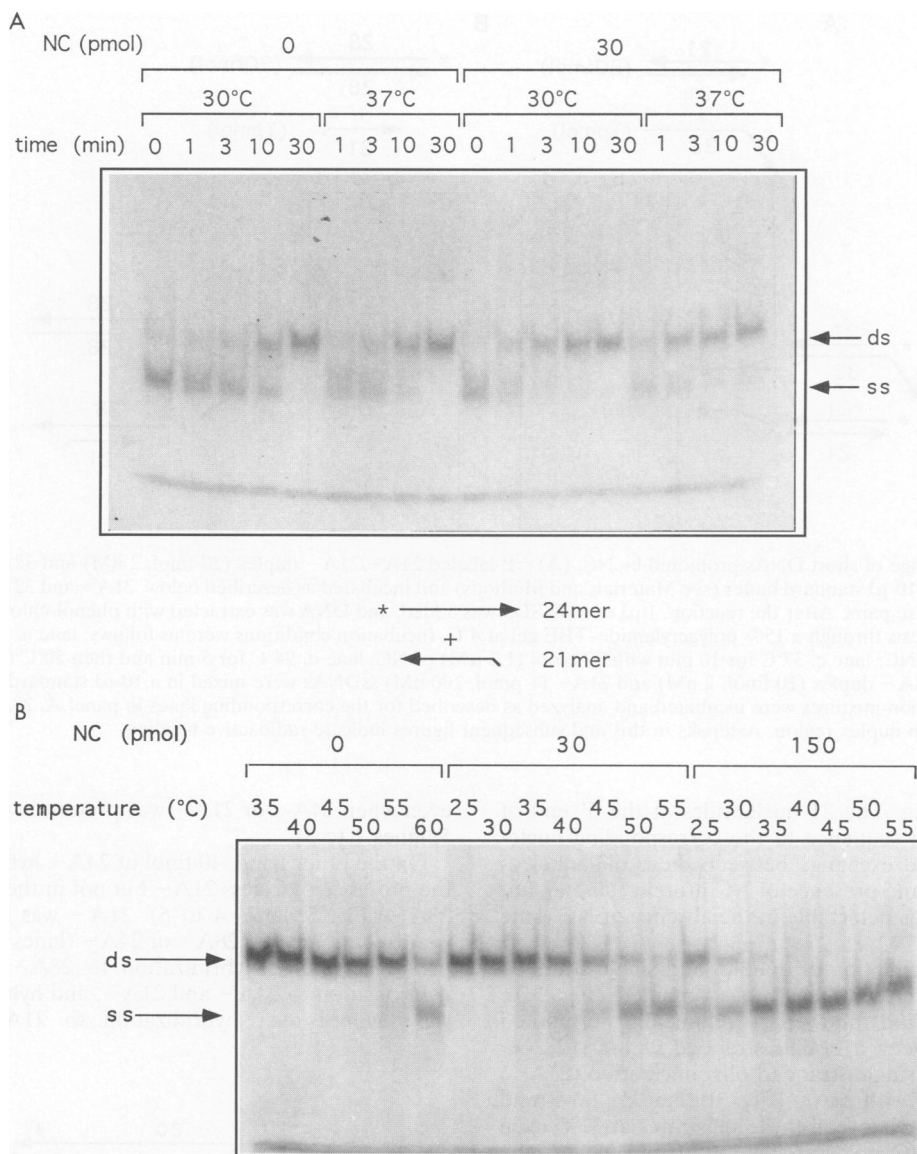


FIG. 2. (A) Effect of NC on annealing of short DNA oligonucleotides. A 10- μ l reaction mixture contained standard reaction buffer with 2 fmol (0.2 nM) of 32 P-labeled 24A+ oligonucleotide and 10 fmol (1 nM) of unlabeled 21A- oligonucleotide with or without 30 pmol (3 μ M) of NC. 24A+ and 21A- can form 20 contiguous base pairs (see Materials and Methods). 21A- was added at the start of the incubation. After the reaction, the samples were treated as described in Materials and Methods and analyzed on a 15% polyacrylamide-TBE gel. (B) Effect of NC on melting of short duplex DNA. A 5- μ l reaction mixture contained a standard buffer and 32 P-labeled 24A+ (1 fmol, 0.2 nM) and unlabeled 24A- (5 fmol, 1 nM). Oligonucleotides 24A+ and 24A- have the potential to form a complete duplex (see Materials and Methods). These two DNAs were incubated together at 30°C for 10 min before addition of NC to allow all labeled 24A+ to form a duplex. Indicated amounts of NC were then added to the reaction mixture, and incubation was carried out successively at 25°C for 5 min, 30°C for 5 min, 35°C for 5 min, 40°C for 5 min, 45°C for 5 min, 50°C for 5 min, 55°C for 5 min, and 60°C for 5 min. After each step of incubation, one test tube was transferred to ice, and 5 μ l of stopping solution was added immediately. Samples were analyzed on a 15% polyacrylamide-TBE gel.

stranded form. To test this hypothesis, excess NC was added to a pair of preannealed complementary 24-mer DNAs (0.2 nM 32 P-24A+ and 1 nM of 24A-), and the temperature was raised in steps of 5°C (Fig. 2B). In the absence of NC, the single-stranded form was detected only above 55°C, but in the presence of 30 pmol (6 μ M) of NC, substantial ssDNA was observed at 40°C, and at 150 pmol (30 μ M) of NC, the single-stranded form was detected at 25°C. Thus, NC can promote denaturation of a dsDNA oligonucleotide.

Strand exchange reaction promoted by NC protein. Since we

found that NC could stimulate both single-strand \rightarrow double-strand (ss \rightarrow ds) and ds \rightarrow ss transitions under different experimental conditions, we tested whether both of these transitions could be promoted by NC under identical conditions, in the same test tube, by monitoring strand exchange between complementary double-stranded and single-stranded DNA oligonucleotides. In the experiment shown in Fig. 3A, a 32 P-labeled 21-mer double-stranded oligonucleotide (32 P-21A+ unlabeled 21A-) was mixed with a 50-fold excess of a single-stranded 32-mer (32A-) and incubated at 37°C in the presence or

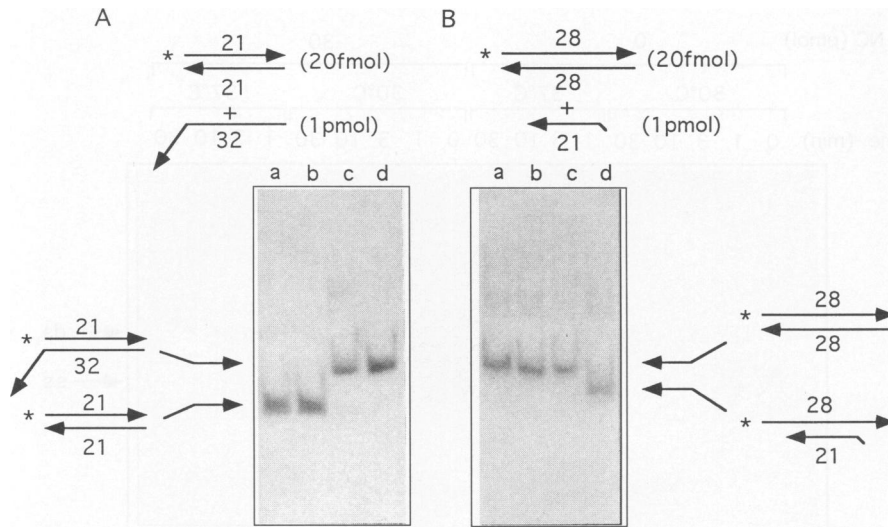


FIG. 3. Strand exchange of short DNAs promoted by NC. (A) ³²P-labeled 21A+/21A- duplex (20 fmol, 2 nM) and 32A- (1 pmol, 100 nM) ssDNAs were mixed in a 10- μ l standard buffer (see Materials and Methods) and incubated as described below. 21A+ and 32A- have the potential to form 21 contiguous base pairs. After the reaction, 1 μ l of 10% SDS was added, and DNA was extracted with phenol-chloroform (1:1) and then subjected to electrophoresis through a 15% polyacrylamide-TBE gel at 4°C. Incubation conditions were as follows: lane a, no incubation; lane b, 37°C for 10 min without NC; lane c, 37°C for 10 min with 12 pmol (1.2 μ M) of NC; lane d, 94°C for 5 min and then 50°C for 5 min without NC. (B) ³²P-labeled 28A+/28A- duplex (20 fmol, 2 nM) and 21A- (1 pmol, 100 nM) ssDNAs were mixed in a 10- μ l standard buffer (see Materials and Methods), and reaction mixtures were incubated and analyzed as described for the corresponding lanes in panel A. 28A+ and 21A- have a potential to form a 20-bp duplex region. Asterisks in this and subsequent figures indicate radioactive labeling.

absence of NC protein. The 21 nucleotides at the 5' end of 32A- are identical in sequence to the corresponding nucleotide of 21A-. Strand exchange between these oligonucleotides was observed in the presence of NC protein (lane c), but no strand exchange was detectable in the absence of NC (lane b).

With a second combination of double- and single-stranded DNAs, a strikingly different result was obtained (Fig. 3B). While heat denaturation followed by reannealing promoted strand exchange between a double-stranded 28-mer (28A+/28A-) and a shorter single-stranded oligonucleotide (21A-) that could form 20 bp with 28A+ (Fig. 3B, lane d), no strand exchange was observed between these substrates at 37°C even in the presence of NC protein (lane c). This excludes the possibility that NC protein's only role in the strand exchange process was to promote denaturation of the dsDNA, with annealing taking place after removal of NC. When the substrates were first heat denatured and then NC was added, labeled 28A+ again hybridized exclusively to 28A- instead of 21A-, although the latter was present in 50-fold excess (data not shown). Thus, NC protein also plays a role in the strand exchange process after the strand separation step.

Selective annealing of complementary oligonucleotides. To further examine the effect of NC protein on the specificity of strand exchange, we tested oligonucleotides of different lengths in competition for annealing with complementary DNA strands. Figure 4 shows a diagram of the substrates used in this experiment. The actual DNA sequences are given in Materials and Methods. 28A- can form a 28-bp duplex with 28A+, while 24A- can form 24 bp and 21A- can form only 20 bp with 28A+. 28X- is a 28-mer, but it can form only 20 bp with 28A+.

Several combinations of these oligonucleotides were tested in competition for annealing with 28A+ (Fig. 5) in the presence of NC protein. When 28A- was present at a concentration of 4 nM, the majority of 28A+ was hybridized to 28A-

even when 24A- or 21A- was present in 25-fold excess (Fig. 5, lanes 1 to 3).

On the other hand, 40 fmol of 24A- hybridized to 28A+ in the presence of excess 21A- but not in the presence of excess 28A- (Fig. 5, lanes 4 to 6). 21A- was unable to compete against either excess 28A- or 24A- (lanes 7 to 9). Thus, in the presence of NC, hybridization to 28A- was favored over hybridization to 24A- and 21A-, and hybridization to 24A- was favored over hybridization to 21A-. To determine

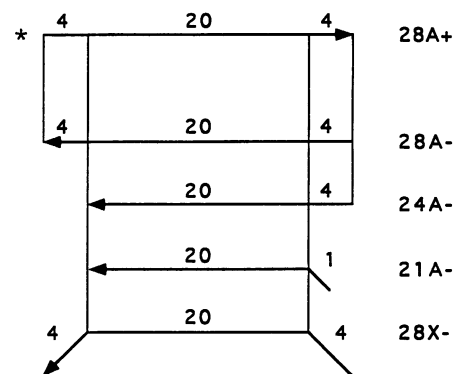


FIG. 4. Substrates for the selective annealing assay. Sequences of these DNA oligonucleotides are described in Materials and Methods. 28A+, 24A+, and 21A have the same polarity, while 28A-, 24A-, 21A- and 28X- have the opposite polarity. The numbers in their names indicate the lengths of these oligonucleotides. 28A+ and 28A-, 24A+ and 24A-, and 21A+ and 21A- can form complete duplex structures. 24A+ is identical to the 24-base sequence at the 3' end of 28A+. Twenty nucleotides of 21A+ are identical to the middle 20 nucleotides of 28A+. Twenty nucleotides in the middle of 28X- are identical to the 20 nucleotides in the corresponding region of 28A-.

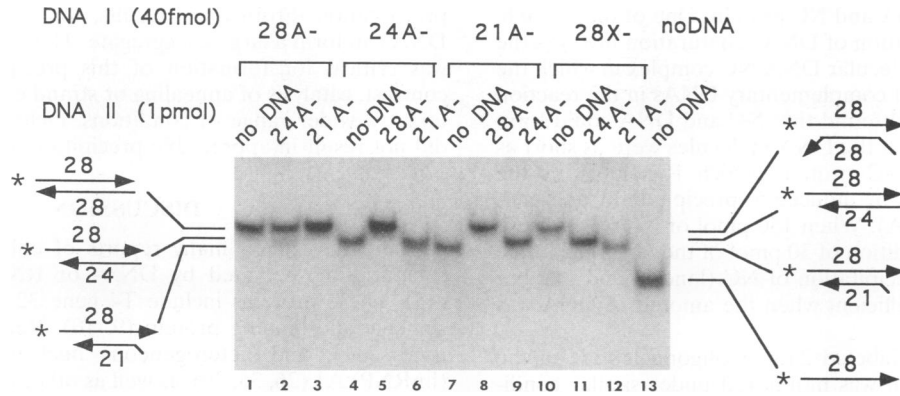


FIG. 5. Effect of the length of the base-paired region on the annealing of competing DNAs in the presence of HIV-1 NC protein. Reaction mixtures (10 μ l) contained 32 P-labeled 28A+ (18 fmol) with different combinations of two competing complementary oligonucleotides (40 fmol and 1 pmol, respectively) in standard annealing buffer with 25 pmol of HIV-1 NC protein. Incubation was at 30°C for 15 min and was followed by addition of 5 μ l of stopping solution (see Materials and Methods). The samples were analyzed by electrophoresis through a 15% polyacrylamide gel in TBE at 4°C.

whether this discrimination reflected an effect of the length of the base-paired region rather than simply an effect of the overall length of the DNA, we used 28X- DNA, which, although 28 nucleotides long, could only form 20 contiguous base pairs with 28A+. Both 24A- and 21A- effectively competed with 28X- for annealing to 28A+, indicating that the selective annealing was determined by the length of the potential base-paired region and not the total length of the oligonucleotides (lanes 10 to 12).

From these results, we propose that NC, by catalyzing the ss \leftrightarrow ds transition and thereby accelerating the approach to equilibrium, differentially enhances annealing between pairs of DNA strands that can form the most stable duplex (in this

example, by forming a longer base-paired region) among competing alternatives.

The effect of temperature on selective annealing by NC was tested as shown in Fig. 6. In the absence of NC, selective annealing of 28A- to 28A+ in the presence of a 25-fold excess of 21A- was observed only at 65°C, which is between the predicted melting temperature of 28A+/28A- and that of 28A+/21A- under these conditions. In the presence of NC, this selectivity was observed at all the temperatures tested, ranging from 0 to 70°C. At the highest temperatures (65 and 70°C), selectivity was reduced, presumably as a result of either partial denaturation of NC or inefficient DNA binding by NC at these temperatures.

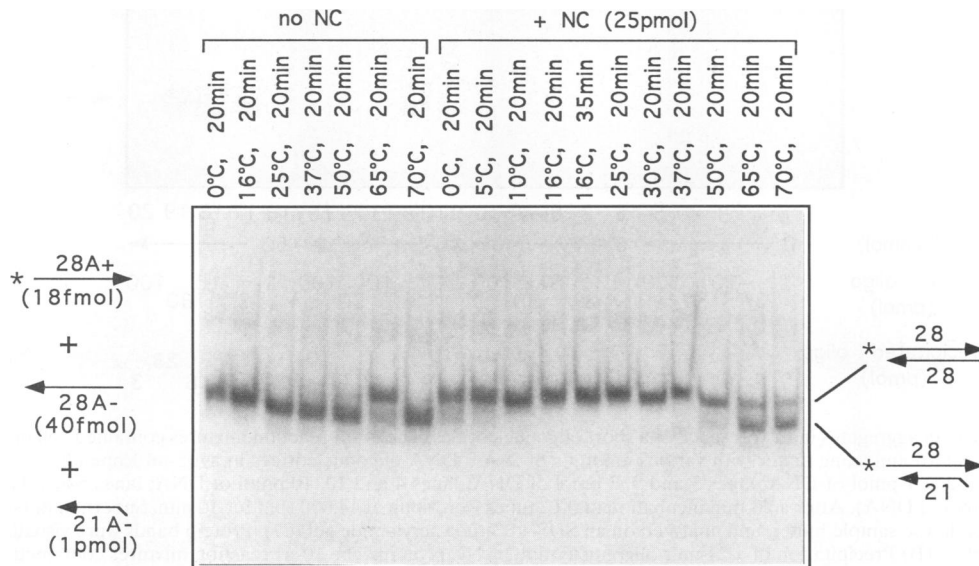


FIG. 6. Effect of NC protein on annealing of an ssDNA oligonucleotide (28A+) with two competing complementary DNAs (28A- and 21A-). The 10- μ l reaction mixtures contained 32 P-5'-end-labeled 28A+ (20 fmol), unlabeled 28A- (40 fmol), and unlabeled 21A- (1 pmol) in standard annealing buffer, with or without 25 pmol of HIV-1 NC protein. Incubation was performed at the indicated temperature and for the indicated length of time. After each reaction, the samples were chilled on ice, 1 μ l of 10% SDS was added, and DNA was extracted with phenol-chloroform (1:1) and analyzed on a 15% polyacrylamide-1 \times TBE gel.

Coaggregation of DNA and NC protein. One of the possible mechanisms for stimulation of DNA renaturation involves the formation of a multimolecular DNA-NC complex in which the overall concentration of complementary DNAs in the reaction is irrelevant. We indeed found that NC and DNA could form a coaggregate even when the DNA molecules were as short as 24 nucleotides long. NC protein, which is soluble in the absence of DNA, could be induced to precipitate by a 24-mer oligonucleotide (Fig. 7A). When 150 pmol of NC was present in 10 μ l of solution, addition of 30 pmol of the oligonucleotide resulted in efficient precipitation of NC (lanes 5 and 11), but precipitation was less efficient when the amount of DNA was greater or less.

Precipitation of the labeled 24-mer oligonucleotide in the presence of NC protein was monitored under similar conditions (Fig. 7B). Ten or 30 pmol of the oligonucleotides in 10 μ l of solution was efficiently precipitated by 150 pmol of NC (78 and 77%, respectively) (lanes 19 and 20), but addition of more DNA inhibited precipitation (lane 20). In the presence of 30 pmol of NC, 1.2 pmol out of a total of 3 pmol of DNA could be precipitated, but addition of more NC did not cause more

precipitation. From these results, we conclude that NC and DNA can form a large coaggregate. The ratio of DNA and NC was critical for formation of this precipitable complex. In contrast, catalysis of annealing or strand exchange could occur under a wider range of conditions, including conditions that did not result in appreciable precipitation.

DISCUSSION

There have been many reports of enhanced nucleic acid renaturation mediated by DNA- or RNA-binding proteins (45). These proteins include T4 gene 32 protein (3), *E. coli* single-strand-binding protein (9, 10), RecA protein (8), histones (11), and heterogeneous nuclear ribonucleoprotein (hnRNP) A1 (26, 35, 39), as well as other proteins. hnRNP A1 is able to accelerate annealing of a 200-bp DNA molecule by several thousand-fold (39), while the rate enhancements promoted by the other proteins cited above are significantly smaller. The extent of stimulation DNA annealing by hnRNP A1 appears to be too great to be explained solely by inhibition of electrostatic repulsion or intrastrand secondary structure formation (39).

A recent report has shown that HIV-1 NC protein increases the annealing rate of a 149-bp DNA fragment by more than 4 orders of magnitude (13). The purified, recombinant HIV-1 NC protein that was used for the experiments described in this report also retained a potent DNA renaturation activity despite addition of Met-(His)₆ sequence to the N terminus. We found that HIV-1 NC can also accelerate annealing of short DNA oligonucleotides. Similar acceleration has subsequently been observed with RNA oligonucleotides (22).

We found that NC protein could form a large coaggregate in the presence of DNA, even when the DNA molecules were rather small (24-mer). Lapadat-Tapolsky et al. also have

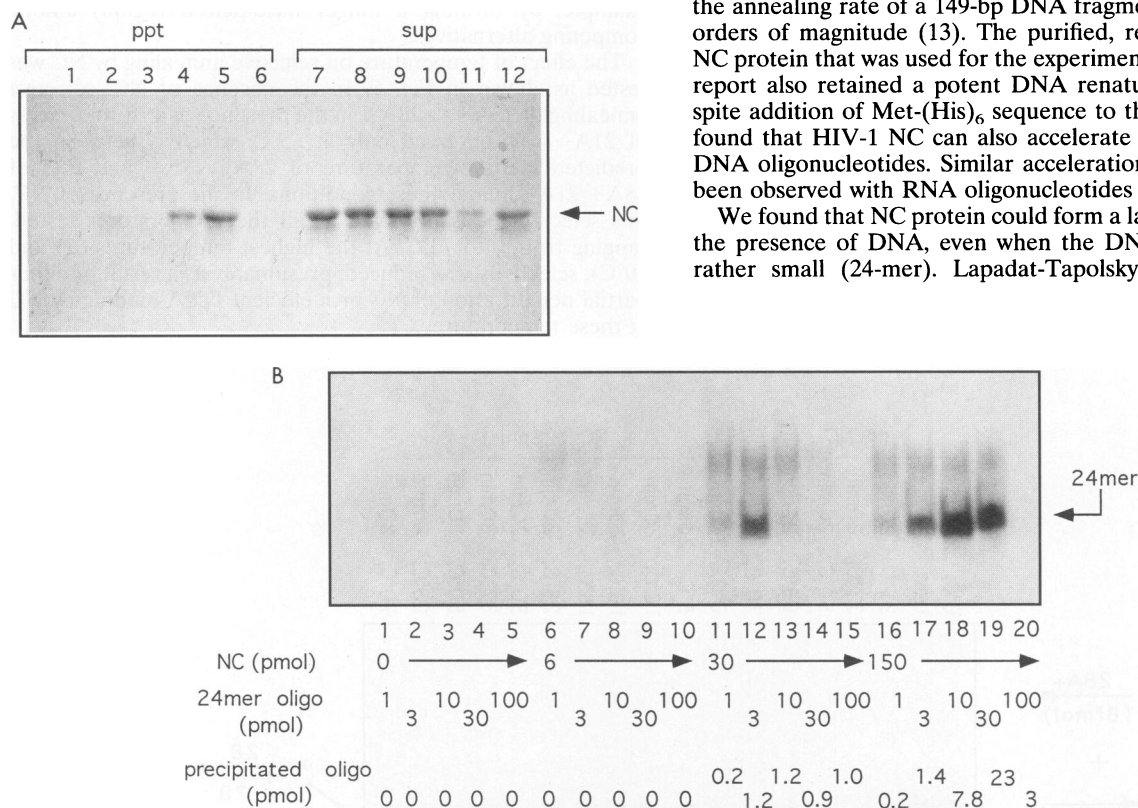


FIG. 7. (A) NC protein aggregates in the presence of a short oligonucleotide. The 10- μ l reaction mixtures contained 150 pmol (15 μ M) of HIV NC protein in the standard annealing buffer with various amounts of 24A+ DNA oligonucleotides in a 1.5-ml Eppendorf test tube (lanes 1 and 7, no DNA; lanes 2 and 8, 1 pmol of DNA; lanes 3 and 9, 3 pmol of DNA; lanes 4 and 10, 10 pmol of DNA; lanes 5 and 11, 30 pmol of DNA; lanes 6 and 12, 100 pmol of DNA). After a 20-min incubation at 0°C, tubes were spun at 14,000 rpm for 10 min. Supernatant (sup) and pellet (ppt) were separated, boiled in the sample buffer, and analyzed on an SDS-18% polyacrylamide gel (27). Protein bands were visualized by staining with Coomassie brilliant blue. (B) Precipitation of a 24-mer oligonucleotide by NC protein. The 10- μ l reaction mixtures contained standard annealing buffer, various amounts of 24-mer oligonucleotide (oligo; 24A+), and NC protein, as indicated. The amount of ³²P-labeled 24A+ in the reaction was kept constant, although the total amount of 24A+ was varied by adding unlabeled oligonucleotide. After 20 min of incubation at 0°C in an Eppendorf Microfuge tube, the samples were centrifuged at 14,000 rpm for 20 min. The pellets were dissolved in 5 μ l of 10 mM Tris-Cl (pH 7.9)-1 mM EDTA, 5 μ l of stopping solution (see Materials and Methods) was added, and the samples were analyzed on a 15% polyacrylamide-TBE gel. The gel was then dried and exposed for autoradiography. Quantitation of the radioactivity was done with a Molecular Dynamics PhosphorImager. The amount of precipitated oligonucleotide was calculated by comparing radioactivities in the pellet and supernatant fractions.

observed coaggregates of NC with longer DNA (plasmid DNA containing a 6-kb region from the HIV-1 genome) (28). Since the DNA molecules in our experiment were short, it is not plausible that a complex of a single DNA molecule and several bound NC molecules would be large enough to be precipitated. This result therefore suggests the possibility of an interaction between NC molecules bound to nucleic acids. Khan and Giedroc have previously observed cooperative binding of NC to nucleic acids, which may reflect the same protein-protein interactions (25). Alternatively, simultaneous binding of single NC proteins to two DNA molecules could provide an explanation for the coaggregation that we observed.

Sikorav and Church have proposed that aggregation of DNA provides a general mechanism for acceleration of DNA renaturation by a variety of methods (45). DNA aggregation in the presence of NC is consistent with such a mechanism playing a role here. There is no direct evidence, however, that this aggregate is an intermediate in DNA renaturation, and the conditions favoring annealing were not identical to those favoring formation of precipitable aggregates.

Some of the activities previously shown for NC, such as promoting dimerization of the retroviral genome or annealing of a tRNA primer to the primer binding site, may largely be due to NC's ability to destabilize secondary structure. In the absence of proteins, these reactions proceed much more rapidly after the nucleic acids are heat denatured (42, 43), suggesting that removal of intramolecular secondary structure is rate limiting. Melting of tRNA by HIV NC protein was demonstrated directly by circular dichroism spectroscopy (25). We were able to show complete melting of short dsDNA at high NC concentrations, although at lower protein concentrations, NC stimulated renaturation. These results suggest that NC is able to stimulate both the ds \rightarrow ss and the ss \rightarrow ds transition, at least under different protein concentrations.

Results from our DNA annealing experiments with competing DNA strands (Fig. 5 and 6) indicate that in the presence of NC protein, these DNAs are in equilibrium, with a rapid rate of ss \leftrightarrow ds transition. hnRNP A1 protein also is able to facilitate a similar approach to equilibrium, albeit at a higher temperature (40). Recent results suggest that NC can play the same role with RNA substrates (22, 50).

What is the significance of these activities of NC protein *in vivo*? The propensity of nucleic acid molecules to form metastable base-paired structures that dissociate slowly can limit the rate and specificity of reactions that involve specific base-pairing (21). One prediction from the results described above is that NC could facilitate correct base-pairing or folding of nucleic acids in a fashion similar to that of protein chaperones (2). When DNA or RNA is misfolded, the typically high energy barrier to abandoning the misfolded state under ordinary conditions at physiological temperature means that the time required to reach the most thermodynamically stable folded conformation can be extremely long. In the presence of NC, this process could be greatly accelerated, although it remains to be determined whether NC can indeed promote folding of a large RNA molecule. Such an activity on RNA conformation has recently been proposed for hnRNPs, including hnRNP A1 (41). This activity need not be limited to correction of misfolded nucleic acids. Since RNA folding could start before completion of transcription, the initial folded conformation of a nascent transcript might be different from that of the most thermodynamically stable structure for the complete RNA. Thus, the ability of NC to reduce the kinetic barriers to structural transformations of nucleic acids could be essential in allowing the transcripts to achieve a mature conformation. Dimerization of the retroviral genome can be

considered an example of such a folding problem. The NC domain of the Gag polyprotein is required for this process *in vivo* (34), and purified NC protein can promote this reaction *in vitro* (42, 43). These conformational changes in viral RNA may be critical for efficient packaging and for subsequent activities of genomic RNA. Involvement of the NC domain of the Gag polyprotein in genomic RNA packaging has been established by genetic experiments (4, 5, 14, 16–20, 32, 33, 38). After dimerization and packaging, a further step in maturation of murine leukemia virus genomic RNA requires the presence of mature NC protein and turns the genomic RNA into a more compact conformation (16).

Genetic evidence suggests that NC protein is also involved in reverse transcription (32). The ability of NC to promote the ds \rightarrow ss transition could play a role in facilitating strand displacement in the final stage of viral DNA synthesis. NC protein might also assist reverse transcription by destabilizing secondary structures in the template DNA. NC has been reported to increase the initial product of reverse transcription, but the basis for this effect remains to be defined (32).

The selective annealing activity of NC may have practical applications. One might be to enhance specificity in PCR. Annealing of primers to incorrect sites can lead to production of spurious PCR products. The ability of NC protein to promote selective annealing might be employed to increase the ratio of correct to incorrect products. This activity might also prove useful in promoting accurate renaturation of complex DNAs in solution, which is required for genomic mismatch scanning (36), or DNA annealing to an immobilized DNA target on a chip (15) or on other materials (e.g., in Southern hybridization or *in situ* hybridization). Whether NC will prove useful in these applications remains to be explored.

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