Bending and Unwinding of Nucleic Acid by Prion Protein†

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ABSTRACT: Nucleic acid induces conformational changes in the prion protein (23−231 amino acids) to a structure resembling its pathological isoform. The prion protein, in turn, facilitates DNA strand transfer and acts as a DNA chaperone which is modulated by the N-terminal unstructured basic segment of the protein. Here we have studied the prion protein induced conformational changes in DNA using oligonucleotides covalently labeled with the energy donor fluorescein and the acceptor rhodamine moieties by fluorescence resonance energy transfer (FRET) and by thermal stability of the unlabeled oligonucleotides. The protein induces a strong FRET effect in the oligonucleotides evidenced from the simultaneous quenching of fluorescence intensity of the donor and increase in the fluorescence intensity of the acceptor, which indicate bending of the oligonucleotides by the prion protein. The energy transfer efficiency induced by the protein is greater for the larger oligonucleotide. The prion protein also induces significant structural destabilization of the oligonucleotides observed from the lowering of their melting temperatures in the presence of the protein. The truncated globular prion protein 121−231 fragment neither induces FRET effect on the oligonucleotides nor destabilizes their structures, indicating that the N-terminal segment of the prion protein is essential for the DNA bending process. Equilibrium binding and kinetics of FRET show that the protein binding to the oligonucleotides and their bending occur simultaneously. The DNA structural changes observed in the presence of the prion protein are similar to those caused by proteins involved in initiation and regulation for protein synthesis.

The major constituent of infectious agent of the fatal neurodegenerative diseases, viz., transmissible spongiform encephalopathies (TSE)[1] or prion diseases, is a β-sheet-rich conformational isoform of noninfectious α-helical soluble prion protein, PrP^C, which remains attached to the outer membrane of the cells through a glycophosphatidyl inositol linkage (*1*, *2*). The infectious agent, termed PrP^{Sc}, can exist as oligomers and amyloid polymers. The biological function of PrP^C remains largely uncertain, but the protein can play a role in maintaining cellular copper concentration, signal transduction, RNA binding, and DNA metabolism (*3*−*5*). Unlike bacterial and viral diseases where nucleic acid transmits the infection, prion disease has been considered to propagate by the conversion of PrP^C by PrP^{Sc} (*1*, *6*). The existence of multiple prion strains has also been attributed to the conformational variations of PrP^{Sc} although the existence of a nucleic acid as a cofactor for infection can explain the strain multiplicity (*1*, *6*, *7*). The amyloid formed from the truncated 90−231 fragment of the mouse recombinant prion protein is found to be infectious and shows strain specificity of the prion disease in the experimental mice overexpressing this protein fragment; however, amyloids obtained from cellular hamster PrP^C have been found to be noninfectious in similar experiments (*8*−*10*). Inoculation of wild-type hamsters with the in vitro generated PK-resistant prion protein form by protein misfolding cyclic amplification has been found to be infectious (*11*). In spite of a large amount of information favoring infectious agent being constituted from the protein and inability to identify disease-specific nucleic acid, the involvement of a host-derived nucleic acid in the prion disease has not been ruled out (*12*−*14*).

A number of different molecules have been found to facilitate the conversion of the full-length prion protein to insoluble aggregates (*15*−*20*). Nucleic acids, DNA, and RNA in solution and *in vitro* can catalyze conversion of recombinant and cellular PrP^C to its β-structured form and produce oligomers and linear and spherical amyloids which are similar in morphology to the prion amyloids found in the prion-diseased brains (*21*−*25*). DNA can also prevent aggregation of the prion protein (*22*). Certain DNA aptamers can specifically bind to PrP^C (*26*). Recent biophysical studies show that both the unstructured N-terminal segment and the globular 121−231 domain of the prion protein form a complex with nucleic acid, and the interaction is mediated mainly through the globular domain (*27*). It was previously shown that nucleic acid unfolds the globular domain of the prion protein, which leads to its polymerization to amyloids

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[1] Abbreviations: PrP^C, cellular prion protein; PrP^{Sc}, infectious prion protein; α-PrP, recombinant human prion protein; FAM, 5,6-carboxyfluorescein; TAMRA, 5-carboxytetramethylrhodamine; FRET, fluorescence resonance energy transfer; Lef-DNA, binding region of transcriptional regulator Lef protein; NC-DNA, 28 base pair oligonucleotide constituting the stem−loop region of the packaging signal of HIV1 gag protein; TSE, transmissible spongiform encephalopathies.

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(*28*). It has been suggested that structural conversion of PrP^{C} to PrP^{Sc} can be catalyzed by nucleic acids that can play a role in the prion diseases although infectivity of the nucleic acid induced prion protein amyloid has not been demonstrated yet *in vivo* (*29*). Although the prion protein is a cell surface protein, there is evidence that the protein can be present in the cytoplasm where it can interact with nucleic acids (*30*, *31*). Cell culture studies have shown that the prion protein can translocate to the nuclei and bind to the chromatin (*32*). It has also been suggested that the prion protein can function as a nucleic acid pattern recognition receptor (*27*). The prion protein induces ordered aggregation of nucleic acids to condensed nucleic acid globules, and unlike other cellular chaperone proteins, the prion protein mimics the retroviral NCp7 protein of HIV1 and chaperones DNA strand transfers required for production of a complete proviral DNA with long terminal repeats of HIV1 (*33*, *34*). The globular 121−231 region of the prion protein does not show DNA strand transfer properties (*34*).

The DNA strand transfer property of the prion protein needs to involve modification of the structure of nucleic acids. Here we have focused our studies on the modification of the structure of DNA by the prion protein by using oligonucleotide labeled with donor and acceptor fluorescent molecules attached at their 5′ ends by the fluorescence resonance energy transfer (FRET) method and by structural destabilization of the unlabeled oligonucleotides by the prion protein (*35*−*39*). The present results show that the prion protein bends and unwinds DNA, which are the earlier steps of the DNA strand transfer properties of the protein.

EXPERIMENTAL PROCEDURES

Isolation and Purification of Recombinant Proteins. Full-length human recombinant prion protein (α-PrP) was isolated by the method described in the literature and was a kind gift from Dr. H. Rezaei (*40*). Details of the cloning of the protein gene and isolation procedure can be found in the above reference. Protein concentration was measured by the optical density at 280 nm using an extinction coefficient value of 56795 M^{-1} cm^{-1}. The purified α-PrP migrated in SDS−PAGE as a single species with an estimated molecular mass of 25 kDa. The molecular mass of the protein was also confirmed by mass spectroscopy. The truncated mouse prion protein C-terminal fragment 121−231 (wild type) was isolated from the plasmid (provided by Dr. R. Glockshuber) using the published procedure (*41*). The protein in the SDS−PAGE experiment showed estimated molecular mass of 13.5 kDa for the 121−231 fragment. The extinction coefficient of 21000 M^{-1} cm^{-1} at 280 nm was used to calculate the concentration of the protein fragment.

Preparation of Double-Stranded Oligonucleotides. The effects of the prion protein on the structure of DNA were studied by the FRET method using the two oligonucleotides shown in Figure 1. The 28 base pair oligonucleotide constitutes the stem−loop region of the packaging signal of HIV1 gag protein and has been termed here as NC-DNA (*42*). The 16 base pair Lef-DNA is the sequence where HMG box proteins bind to the DNA (*35*). The unlabeled complementary strands were obtained from QIAGEN. Concentrations of single strands and duplexes were determined from absorbance at 260 nm of nucleotides. The single-stranded

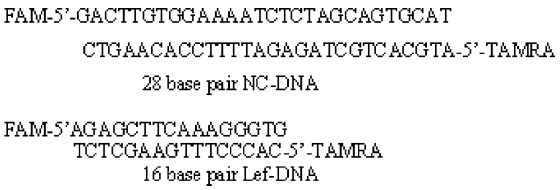

FIGURE 1: Sequences of the 28 bp NC-DNA and 16 bp Lef-DNA duplexes labeled at the 5′ ends with FAM [(−) strand] and TAMRA [(+) strand] used in the FRET experiments to observe the change in DNA conformation induced by the prion protein.

nulceotides labeled at the 5′ end by 5,6-carboxyfluorescein (FAM) and 5-carboxytetramethylrhodamine (TAMRA) were also obtained from QIAGEN. DNA duplexes were prepared by mixing the complementary single-stranded oligonucleotides in equimolar amounts (1:1) in 10 mM Hepes−KOH (pH 7.5), 100 mM NaCl, and 10 mM $MgCl_2$ buffer and hybridized by cooling slowly from 90 to 20 °C over 1 h (*43*). Finally, the solutions of DNA duplexes for the experiments were prepared by extensive dialysis either in 10 mM potassium phosphate buffer containing 100 mM KCl, pH 6, or in 0.1 M Tris-HCl buffer, pH 7.2.

Fluorescence Resonance Energy Transfer. FRET is a distance-dependent interaction between the electronic excited states of two dye molecules where excitation energy is transferred from a donor molecule to an acceptor molecule without emission of a photon. This transfer of energy leads to a reduction in the donor's fluorescence intensity and excited-state lifetime and to an increase in the acceptor's emission intensity. In the case of the protein−DNA interaction, FRET measurements allow the determination of the extent of DNA bending that brings the ends of the DNA in close proximity after ligand binding, and we have used FRET as an indication of the bending of the oligonucleotides by the prion protein in this study (*38*). We have mostly followed the procedure of Dragan et al. to study bending of the oligonucleotides by various proteins (*35*). The majority of the FRET experiments have been carried out in 10 mM potassium phosphate buffer containing 100 mM KCl, pH 6, to compare the present FRET results with the data of 16 bp Lef-DNA with HMG proteins (*35*). A few results of the FRET studies at pH 7.2 in 0.1 M Tris-HCl buffer have also been presented in order to compare with the results obtained at pH 6. The steady-state fluorescence measurements were carried out in a Hitachi 4500 spectrofluorometer fitted with a thermostat at 20 °C. The concentrations of the nucleotides were 5 nM.

We have determined the transfer efficiency (TE) from FRET experiments from the decrease in the fluorescence intensity of donor fluorescein attached to the FAM−Lef-DNA−TAMRA and FAM−NC-DNA−TAMRA by using the equation (*43*):

$$TE = (I_{DO} - I_D)/I_{DO} \quad (1)$$

where I_{DO} and I_D are the fluorescence intensities of the fluorescein group attached to the nucleotides in the absence of protein (buffer) and in the presence of increasing concentrations of protein at 515 nm upon excitation by 490 nm radiation (excitation maximum of the fluorescein moiety attached to the oligonucleotides) (*39*).

Binding Measurements. Equilibrium binding between α-PrP and the above DNAs was carried out by monitoring the changes in the fluorescence polarization of intrinsic tryptophan groups of the protein situated at the unstructured 23−232 segment of the molecule with increase in the DNA concentrations. The fluorescence polarization measurements were carried out in the above spectrofluorometer equipped with an accessory for steady-state polarization measurements. The fluorescence polarization for the intrinsic tryptophan groups was monitored at 350 nm, exciting the solution at 280 nm. The fluorescence polarization (*P*) values were calculated by the equation:

$$P = I_{||} - GI_{\perp}/I_{||} + GI_{\perp} \quad (2)$$

where || and ⊥ are respectively the intensities of the emitted light when the emission polarizers are aligned parallel and perpendicular to the polarization of the excited light. *G* is a correction factor of the instrument accounting vertically versus horizontally polarized light. A 220 nM protein concentration was used for tryptophan polarization experiments.

Kinetics of the Interaction between the Prion Protein and the Labeled Oligonucleotides. Kinetic experiments were carried out using a stopped-flow apparatus (SFA-20 rapid kinetics stopped-flow accessory, Hi-tech, dead time <2 ms) attached to the spectrofluorometer (SFA-20 rapid kinetics stopped-flow accessory). Oligonucleotides at 10 nM concentrations were used so that the concentrations for NC-DNA and Lef-DNA phosphates were 560 and 320 nM, respectively. The prion protein concentrations for kinetic experiments were 450 and 260 nM for NC-DNA and Lef-DNA, respectively, to maintain α-PrP to DNA-ph ratios at 0.8. The experiments were performed at pH 6 at 20 °C.

Structural Stability of Nucleic Acids in the Presence of the Prion Protein. Structural stability of the unlabeled Lef- and NC-oligonucleotides and a plasmid pxNnlslacZ (18.8 kb) in the presence of the prion protein and its fragment was studied in 0.1 M Tris-HCl buffer, pH 7.2, from the change of the absorbance of the nucleic acid bases at 260 nm as a function of temperature in a JASCO V-530 spectrophotometer fitted with a Peltier thermostat for temperature control. The nucleotide−phosphate concentrations were 1.5 μM, and protein to phosphate ratios in the experiments were maintained at 0.15. The increase in temperature was controlled at 0.5 °C/min. The stability of the nucleotides and plasmid DNA against temperature was obtained either from the temperature where 50% change in their absorbance at 260 nm occurred or from the peak of the plot of the derivative of the increase in the absorbance with temperature against the temperature of the solution.

RESULTS

The fluorescence spectra of the 16 bp FAM−Lef-DNA−TAMRA when excited by 490 nm (excitation wavelength of the donor fluorescein group) in buffer at pH 6 and 7.2 showed the major emission maximum of fluorescein at 515 nm and another peak at 570 nm characteristic of rhodamine (acceptor moiety) (Figure 2a,b). At pH 7.2, the acceptor peak appeared as a shoulder (Figure 2b). Similar results were also observed with NC-DNA (Figure 2c,d). It is to be noted that the fluorescence intensity throughout the spectrum is greater at pH 7.5 than what was observed at pH 6 when same concentration of the oligonucleotides (5 nM) was used (Figure 2). The ratio of fluorescence intensities of donor fluorescein at 515 nm to that of the acceptor rhodamine at 570 nm of the Lef-DNA (spectrum 1, Figure 2a) in buffer was in good agreement with the results for this oligonucleotide reported by Dragan et al. (*35*).

The addition of α-PrP decreased the fluorescence intensity of both the donor and acceptor groups attached to the oligonucleotides to different extents for the two DNAs which also depended upon the pH of the solution (Figure 2). Decrease in both the donor and acceptor fluorescence intensities of the oligonucleotides labeled with fluorescein and rhodamine has been observed when human TATA-binding protein binds to and bends the TATA sequence (*36*). In order to observe the FRET effects on the acceptor rhodamine after the addition of the prion protein, we have normalized the spectra of the fluorescent DNAs in the presence of the protein with respect to the fluorescent intensity of the donor fluorescein at 515 nm in buffers, and these are shown in the insets of Figure 2. This normalization procedure has been used for comparison of FRET effects in various systems (*36*, *44*, *45*). Normalized spectra in the insets of Figure 2 show pronounced rhodamine fluorescence spectra and the saturation of the FRET effect above 0.2 μM α-PrP.

At saturation binding, there was ∼80% and 60% quenching of the donor fluorescence at pH 7.2 and 6, respectively, for the Lef-DNA. The corresponding increases in the fluorescence intensities of the acceptor rhodamine (based on normalized spectra) were 70% and 55%, respectively (Figure 2a,b and the insets). In the presence of a saturating concentration of α-PrP to the NC-DNA, ∼85% and 70% quenching of the fluorescence intensities of the donor fluorescein occurred at pH 7.2 and 6, respectively (Figure 2c,d). At pH 6, unlike other titration results, we observed a major reduction in the fluorescence intensity when 40 nM protein was added to the NC-DNA (spectrum 2, Figure 2c). The increases in the acceptor rhodamine fluorescence intensities were ∼70% and 50% at pH 6 and 7.2, respectively, at saturation of the reaction. The results of Figure 2 showed that the degree of donor fluorescence quenching and increase in the acceptor fluorescence are greater (∼20%) at neutral pH than the same at pH 6 when the prion protein was added to the nucleotides. The addition of 350 nM α-PrP to equimolecular mixtures of single-stranded FAM-Lef and Lef-TAMRA (corresponding to the protocol of trace 8, Figure 2a) did not show any increase in the fluorescence of TAMRA when the solution was excited by donor (fluorescein) excitation at 490 nm (*43*). Similar results were obtained with mixtures of single-stranded FAM-NC and NC-TAMRA in the presence of 480 nM prion protein (corresponding to trace 7, Figure 2c). These experiments demonstrated that the observed FRET effects arose only from the conformational changes of the double-stranded nucleotides.

The human and mouse prion protein 121−231 fragments have 95% sequence homology, and studies have shown that they resemble each other in their secondary structures and structural stability (*41*). Therefore, we have considered that the results obtained from the mouse protein fragment would reflect the property of the corresponding human prion protein fragment and can be compared with the results of the human full-length prion protein 23−231. The addition of the

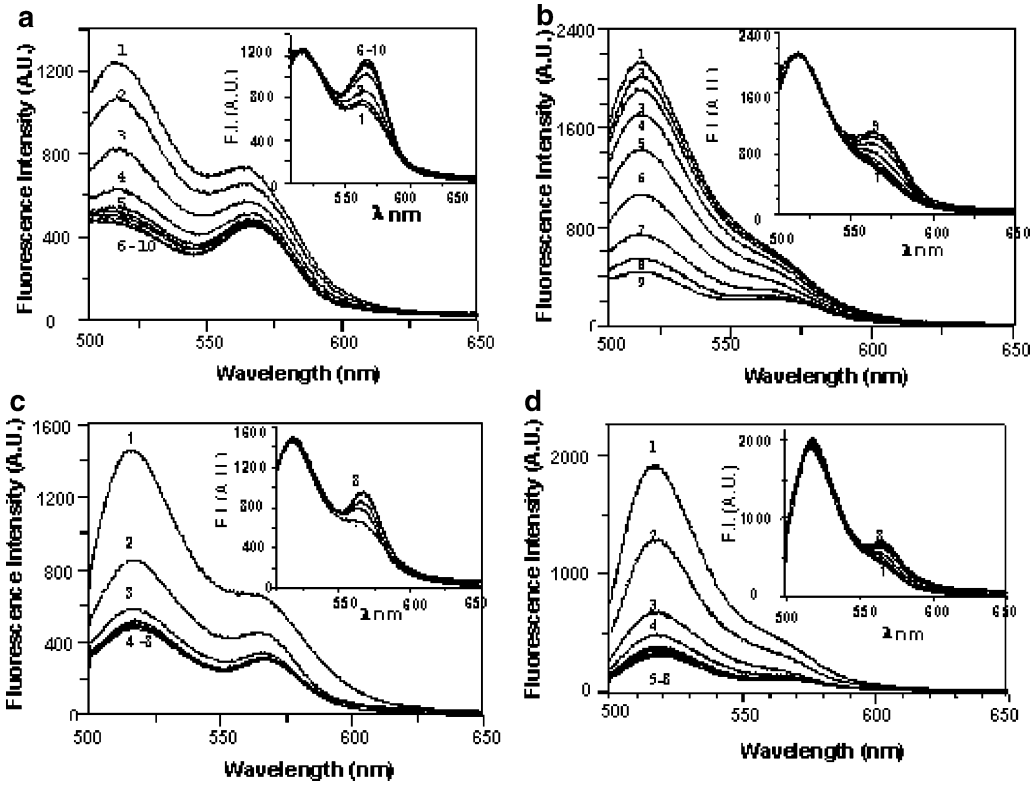

FIGURE 2: Quenching of fluorescence spectra of 16 bp FAM−Lef-DNA−TAMRA with the addition of increasing concentrations of α-PrP at pH 6 (panel a**)** and pH 7.2 (panel b). Similar results with labeled 28 bp FAM−NC-DNA are shown in panels c and d at pH 6 and 7.2, respectively. The measurements were carried out at 20 °C. The spectra in the presence of protein were corrected for dilution resulting from the addition of protein solution. Traces numbered 1 in the panels represent spectra of the double-labeled DNAs (5 nM) in buffers in the absence of the protein. The excitation wavelength was 490 nm. The peaks at 518 and 570 nm represent the fluorescence maxima of FAM (fluorescence donor) and TAMRA (rhodamine, energy acceptor), respectively. Traces 1−9 are the fluorescence spectra of the DNAs in the presence of 0, 20, 40, 80, 120, 190, 250, 450, and 700 nM α-PrP, respectively. Spectra 2−9 after normalization (with respect to the fluorescent intensity at 518 nm of spectrum 1) are shown in the insets of panels a and b. Titration results of NC-DNA at pH 6 and 7.2 by α-PrP are shown in panels c and d, respectively, where traces 1−8 represent the fluorescence spectra of the labeled NC-DNA in the presence of 0, 40, 80, 120, 190, 250, 480, and 700 nM α-Prp. The normalized spectra determined as above are shown in the insets of the panels, where 1 is the spectra in the absence of protein and 8 is the spectra in the presence of 700 nM protein in panels c and d. Bandwidths were 10 and 20 nm for excitation and emission, respectively.

globular fragment of the prion protein moPrP121−231 was found to reduce the fluorescence intensity of the oligonucleotide spectra to a much smaller extent than what was observed when the full-length protein was added to the Lef-DNA and NC-DNA (panels a and b of Figure 3, respectively). In fact, the decrease in the fluorescence intensity (∼10%) in the presence of ∼1.2 μM moPrP121−231 resulted from the dilution due to the increase of the volume of the experimental solution from 500 to 540 μL (∼8% increase) due to the addition of the protein solution. Correction of these spectra for dilution showed that this prion protein fragment did not induce any FRET effect in the oligonucleotides, suggesting the inability of this globular prion protein fragment to induce any measurable structural change on the DNA (insets, Figure 3). These results show that the unstructured N-terminal segment comprising 23−120 residues of the full-length prion protein is essential for inducing the observed FRET effect on the oligonuclotide.

We have presented the transfer efficiency of FRET for the prion protein on the labeled Lef-DNA and NC-DNA based on eq 1 in panels a and b of Figure 4, respectively. Although the TE values for the Lef-DNA attained a saturation value above the protein to oligonucleotide mole ratio approximately at 1.5 at pH 6, the TE value did not show saturation even above a mole ratio of 3 of protein to nucleotide at neutral pH. At equal molar ratio of the protein to Lef-DNA, TE values are larger at neutral pH than those at pH 6. The comparison also shows that the TE values at lower protein to nucleotide ratio are nearly identical and have similar slopes for the increase in the TE values. The results of the TE values for the NC-DNA showed that, at both pHs, TE values attain saturation above a protein to nucleotide mole ratio of 1. However, the TE values are greater at pH 7.2, and the slopes of TE increase, with increase in protein concentration, are larger at neutral pH to a small extent.

Binding Measurements. We have studied the binding between α-PrP and the oligonucleotides from the changes in the fluorescence polarization of the intrinsic tryptophan groups (seven in number) present in the unstructured N-terminal segment of the protein (Figure 5). The dissociation constants based on a single nucleotide concentration using the Hill plot are 1.25 ± 0.01 and 1.11 ± 0.01 μM for Lef-DNA and NC-DNA, respectively.

Kinetics of the Interaction between α-PrP and the Fluorescent Oligonucleotides. The decrease in the fluorescence intensities (after normalization) of the donor fluorescein attached to Lef-DNA and NC-DNA (each of 10 nM DNA concentration) after the addition of α-PrP as a function of time is shown in Figure 6. The molar ratio of protein to DNA−phosphate was maintained at 0.8 where the FRET

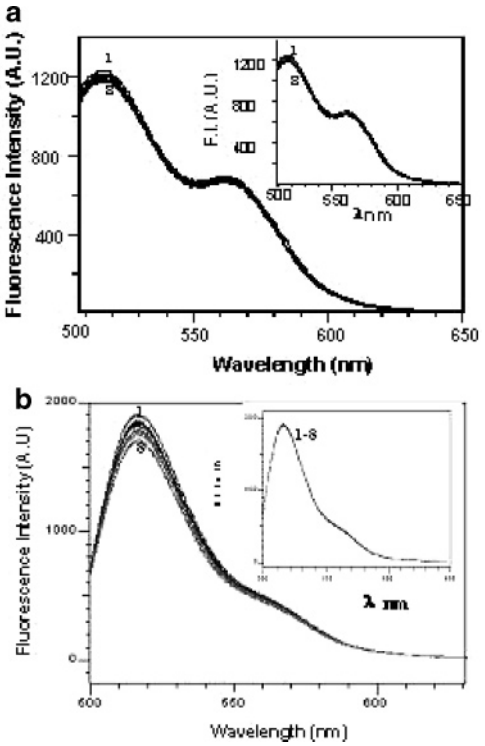

FIGURE 3: Effect of the moPrP121−231 prion protein fragment on spectra of FAM−Lef-DNA−TAMRA at pH 6 (panel a) and FAM-NC-DNA-TAMRA at pH 7.2 (panel b). Concentrations of the DNAs were 5 nM, and other experimental conditions were the same as in the full-length protein (Figure 2). There is no significant quenching of the donor fluorophore (FAM) at 518 nm by moPrP121−231 (0−500 nM, traces 1−8). Spectra after correction for dilution due to the addition of protein solution are shown in the insets of panels a and b and indicate that spectral properties of the DNAs do not change in the presence of the protein fragment. The solutions were excited by 490 nm light with slit widths 10 and 20 nm for excitation and emission, respectively. The temperature was 20 °C.

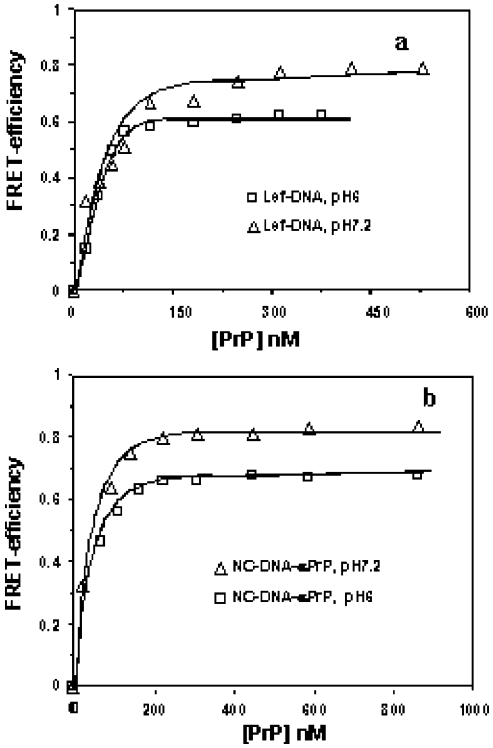

FIGURE 4: Dependence of FRET efficiency (eq 1) on α-PrP concentration at pH 6 and 7.2: (a) FAM−Lef-DNA−TAMRA; (b) FAM−NC-DNA−TAMRA. At both pHs, the FRET efficiency is greater for the NC-DNA albeit to a smaller extent, and the efficiency is more at pH 7.2 than that at pH 6. The results have been calculated from the experiments reported in Figure 2.

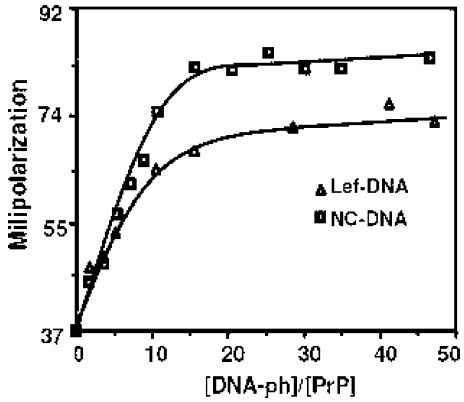

FIGURE 5: Binding measurements of unlabeled NC-DNA (squares) and Lef-DNA (triangles) with the prion protein by changes in the fluorescence polarization of intrinsic tryptophan groups of the protein. Increasing amounts of nucleotides were added to the protein in 10 mM potassium phosphate buffer containing 100 mM KCl, pH 6. The α-PrP concentration was 220 nM. Fluorescence was measured at 350 nm after exciting the protein solution at 280 nm. The tryptophan polarization values during 30 min for the samples (mole ratios of DNA−phosphate of 5 and 10 for both of the nucleotides) remained unchanged when turbidity increased between 2% and 5%, indicating that turbidity did not affect the fluorescence polarization. The temperature was 20 °C.

value attained saturation for both of the DNAs (Figures 2a,c and 4). Both of the kinetic traces show a monophasic decrease in the fluorescence intensity of the fluorescein without any detectable lag phase. The kinetic curves of association are real time processes where the free DNA population shifts to nearly fully formed complexes with the prion protein (*36*). The half-lives for these association reactions are ∼3 and 1.5 s for Lef-DNA and NC-DNA, respectively. At the completion of the reaction (>15 s), the decrease in the fluorescence intensities of the fluorescein groups is in good agreement with those observed in steady-state equilibrium measurements (not shown), which demonstrates that Lef- and NC-DNA are bound and bent by α-PrP in real time. Taken together, these observations provide evidence that full bending of DNA is achieved concurrently with its binding to the prion protein. The reaction is also about two times faster, and the decrease in fluorescence quenching was ∼20% more, probably indicating that the higher order structural change of NC-DNA occurs compared to Lef-DNA in identical reaction conditions (*43*).

Structural Stability of Nucleic Acids. We have compared the structural stability of nucleic acids in the presence of α-PrP and the structured 121−231 region since there were differences in the FRET effects of these two molecules. The effect of α-PrP on the structure of different oligonucleotides and plasmid DNA is presented in Figure 7. The ratio of [protein]/[DNA-ph] for the experiments in Figure 7 was kept at 0.85 where the FRET efficiency attained saturation with α-PrP (Figure 4b). Lef-DNA in Tris-HCl buffer, pH 7.2, shows a midpoint of structural transition at 54 °C in the buffer, which in the presence of 10.8 μM prion protein

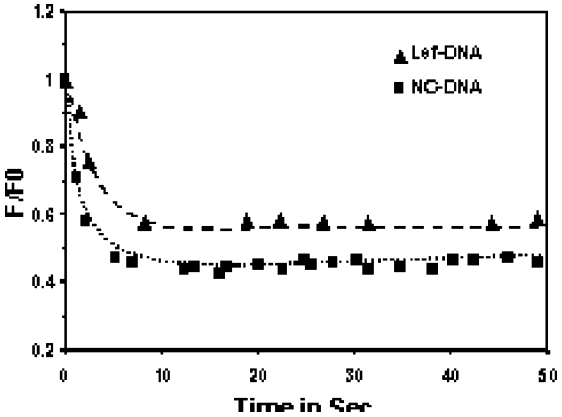

FIGURE 6: Kinetics of binding of α-PrP to the 16 bp Lef-DNA (upper trace) and 28 bp NC-DNA (lower trace) and bending of the nucleotides (normalized). In both of the experiments, 10 nM DNA was used for the kinetic study. For the Lef- and NC-DNA the corresponding phosphate concentrations were 320 and 560 nM, respectively, and the corresponding concentrations of the protein used were 260 and 450 nM, respectively. The experiments were performed in 10 mM potassium phosphate buffer containing 100 mM KCl, pH 6. The temperature was 20 °C.

decreases to 45 °C, indicating destabilization of the DNA (traces1 and 2, respectively, Figure 7a). The profile of structural changes in the NC-DNA in the presence of the prion protein with temperature was broad, and we have presented the results as derivatives of changes of absorbance at 260 nm against the temperature of the solution in Figure 7b. The plot (trace 1) shows a single peak with a T_m at 63 °C in Tris-HCl buffer for NC-DNA. In the presence of 10.8 μM α-PrP, the major peak shows a T_m value of 53 °C, indicating destabilization and unwinding of the NC-DNA similar to those observed for the Lef-DNA above (trace 2, Figure 7b). The trace also shows two shoulders at 63 and 68 °C, the origins of which are not clear. In the presence of PrP121−231, the temperature profile of the OD change of the NC-DNA was similar to that in buffer (trace, 3), indicating that this globular segment of the prion protein does not influence the structural stability of NC-DNA. The stability of a plasmid, pXNnlslacZ, however, was not perturbed by α-PrP (10.8 μM) using the same [protein]/[DNA-ph] ratio of 0.85 shown in Figure 7c, where traces 1 and 2 are the temperature profiles of structural changes of the plasmid DNA in buffer and in the presence of α-PrP, respectively.

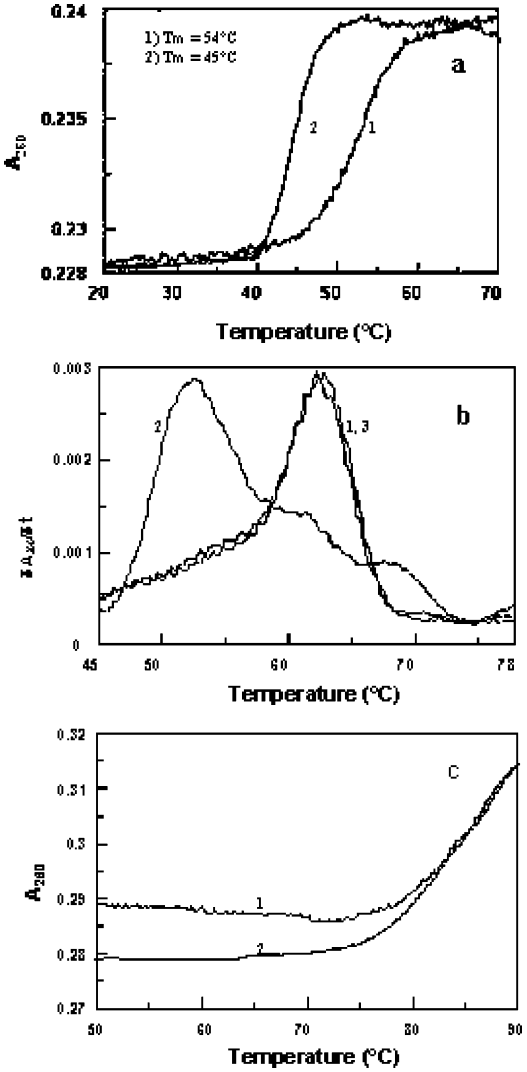

FIGURE 7: Effect of α-PrP on the stability of DNA structure measured from the change in the absorbance of nucleic acid at 260 nm, pH 7.2, in Tris-HCl buffer, 0.1 M. (a) Trace 1, Lef-DNA in buffer, and trace 2, in the presence of the prion protein. (b) Traces 1 and 2, temperature profiles of NC-DNA in buffer and in the presence of 10.8 μM prion protein respectively; trace 3, temperature profile of the absorbance change in the presence of the globular fragment moPrP121−231. (c) Effect of the prion protein on the thermal denaturation of pXNnlslacZ (trace 1) in buffer and (trace 2) in the presence of protein. The protein to nucleotide phosphates in the experiments were maintained as 0.15. The temperature increment was 0.5°/min.

DISCUSSION

The effect of α-PrP on the FRET of the oligonucleotides shows that the protein can bend both the 16 bp Lef-DNA and the 28 bp NC-DNA, and the bending process is complete within 20 s of the mixing of the protein with the DNAs (Figure 6). Comparisons of the results of steady-state FRET, kinetics of the FRET effect, and binding of the prion protein with the oligonucletides suggest that binding of the protein to nucleic acid and the bending of the latter proceed simultaneously. Similar simultaneous DNA binding and bending have also been observed with *Eco*RI methyltransferase (*37*). The increased FRET efficiency for the 28 bp NC-DNA suggests that the prion protein is more effective in bending this oligonucleotide than the smaller 16 bp Lef-DNA (Figure 4). These observations are in apparent contradiction of the prediction from FRET theory since this effect decreases with increase in the size of the oligonucleotides (*38*). The interaction of the prion protein with the oligonucleotide is sterically defined, which may explain the observed discrepancy (*46*). However, it is also possible that the observed fluorescent property changes in the presence of the prion protein may arise from the aggregation between DNA molecules in the presence of the protein. The absence of FRET effect when the prion protein was added to the mixture of donor- and acceptor-labeled single strands of the oligonucleotides suggests that the observed FRET effect arises from the conformational change of the double-stranded oligonucleotides in the presence of the prion protein.

Comparison of the results of Figures 4 and 5 shows that the FRET effect is complete before the saturation of binding

of the protein to DNA measured from Trp polarization of the protein. The FRET effect shows the change in the DNA conformation when a ligand binds to it, and our results show that the bending of the oligonucleotides is complete before the attainment of saturation of the binding of the protein to the oligonucleotides.

The present result also shows that the globular protein fragment 121−231 is unable to show the bending properties of the full-length α-PrP. Both NMR and small-angle X-ray scattering results demonstrate that DNA interacts and forms a stable complex with the globular fragment of the protein. NMR results also show that the unstructured N-terminal segment contributes to the formation of the consolidated complex of the full-length protein with DNA (*27*). Further, DNA binds to the full-length prion protein and the globular 121−231 fragment with comparable affinities (*27*). In spite of these observations, our results show that the N-terminal positively charged unstructured basic peptide segment of the protein is essential to induce bending of the DNA.

The increased strain due to the prion protein induced bending of Lef-DNA and NC-DNA is expected to facilitate dissociation and unwinding of the DNA strands, thereby destabilizing its structure. This we find in Figure 7a,b where the presence of α-PrP lowers the melting temperature of both of the DNAs. The high mobility group domain of the Lef-1 protein also bends and unwinds DNA (*47*). The globular C-terminal 121−231 prion protein fragment which does not bend the DNA (Figure 3) also does not have any effect on the melting of the DNA under identical experimental conditions (Figure 7b, trace 3). This fragment has been found to be unable to induce DNA strand transfer observed with the full-length protein (*34*). Therefore, we find that although the full-length prion protein and its globular 121−231 fragment have comparable affinities for DNA, the positively charged N-terminal segment of the prion protein bends and unwinds DNA, which are related to the DNA strand transfer properties of the prion protein (*27*, *34*). It has been reported that the binding energy (affinity) of proteins to DNA does not correlate with the DNA unwinding properties of proteins (*48*).

The 28 bp NC-DNA constitutes the stem−loop region of the HIV-1 genome. The HIV-1 nucleocapsid protein NCp7 has been found to dissociate this short DNA duplex (NC-nucleotide used here) in the stem−loop region in order to form a longer helix with a competing complementary single strand (*49*). The prion protein mimics these properties of the NCp7 protein (*34*). We consider that the destabilization of NC-DNA by the prion protein observed in this study results from the bending of the duplex by the protein. The destabilization of the DNAs in this study by the prion protein would also suggest that the protein possesses greater affinity for the single-stranded DNA (*49*). The NCp7 protein, which is very similar to the prion protein in its DNA strand transfer and DNA chaperoning properties, has been postulated to bind to the high-affinity TG sequence present in the 3′ and 5′ ends of NC-DNA (Figure 1) (*49*). It is a matter of speculation that the prion protein may also have high affinity for the TG sequence since Lef-DNA also has this sequence (Figure 1) and the protein also destabilizes the structure of this oligonucleotide (Figure 7a).

The addition of α-PrP to a plasmid, pXNnlslacZ (18.8 kb), did not destabilize the structure of this DNA (Figure 7b). The nucleocapsid protein, NCp7, is also without any effect on the structure of large DNA (*49*). The instability of the NC- and Lef-DNA may arise from end effects with oligonucleotides where the protein might induce "opening−breathing" of the small oligonucleotides similar to what has been suggested for the destabilization of NC-DNA by NCp7 (*49*). It has been suggested that the reduced stability of the nucleic acid and electrostatic interaction of the basic NCp7 protein with it are probably responsible for nucleic acid chaperoning properties of NCp7 (*50*). A similar mechanism may be responsible for the nucleic acid chaperoning property of the prion protein.

The results in the literature show that the proteins which are involved in initiation and regulation of eukaryotic or prokaryotic protein synthesis simultaneously bind and bend the DNA. The agreement between the results of the decrease in the fluorescence intensity obtained from stopped-flow (Figure 6) and steady-state experiments (not shown) in the present study suggests that the prion protein behaves similarly to these proteins (*36*, *43*, *51*, *52*). Bending of DNA is also observed in the process of mismatch repair and gene transcriptional regulation (*37*). It has been demonstrated that transcription of most eukaryotic genes is regulated through the cooperative action of multiple transcription regulatory proteins which can be achieved by DNA bending, leading to the exposure of nucleic acid bases to the solvent, thereby facilitating binding of these proteins to different DNA recognition sites (*52*). The bending of DNA by the prion protein may also lead to exposure of binding sties for transcription regulatory proteins that can be further facilitated by unwinding of the DNA (*47*). Therefore, in addition to the already observed polymerization of the prion protein to amyloid polymers and DNA strand transfer properties observed when DNA and the prion protein interact, the present study shows that the prion protein also modifies the DNA structure similar to the cellular proteins involved in transcriptional regulation. This property of the prion protein needs further elaboration, which is expected to provide a better understanding of the biology of the prion protein.

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