Analysis of nucleic acid chaperoning by the prion protein and its inhibition by oligonucleotides

Cécile Guichard¹, Roland Ivanyi-Nagy¹, Kamal Kant Sharma², Caroline Gabus¹, Daniel Marc³, Yves Mély² and Jean-Luc Darlix¹,*

¹Unité de Virologie Humaine INSERM, ENS, IFR 128, 46 allée d’Italie, 69364 Lyon, ²UMR 7213 CNRS, Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie, Université de Strasbourg, 74 route du Rhin, 67401 Illkirch and ³INRA, Nouzilly, TOURS, France

ABSTRACT
Prion diseases are unique neurodegenerative illnesses associated with the conversion of the cellular prion protein (PrPC) into the aggregated misfolded scrapie isoform, named PrPSc. Recent studies on the physiological role of PrPC revealed that this protein has probably multiple functions, notably in cell–cell adhesion and signal transduction, and in assisting nucleic acid folding. In fact, in vitro findings indicated that the human PrP (huPrP) possesses nucleic acid binding and annealing activities, similarly to nucleic acid chaperone proteins that play essential roles in cellular DNA and RNA metabolism. Here, we show that a peptide, representing the N-terminal domain of huPrP, facilitates nucleic acid annealing by two parallel pathways nucleated through the stem termini. We also show that PrP of human or ovine origin facilitates DNA strand exchange, ribozyme-directed cleavage of an RNA template and RNA trans-splicing in a manner similar to the nucleocapsid protein of HIV-1. In an attempt to characterize inhibitors of PrP-chaperoning in vitro we discovered that the thioaptamer 5'-GACACAAGC CGA-3' was extensively inhibiting the PrP chaperoning activities. At the same time a recently characterized methylated oligoribonucleotide inhibiting the chaperoning activity of the HIV-1 nucleocapsid protein was poorly impairing the PrP chaperoning activities.

INTRODUCTION
Transmissible spongiform encephalopathies (TSEs) such as Creutzfeldt-Jakob disease (CJD), kuru and familial insomnia (FFI) in humans, scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle are a group of fatal neurodegenerative diseases (1–3). A major molecular characteristic of TSEs is the accumulation of a misfolded, aggregated, partially protease-resistant prion protein (PrP), named PrPres, in the central nervous system (CNS) (1–3). Accumulation of PrPres appears to take place by recruitment and templated transconformation of the normal cellular prion protein (PrPC) by PrPres (4–7), and is thought to induce functional damages to the CNS. In support of this, generation of spongiform encephalopathy requires the presence of both PrPres and PrPC since mice devoid of PrPC are resistant to challenge with the infectious prion agent (8).

The PrPC is highly conserved in mammals and abundantly expressed in cells of the nervous and lymphoreticular systems but its physiological role has remained for a long time a matter of speculation (2,9). In fact, PrP null mice were found to develop and reproduce normally (10), or manifested only subtle phenotypic effects [reviewed in (11)], suggesting that PrPC has no essential function(s) that could not be compensated by proteins with overlapping activities. Nevertheless, several functions have been proposed for PrPC, including superoxide dismutase activity (12,13), participation in copper metabolism (14), signal transduction (15) and neuroprotection [(16,17), and references therein]. Recent studies revealed that PrPC has a crucial role in cell–cell adhesion and in signal transduction mediated by Src-related kinases in the zebrafish animal model (18). In addition, there are clear indications that PrPC assists nucleic acid folding and interactions in a manner similar to cellular and viral nucleic acid chaperones in vitro (19–21), and might well restrict retrovirus replication (22,23).

In fact, there are many different nucleic acid binding proteins that recognize DNA and RNA with a broad...
sequence specificity in any given cell. Among these ubiquitous nucleic acid binding proteins (NABP) there exists a class named nucleic acid chaperones, which provide assistance to the folding of DNA and RNA by preventing and resolving misfolding, and by chaperoning DNA/RNA interactions (24,25). Thus nucleic acid chaperones are considered to be essential co-factors for many basic biological processes including nucleic acid maintenance, RNA splicing, transport and translation (24,25) and PrPC would be one of these proteins (19–21).

In an attempt to better understand the relationship between the PrP and nucleic acids and its possible role in nucleic acid metabolism, we investigated the nucleic acid chaperoning activities of the recombinant human and ovine PrP in vitro. Using fluorescence resonance energy transfer (FRET), we report that a prion peptide representing the N-terminal domain of huPrP is very active in facilitating nucleic acid annealing. Furthermore, by using established nucleic acid chaperoning assays, notably DNA strand transfer (26), ribozyme cleavage of an RNA substrate (27,28) and RNA trans-splicing (29,30), we show that the human and ovine PrPs are potent nucleic acid chaperones. Recently we have been able to characterize a potent oligonucleotide (ODN) inhibitor of the nucleic acid chaperoning activity of the HIV-1 nucleocapsid protein (NC) in vitro and its inhibitory effect on virus replication in primary human cells (31). This prompted us to search for ODNs capable of inhibiting the nucleic acid chaperoning activity of PrP. We discovered that a previously identified 5'-GACACAAGCGA-3' thioaptamer binding to Syrian hamster (ShA) and human PrP (32) was a potent inhibitor of PrP-chaperoning activity in vitro, while the non-modified ODN was not, and a methylated ODN specific for HIV-1 NC was slightly inhibitory.

MATERIALS AND METHODS

Recombinant proteins

Recombinant human PrP (huPrP from residues 23–231), the N-terminal region [huPrP(23–145)] and the C-terminal region [huPrP(222–231)] were expressed in Escherichia coli and purified to homogeneity (19). The N-terminal region 23–110 of huPrP was synthesized by fmoc chemistry and purified to homogeneity by HPLC (19). The ovine PrP (ovPrP, residues 25–234) was produced in E. coli and purified to homogeneity (33). HIV-1 nucleocapsid protein NCp7 and NC(12–53) lacking the N- and C-terminal regions were obtained as pure proteins as previously described (19,34). Proteins were dissolved at 1 mg/ml in buffer containing 30 mM HEPES pH 6.5, 30 mM NaCl and 0.1 mM ZnCl2. HnRNP A1 and YB-1/p50 were provided by Christiane Branlant (France) and Lev Ovchinnikov (Russia), respectively.

Plasmid DNAs and RNAs

Plasmids pS14, pS20 and pR3, for the ribozyme assays, were provided by E. Bertrand (Montpellier) (28) and plasmids H1 and H2 for the trans-splicing assays by Renée Schroeder (Vienna, Austria). All plasmid DNAs were amplified in E. coli Rec A- cells and purified by affinity chromatography (Qiagen, USA). H1 DNA (549 nt of exon 1 and 131 nt of the 5' part of the intron) was linearized with SalI and H2 DNA (147 nt of the 3' half of the intron and 23 nt of exon 2) was linearized with BamHI, then transcribed. Templates pS14, pS20 and pR3 were digested by Pst I, treated by Klenow polymerase to remove the 3' strand overhang, and then the substrate RNA and the ribozyme were generated by in vitro transcription with modifications: for substrate RNA UTP was at 10 μM and 50 μCi 32P-UTP (Amersham, UK) was added. For the ribozyme, UTP was at 100 μM with 10 μCi of 32P-UTP. For the trans-splicing assays, RNAs H1 and H2 were prepared by in vitro transcription with T7 RNA polymerase according to the manufacturer's instructions (Promega) and labelled by incorporation of 35S-UMP during transcription.

Following RNA synthesis, the DNA template was removed by treatment with RNase-free DNase I (Promega) for 20 min at 37°C, followed by phenol extraction and ethanol precipitation. All RNAs were purified by 8% PAGE in 7 M urea, 0.5× TBE. RNAs were recovered by elution in 0.3 M sodium acetate, 0.1% SDS, for 4 h at 37°C and ethanol precipitated. RNAs were dissolved in sterile H2O and their integrity was verified by PAGE–urea.

TAR DNA

ODNs used for DNA annealing corresponded to the HIV-1 TAR sequences, in the sense and antisense orientations, respectively. ODNs were purchased from Eurogentec (Belgium). TAR ODNs are 56 nt in length.

TAR(+) (sense):
5'-GGTCTCTCTTGTTAGACCAGGTCCGCGGGAGCTCTCTGGCTAGCAAGGAACCC-3'

TAR(-) (antisense):
5'-GGGTTCTCTGTAGCCAGAGCTCCCAGGCTCGACCTGGTCTAACAAGAGACC-3'

ODNs used for DNA strand transfer assays corresponded to the HIV-1 repeated R sequences, in the sense and antisense orientations, already described in (35,36).

TAR(-) and R(+) ODN were 32P-labelled with 50 μCi of 32P-ATP using T4 polynucleotide kinase. Labelled ODNs were purified by 10% PAGE and 20% ethanol precipitation. For the ribozyme, UTP was at 100 μM with 10 μCi of 32P-UTP. For the trans-splicing assays, RNAs H1 and H2 were prepared by in vitro transcription with T7 RNA polymerase according to the manufacturer's instructions (Promega) and labelled by incorporation of 35S-UMP during transcription.

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TAR(-) and R(+) ODN were 32P-labelled with 50 μCi of 32P-ATP using T4 polynucleotide kinase. Labelled ODNs were purified by 10% PAGE, 7 M urea in 50 mM Tris borate, 1 mM EDTA, pH 8.3 (0.5× TBE) and recovered, ethanol precipitated and dissolved in sterile H2O before use.

Oligonucleotides

The 12- nt long thioaptamer (GA-12; 5'-GACACAAGCGA-3'), with five modifications S' to the dA residues, mODN-11 (5'-GGUUUUUGUGU-3' with a 2'O-methyl modification at each residue) and ODN-11 (5'-GGTTTTTGTGT-3', without modifications) were obtained in a highly pure form from Eurogentec (Belgium). Ct-12 corresponds to GA-12 without the modifications.
Destabilization assay

To monitor the nucleic acid destabilizing properties of huPrP, cTAR DNA was labelled either by carboxytetramethylrhodamine (TMR) or ethyl 2-[3-(ethylamino)-6-ethyl]-2,7-dimethylxanthen-9-yl]benzoate hydrochloride (Rh6G) at its 5'-end, while the 3' terminus was labelled with either 4-(4'-dimethylaminophenylazo) benzoic acid (Dabcyl) or 5(and 6)-carboxyfluorescein (Fl). The dyes form a non-fluorescent heterodimer when the cTAR stem is closed, while melting of the stem restores the fluorophore fluorescence. Thus, the destabilizing ability of HuPrP peptide (23–110) was evaluated from the ratio of the fluorescence intensity in the presence versus the absence of the peptide. Experiments were performed by adding huPrP (23–110) at increasing peptide to oligonucleotide ratio to 50 nM doubly labelled cTAR, in 25 mM Tris, 30 mM NaCl and 0.2 mM MgCl₂, pH 7.5.

Kinetics of cTAR-dTAR annealing with the huPrP peptide (23–110)

The real-time annealing kinetics of cTAR with dTAR was investigated by mixing Rh6G-5'-cTAR-3'-Dabcyl with an excess of non-labelled dTAR in conditions already published (35,37). Formation of the 55-bp cTAR/dTAR extended duplex (ED) strongly increases the interchromophore distance, leading to a full recovery of Rh6G emission. The dependence of cTAR-dTAR annealing kinetics on temperature was performed at six different temperatures, viz. 15, 20, 25, 30, 35 and 40°C, as previously described (37).

The TAR(−)/TAR(+) annealing assay

Tar(+) and 32P-Tar(−) ODNs were incubated (0.03 pmol each) in 10 μl of buffer A (35,37) in the presence of increasing protein concentrations. Reactions were performed at 37°C for 5 min except for the positive control which was incubated at 65°C. To stop the reaction and denature the protein, we added 5 μl of a solution containing 20% glycerol, 20 mM EDTA, pH 8.0, 2% SDS, 0.25% bromophenol blue and 0.4 mg/ml calf liver tRNA. Samples were resolved by 8% native PAGE in 50 mM TBE at 4°C. The level of strand exchange as a function of time was quantified by PhosphorImaging.

Effect of inhibitors on the kinetics of cTAR-dTAR annealing with the N-terminal huPrP

Inhibition of huPrP(23–110)-promoted cTAR-dTAR annealing was performed by adding a 5-fold molar excess of GA-12, mODN-11 or Ct-12 relative to cTAR/dTAR. The cTAR (or dTAR)-huPrP(23–110)-ODN mixtures were incubated for 5 min to ensure that the binding equilibrium was reached. Then, cTAR-dTAR hybridization was initiated by manual mixing of cTAR-huPrP (23–110)-ODN mixture with dTAR-huPrP(23–110)-ODN mixture. To avoid the effects of local concentration fluctuations on the annealing kinetics, equal volumes of the mixtures were used. Experiments were performed in pseudo-first order conditions by mixing 10 nM of doubly labelled cTAR with 300 nM of non-labelled dTAR in presence of huPrP(23–110) at a peptide/oligonucleotide molar ratio of 1:1. To this mixture, inhibitors were added in increasing concentrations (from 0.1- to 10-fold) to follow their concentration dependent inhibitory effect.

All experiments were performed in 25 mM Tris–HCl (pH 7.5), 30 mM NaCl, 0.2 mM MgCl₂ at 20°C.

DNA strand transfer assay

32P-labelled R(+) wt, non-labelled R(−) 3’-modified and R(−) wt were heat denatured (2 min at 90°C) and chilled on ice. All components were kept on ice. An amount of 0.03 pmol each of R(+) wt and R(−) 3’-modified were mixed with reaction buffer to a final concentration of 20 mM Tris–HCl, pH 7.0, 30 mM NaCl, 0.1 mM MgCl₂, 10 μM ZnCl₂ and 5 mM DTT in 5 μl final volume, incubated for 30 min at 65°C and chilled on ice. Subsequently, 0.03 pmol R(−) wt was added together with the chaperone protein at a final protein to nucleotide molar ratio as indicated in figure legends. Reactions were left to proceed for 1–5 min at 37°C. The mixtures were then chilled on ice and reactions stopped with 2.5 μl of 20% glycerol, 20 mM EDTA pH 8.0, 0.2% SDS, 0.25% bromophenol blue and 0.4 mg/ml calf liver tRNA. Samples were resolved by 6% native PAGE in 50 mM TBE at 4°C. The level of strand exchange as a function of time was quantified by PhosphorImaging.

Hammerhead Ribozyme cleavage assay

Ribozyme and substrate RNAs were independently heated for 1 min at 90°C in H₂O. The reaction buffer was added to yield final concentrations of 5 mM MgCl₂, 100 mM NaCl, 20 mM Tris–HCl, pH 7.5. After slowly cooling down to 37°C, RNAs were further incubated for 5 min at 20°C. An amount of 0.1 pmol of ribozyme and 0.02–2 pmol of RNA substrate were then combined in a final volume of 10 μl, each protein was added at final concentrations as indicated in the figure legend and incubations were for 1–30 min at 37°C. Reactions were terminated by adding 20 μl of stop solution (0.3% SDS, 15 mM EDTA), and RNAs were extracted with 30 μl of phenol and 15 μl of chloroform. The aqueous phase was precipitated with ethanol and the pellet resuspended in 45% formamide, 0.5× TBE, and 0.1% dye. RNAs were then analysed on 8% PAGE in TBE. All quantitative measurements were done by PhosphorImaging.

Trans-splicing assay

An amount of 2 × 10⁻⁸ M each of 35S-labelled RNAs were incubated for 1 min at 95°C in H₂O and cooled down to 37°C. Then, the splicing buffer (40 mM Tris–Cl at pH 7.4, 3 mM MgCl₂, 0.4 mM spermidine, 4 mM DTT), 8 U RNAsin (Promega) and 32P-GTP were added with the nucleic acid chaperone, at protein to nucleotide molar ratios as indicated in the figure legend, at 37°C for 30 min in 10 μl. Reactions were stopped by adding a final concentration of 40 mM EDTA and 300 μg/ml of tRNA. Proteins were removed by phenol–chloroform.
RESULTS

In order to examine the nucleic acid chaperoning (24,25,38) properties of the PrP, we have used advanced assays [reviewed in (39)] that have been developed for the characterization of key chaperone proteins implicated in the metabolism of cellular RNAs such as hnRNP A1 and FMRP proteins (28,40–42), and of retroviral RNA, like the NC protein of retroviruses [(26,28,42), reviewed in (43,44)]. Although the details of their mechanism of action is incompletely understood, nucleic acid chaperones are thought to facilitate RNA (and DNA) conformational rearrangements by a variety of ATP-independent mechanisms, including destabilization of preformed helices, charge neutralization and matchmaker activity (24,25,45,46).

First, we have analysed the helix destabilizing activity of the N-terminal peptide (23–110) of huPrP. Using the doubly labelled cTAR DNA stem-loop (SL), there was no evidence of helix destabilizing activity of huPrP(23–110) in the present conditions, as observed by FRET (Figure 1A and Supplementary Data). This differs from HIV-1 NC protein, which shows significant helix destabilizing activity (47–49) (data not shown), but is similar to the behaviour of the core protein of hepatitis C virus (HCV), another well-characterized RNA chaperone with limited cTAR melting activity (37). Next, we monitored the impact of huPrP(23–110) on the annealing kinetics of cTAR/dTAR by FRET.

Kinetics of cTAR-dTAR annealing facilitated by the N-terminal peptide of huPrP

The real-time annealing kinetics of cTAR to dTAR was investigated in pseudo-first order conditions by mixing Rh6G-5′-cTAR-3′-Dabcyl with an excess of non-labelled dTAR (35,37). Formation of the 55-bp cTAR/dTAR ED strongly increases the interchromophore distance, leading to a full recovery of Rh6G emission. In the absence of huPrP(23–110), the annealing of cTAR with dTAR was very slow, involving two distinct kinetic components with second-order rate constants. In the presence of huPrP(23–110) at a peptide/TAR ratio of 1/1, the same fluorescence plateau was observed as in the absence of peptide, indicating that ED formation went to completion. The huPrP(23–110) strongly accelerated the annealing reaction, since it was completed in ~30 min, instead of >1 day without the peptide. An adequate fit of the annealing kinetic traces was obtained using a bi-exponential function:

\[ I(t) = I_f - (I_f - I_0) \left( a e^{-k_{obs1} (t-t_0)} + (1-a) e^{-k_{obs2} (t-t_0)} \right) \]  

(1)

where \( t_0 \) is the dead time, \( k_{obs1,2} \) are the observed kinetic rate constants, \( a \) is the amplitude of the fast component, and \( I_0 \) and \( I_f \) are the fluorescence intensities of the SL and the ED, respectively.

We already observed a fast and full fluorescence increase at the lowest tested oligonucleotide concentrations (10 nM cTAR and 100 nM dTAR) (Figure 1), suggesting that the peptide probably binds to the TAR sequences with a high affinity (>10^7 M^-1). Indeed, if the binding constant would be <10^6 M^-1, and if we assume that, as for other nucleic acid chaperones, PrP does not exhibit any catalytic activity, most of the oligonucleotides would be free and would anneal at the low speed observed for cTAR and dTAR in the absence of protein.

Annealing as a function of the dTAR concentration indicated a saturation behaviour of both \( k_{obs1} \) and \( k_{obs2} \) values as well as a constant value for their amplitudes (Supplementary Figure S1B and Supplementary Data). Moreover, the values of the intercept for both \( k_{obs1} \) and \( k_{obs2} \) curves were very low, indicating that huPrP(23–110) is unable to dissociate the ED. Based on these kinetic data, a reaction mechanism with two parallel pathways can be proposed (scheme 1), as for the HCV core-promoted cTAR/dTAR annealing (37). Both the fast and slow pathways are based on a distinct cTAR/huPrP(23–110)
complex (cTAR1,2) and involve a rate-limiting interconversion step \( (k_{n.2}) \) coupled to a much faster, preceding binding step, governed by an equilibrium constant \( (K_{M,1,2}) \).

To further characterize the two pathways, the effect of the presence of the huPrP(23–110) on the annealing reaction was investigated. In this respect, dTAR was first substituted by the dTAR-T-L mutant where the 6 nt of the loop were changed to T residues, thus preventing its base-pairing with the cTAR loop. These nucleotide substitutions did not significantly change the time course of ED formation, indicating that loop–loop interactions do not play a significant role (Supplementary Figure S1B).

The nature of the two pathways was further investigated by analyzing the temperature dependence of the \( k_{obs} \) values (Supplementary Figure S1D and Supplementary Data). Furthermore, to investigate the role of the cTAR stem in the annealing reaction, we used the cTAR1,2 derivative where bases complementary to the bulged bases at Positions 49 and 52 have been introduced in order to stabilize the lower half of the stem. The annealing of this mutant to dTAR in the presence of huPrP(23–110) was extremely slow (Supplementary Figure S1C and Supplementary Data), indicating that both kinetic pathways of the huPrP(23–110)-promoted cTAR/dTAR annealing kinetics are nucleated through the stems of cTAR and dTAR.

The temperature dependence of huPrP(23–110)-promoted cTAR/dTAR annealing reaction was performed at 30°C from one to several minutes with NC, huPrP and ovPrP proteins at a protein to nucleotide molar ratio of 1/12 nt and 1/6 nt (lanes 7 and 8, respectively). Mutant NC(12–53) lacking the N- and C-terminal amino acids has only limited strand exchange activity (lanes 9 and 10). HuPrP was found to strongly activate the strand exchange activity of the chaperone protein facilitating formation of the perfect double-stranded DNA ([(+)-wt: (--)-wt]) molecules at the expense of [(+)-wt:(--)-mut] molecules with mismatches (Figure 2A). If the recombinant PrPs have general nucleic acid chaperoning activities, they should facilitate formation of the most stable duplex and thus activate the substitution of DNA (--)-wt for (--)-mut in the double-stranded nucleic acid molecule (Figure 2A).

The 32P-labelled DNA (+)-wt is shown in lane 1 while control double-stranded DNA [(+)-wt:(--)-wt] and [(+)-wt:(--)-mut] are shown in lanes 3 and 4, respectively (Figure 2B). Addition of DNA (--)-wt to double stranded [(+)-wt: (--)-mut] and incubation for 30 min at 0°C (lane 5) or 37°C (lane 6) did not result in any strand exchange. Addition of HIV-1 NCp7 together with DNA (--)-wt resulted in a nearly complete strand exchange under the conditions where NCp7 to nucleotide molar ratios were 1/12 nt and 1/6 nt (lanes 7 and 8, respectively). Mutant NC(12–53) lacking the N- and C-terminal amino acids has only limited strand exchange activity (lanes 9 and 10). HuPrP was found to strongly activate the strand exchange (lanes 11 and 12). A polypeptide representing the N-terminal disordered region of huPrP, huPrP(23–145) (52), also exhibited a strong strand exchange activity (lanes 13 and 14), whereas the polypeptide huPrP(122–231), representing the C-terminal, well-folded region of huPrP (52,53), had little activity (lanes 15 and 16). The recombinant ovine PrP (ovPrP) behaved in a manner similar to huPrP, showing a strong strand exchange activity (lanes 17 and 18) at protein to nucleotide ratios of 1:12 and 1:6.

For kinetic analysis, DNA strand exchange assays were performed at 30°C from one to several minutes with NC, huPrP and ovPrP proteins at a protein to nucleotide molar ratio of 1/12 nt (see ‘Materials and Methods’ section). For the wild-type NCp7, the exchange rate at 30°C was 0.031 mol of DNA exchanged per mole of protein per minute. For the mutant NC(12–53), this value was reduced about nine times, but still was about three times higher than that observed in the absence of NC protein. The exchange rates for the recombinant PrPs were very close to that for NCp7 (0.025, 0.03 and 0.03 mol of DNA...
per mole of protein per minute at 30°C for huPrP, huPrP (23–145) and ovPrP, respectively. For huPrP (122–231), the value was 10 times lower than that for huPrP (23–145).

Facilitation of ribozyme-directed cleavage of an RNA substrate by PrP

Next, we used the hammerhead ribozyme-directed cleavage of an RNA substrate to examine both the RNA annealing and unwinding activities of nucleic acid chaperones (27,28). Nucleic acid chaperones are thought to enhance the rate of ribozyme cleavage by activating the annealing of the substrate RNA to the ribozyme (Figure 3A, step 1) and the unwinding and release of the cleaved RNA products (Figure 3A, step 3), thus allowing recycling of the ribozyme. The ribozyme cleavage assay aims at examining whether PrP accelerates ribozyme cleavage of an RNA substrate in a manner similar to the viral NCp7 chaperone (Figure 3A) (27,28). We selected the R3 hammerhead ribozyme and two RNA substrates, namely S14, with a 14 nt substrate–ribozyme duplex length (7 nt either side of the cleavage site) and S20, with 10 nt either side of the cleavage site (28). The above RNA substrate model system was chosen due its likely biological relevance as evidenced by the similarity of data obtained both in vitro and in cultured cells (28). 32P-labelled RNA S14, the ribozyme and PrP were mixed and incubated for 30 min. RNAs were deproteinized, recovered and analysed by PAGE under denaturing conditions. In the absence of
Figure 3. PrPs facilitate ribozyme cleavage of an RNA. (A) Assay schematic. A hammerhead ribozyme and a \(^{32}\)P-labelled RNA substrate were generated by \textit{in vitro} transcription and gel purified. Cleavage of the \(^{32}\)P-RNA by the ribozyme appears to first necessitate hybridization of the ribozyme to the substrate (step 1). After RNA cleavage (step 2), the products must be released to allow recycling of the ribozyme (step 3). At the end of the reaction RNAs were deproteinized and analysed by PAGE under denaturing conditions to visualize the \(^{32}\)P-RNA products. In the absence of a nucleic acid chaperone, hybridization of the RNA to the ribozyme and release of the products appear to be slow. Addition of a nucleic acid chaperone will accelerate hybridization of the substrate to the ribozyme and dissociation of the products and thus ribozyme turnover. Base pairing between the RNA substrate and ribozyme R3 are underlined on the substrate sequence. Ribozyme mediated cleavage occurs on the 3' side of A (space). For RNA S14, ...GAUUAAGUAGUAGAGUGUCUGCA3', for RNA S20, ...GAUUAAGUAGUAGAGUGUCUGCA3'. (B) Ribozyme-directed cleavage of RNA substrate S14. A 1 \times 10^{-8} \text{ M} of ribozyme R3 and 5 \times 10^{-8} \text{ M} of RNA S14 were incubated as described in the methods. \(^{32}\)P-RNA substrate (S14) and product (\(\Delta S14\)) were analysed by denaturing 8% PAGE. Lanes 1 and 2: R3 and S14 at 4 or 37°C. Lanes 3 and 5: HIV-1 NCp7 at NC concentrations of 2 \times 10^{-8}, 4 \times 10^{-8} and 8 \times 10^{-8} \text{ M} (protein/nt molar ratios of 1/20, 1/10 and 1/5). Lanes 6–8: NC(12–53) at concentrations of 1.6 \times 10^{-7}, 3.2 \times 10^{-7} and 6.4 \times 10^{-7} \text{ M} (molar ratios of 1/2.5, 1/1.2 and 1/0.6). Lanes 9–11: ovPrP at 2 \times 10^{-8}, 4 \times 10^{-8} and 8 \times 10^{-8} \text{ M}, respectively. Lanes 12–14: huPrP at concentrations of 2 \times 10^{-8}, 4 \times 10^{-8} and 8 \times 10^{-8} \text{ M}, respectively. Lanes 15–17: huPrP(23–110) at concentrations of 2 \times 10^{-8}, 4 \times 10^{-8} and 8 \times 10^{-8} \text{ M}, respectively. Lanes 18–20: huPrP(23–145) at concentrations of 2 \times 10^{-8}, 4 \times 10^{-8} and 8 \times 10^{-8} \text{ M}, respectively. Lanes 21–23: huPrP(122–231) at concentrations of 2 \times 10^{-8}, 4 \times 10^{-8} and 8 \times 10^{-8} \text{ M}, respectively. R3, S14 and the 5' sequences of S14 (\(\Delta S14\)) are identified on the right. Markers are on the left. Arrow shows direction of electrophoresis. Note that ribozyme-directed cleavage of S14 or S20 did not occur at 4°C (lane 1). Note that enhanced ribozyme cleavage by PrPs is probably via their match-maker activity for the annealing of S14 RNA to the hammerhead ribozyme (step 1) and their destabilizing activity upon S14 RNA cleavage (step 3) [see ref. (25)].
a chaperone, ribozyme-directed cleavage of the RNA substrate occurred only slowly at 37°C (Figure 3B, lanes 1 at 4°C and 2 at 37°C; 32P-RNA substrate is S14 and cleaved product ΔS14). In agreement with previous reports (28), HIV-1 NCp7 facilitated ribozyme cleavage of RNA S14 to reach completion at a concentration as low as 2 × 10⁻⁸ M (lanes 3–5). On the other hand, NC(12–53) was relatively inactive even at a concentration above 1 × 10⁻⁷ M (lanes 6–8). Interestingly, PrP of ovine or human origin showed strong enhancement of ribozyme cleavage at 2–4 × 10⁻⁸ M (lanes 9–11 and 12–14, respectively). The N-terminal region of huPrP was very active (lanes 15–17 and 18–20) while the C-terminal region was very poorly active (lanes 21–23).

We also examined the effect of PrP using the RNA substrate S20, capable of forming an ED of 20 nt with the hammerhead ribozyme (Figure 3A) which precludes activation of ribozyme cleavage by HIV-1 NCp7 or hnRNP A1 (28). In the absence of NCp7 or PrP, only minimal ribozyme-directed cleavage of RNA S20 was observed at 37°C (Figure 3C, lane 2; 32P-RNA substrate is S20 and product ΔS20) as seen with RNA S14 (Figure 3B, lane 2). The enhancement of ribozyme cleavage of RNA S20 by NCp7 remained very modest even at NCp7 concentration of 4 × 10⁻⁸ M (Figure 2C, lanes 3–4), in agreement with reported data (28). Mutant NC (12–53) was also very poorly active (lanes 5–6). The ovPrP and huPrP also exhibited very little, if any, activity using RNA S20 [lanes 7–8, 9–10, 11–12, and 13–14, for ovPrP, huPrP, huPrP(23–145) and huPrP(122–231), respectively]. Kinetic assays of ribozyme cleavage of RNA S14 were performed with NCp7, huPrP and ovPrP at a concentration of 4 × 10⁻⁸ M and cleavage monitored for up to 30 min. Initial rates were 10–12 μmol of RNA S14 cleaved at 37°C/min/mole of NCp7 and ovPrP. For huPrP, the observed rate was 3-fold slower, while it was approximately 100-fold slower for NC(12–53) at concentrations above 1 × 10⁻⁷ M, although two to three times above rates obtained in the absence of NC or PrP. Similar results were obtained at huPrP or NC concentration of 1 × 10⁻⁷ M (data not shown).

HuPrP and ovPrP facilitate RNA trans-splicing

To further examine the RNA chaperoning activity of the mammalian PrPs, we used the previously established trans-splicing assay (29,30) (Figure 4A), where the pre-mRNA of the thymidylate synthase (td) gene containing a group I intron was split into two halves. The first RNA transcript, H1, corresponds to the 5′ exon sequence of 549 and 131 nt of the intron while the second RNA transcript, H2, represents the 3′ part of the intron (147 nt) and part of exon 2 (23 nt) (Figure 4A). The two RNAs were 35S-UMP labelled during transcription and were incubated together. Reaction was started by adding 32P-GTP, so that the resulting spliced RNA was doubly labelled, internally and at the 5′-end with 32P-GTP (Figure 4A). In the absence of protein, the reaction was carried out at 55°C to allow a productive interaction between H1 and H2 RNAs. Trans-splicing was indeed found to take place at 55°C but only to a limited extend [data not shown and (36)] but not at 37°C according to the accumulation of the 32P-GTP-I1 final product (lane 1 in Figure 4B and C). As previously reported (36) HIV-1 NCp7 strongly stimulated accumulation of 32P-GTP-I1 and therefore trans-splicing (lanes 2–5), with an optimal enhancement at a concentration of 8 × 10⁻⁷ M (panel B, lane 4). Mutant NC(12–53) was found to be poorly active even at 2 × 10⁻⁸ M (panel B, lane 7).

The recombinant huPrP was found to be a strong activator of trans-splicing (Figure 4B, lanes 8–11), with an optimal activation at a concentration of 4 × 10⁻⁷ M (lanes 10 and 11). Interestingly the N-terminal huPrP peptide (23–144) also activated trans-splicing (Figure 4B, lanes 9–12) at concentrations of 2–4 × 10⁻⁷ M (lanes 13 and 14) while the C-terminal domain (123–231) was clearly inactive (lanes 16 and 17) under the present conditions. The ovine PrP was already active at a concentration of 2 × 10⁻⁷ M (lanes 7–11 in panel C) and the N-terminal huPrP peptide (23–110) was as active as the full length huPrP (compare lanes 2–6 and 12–16 in panel C).

Inhibition of huPrP chaperoning activity by a thioaptamer

In a search for PrP-chaperoning inhibitors, we used a 12-nt long thioaptamer (GA-12; GACACAGCAGGA), with five modifications 5′ to the dA residues, since it was found to bind with high affinity to Syrian hamster, bovine and human PrPs in vitro (32). We also selected a 11-nt 2′-O-methyl oligoribonucleotide (mODN-11; GGUUUUG UGU) because it was shown to be a potent inhibitor of the nucleic acid chaperoning activity of the HIV-1 nucleocapsid protein (31).

We examined the ability of these two selected oligonucleotides, namely GA-12 and mODN-11, and of a control ODN with the non-modified sequence GACACAAGCCGA (Ct-12) to inhibit the annealing activity of huPrP (Figure 5A and B). As shown in lanes 7–9, only GA-12 at 100–200 nM was able to completely inhibit huPrP annealing activity, while mODN-11 (lanes 4–6) and ODN Ct-12 (lanes 10–12) were poorly active. The impact of such ODNs on the hammerhead ribozyme directed cleavage of an RNA substrate was monitored under the same conditions. Again the thioaptamer GA-12 at 50–100 nM was found to extensively inhibit the RNA chaperoning of huPrP (lanes 7–9) while mODN-11 at 100–200 nM had moderate effect on huPrP (lane 6) and ODN Ct-12 had no effect (lanes 10–12). Last, we monitored the impact of these three ODNs on the trans-splicing reaction facilitated by huPrP, and again the thioaptamer GA-12 was efficiently (lanes 7–9) inhibiting trans-splicing activation by huPrP, while Ct-12 and mODN-11 were inactive (lanes 4–6 and 10–11). Results obtained with ovPrP were very similar, namely an inhibition of ovPrP chaperoning activity by GA-12 at 100 nM but not by mODN-11 and Ct-12 (data not shown).

We also examined the effect of GA-12 and mODN-11 on huPrP(23–110)-promoted cTAR/dTAR annealing using FRET. Both GA-12 and mODN-11 decreased the kinetics of huPrP(23–110)-promoted cTAR/dTAR annealing but the decrease was more pronounced with
1. H1
2. GTP
3. GTP-
4. RNA products by PAGE analysis:

B
C

Figure 4. Facilitation of RNA trans-splicing by PrPs. (A) Schematic representation of the trans-splicing assay: the two RNA constructs H1 of 680 nt in length (encoding exon 1 of 549 nt and the 5' part of the intron of 131 nt) and H2 of 170 nt in length (encoding the 3' part of the intron of 147 nt and exon 2 of 23 nt) have to fold into a splicing competent structure (step 1). The splicing reaction was started upon addition of the 32P-labelled GTP. The recombinant PrP was added at step 2, where indicated. The final RNA products are represented in step 4. All splicing rates were monitored based on the levels of the final product 32P-labelled GTP-I1 [see (B)]. (B and C) Assays where the H1 and H2 RNAs were incubated with or without a nucleic acid chaperone, namely NCp7 or PrP. At the end of the reaction RNAs were phenol treated to remove the chaperone and analysed by PAGE in denaturing conditions (see ‘Materials and Methods’ section). The RNA substrates (H1, H2), the ligated exons (E1-E2) and the product (guanosine-5'-intron G-I1) are indicated. Panel B. Lane 1: trans-splicing reaction at 37°C with RNA alone at a concentration of 4 × 10⁻⁸ M. Lanes 2–5: HIV-1 NCp7 at concentrations of 2.5, 5, 10 and 20 × 10⁻⁷ M, corresponding to protein to nucleotide ratios of 1:128, 1:64, 1:32 and 1:16. Lanes 6 and 7: NC(12–53) at concentrations of 1 and 2 × 10⁻⁶ M, corresponding to peptide to nucleotide ratios of 1:32 and 1:16. Lanes 8–11: recombinant huPrP(23–231) at concentrations of 2, 4, 8 and 16 × 10⁻⁷ M, corresponding to PrP to nucleotide molar ratios of 1:160, 1:80, 1:40 and 1:20. Lanes 12–15: N-terminal huPrP(23–144) at the same protein to nucleotide ratios as for the huPrP(23–231). Lanes 16 and 17: C-terminal huPrP(123–231) at protein to nt molar ratios of 1:40 and 1:20. Panel C. Lane 1: splicing reaction at 37°C with RNA alone at a concentration of 4 × 10⁻⁸ M. Lanes 2–6: huPrP at concentrations of 1, 2, 4, 8 and 16 × 10⁻⁷ M. Lanes 7–11: recombinant ovPrP at concentrations of 2, 4, 8, 16 and 32 × 10⁻⁷ M. Lanes 12–16: huPrP(23–110) at concentrations of 1, 2, 4, 8 and 16 × 10⁻⁷ M. Note that HIV-1 NCp7, huPrP and ovPrP strongly activated trans-splicing at protein to nt molar ratios of 1:20 to 1:40. Peptides NC(12–53) and C-terminal huPrP(122–231) were very poorly active (lanes 6, 7, 16; in panel B).
Figure 5. Thioaptamer inhibition of huPrP chaperoning activities in vitro. (A) Annealing of TAR(+)TAR(−). The annealing conditions were exactly as described in the ‘Materials and Methods’ section with the TAR(+) and TAR(−) DNA. PAGE analyses are described in ‘Materials and Methods’ section. Lane 1: Control at 65°C for 30 min; lane 2: at 37°C for 30 min. Lanes 3–12: with huPrP for 5 min at 37°C. Lane 3: control showing the complete hybridization of TAR(+) and TAR(−) DNA. Lanes 4–6: with mODN-11 added at the start of the incubation period at 50, 100 and 200 nM. No inhibition of huPrP annealing activity was observed. Lanes 7–9: with ODN GA-12 at 50, 100 and 200 nM. Note that ODN GA-12 causes a complete inhibition of huPrP chaperoning at 200 nM. Lanes 10–12: with control ODN Ct-12 at 50, 100 and 200 nM. (B) Ribozyme mediated cleavage of an RNA substrate. The conditions for the ribozyme cleavage were exactly as described in the ‘Materials and Methods’ section with hammerhead ribozyme and the RNA substrate. PAGE analyses under denaturing conditions are described in the ‘Materials and Methods’ section. Lane 1: Control at 0°C for 30 min; lane 2: at 37°C for 30 min without PrP. Lanes 3: control with huPrP for 15 min at 37°C showing a complete cleavage. Lanes 4–6: with mODN-11 added at the start of the incubation period at 50, 100 and 200 nM. Some inhibition of huPrP activity was observed at 100 nM. Lanes 7–9: with ODN GA-12 at 50, 100 and 200 nM. Note that ODN GA-12 causes a nearly complete inhibition of huPrP at 100 nM. (C) The RNA trans-splicing reaction. The conditions for the trans-splicing reactions were exactly as described in the ‘Materials and Methods’ section with the H1 and H2 RNAs. PAGE analyses under denaturing conditions are described in the ‘Materials and Methods’ section. Lane 1: Control at 0°C for 30 min; lane 2: at 37°C for 30 min without PrP. Lanes 3: control with huPrP at 8 × 10⁻⁷ M for 30 min at 37°C showing a complete cleavage. Lanes 4–6: with mODN-11 added at the start of the incubation period at 50, 100 and 200 nM. No inhibition of PrP activity was observed. Lanes 7–9: with ODN GA-12 at 50, 100 and 200 nM. Note that ODN GA-12 causes a nearly complete inhibition of huPrP at 100 nM. Lanes 10–12: with control ODN Ct-12 at 50, 100 and 200 nM.
GA-12 (Figure 6A). Finally, we analysed the concentration dependence of the inhibition of GA-12 on the huPrP-promoted annealing of cTAR/dTAR (Figure 6A). The inhibitory effect of GA-12 increased with its concentration and appeared already when the GA-12 concentration was one order of magnitude lower as compared to cTAR and dTAR concentrations. All kinetic traces in the presence of GA-12 were adequately fitted with Equation (1) and showed a decrease in both $k_{\text{obs1}}$ and $k_{\text{obs2}}$ values (Supplementary Table S1a and S1b). These data are consistent with a tight binding of GA-12 to huPrP, which reduces the concentration of huPrP available for promoting the cTAR/dTAR annealing reaction. The inhibitor was also found to decrease the amplitude of the fast pathway, further substantiating the hypothesis of a huPrP sequestration by the inhibitor, since a similar decrease in the amplitude of the fast pathway with the concentration of protein was observed with the HCV core protein (37).

Taken together these results show that the GACACAAGCCGA thioaptamer is a potent inhibitor of the huPrP nucleic acid chaperoning activity in vitro and this appears to rely on the backbone modifications.

DISCUSSION

Collectively the data presented herein clearly show that the human and ovine PrPs can assist the folding of RNA and DNA molecules in physiological conditions and at concentrations of $10^{-7}$ M or below (Figures 1–5). Thus, these two mammalian PrPs have the hallmark of bona fide nucleic acid chaperones (24,25). In addition, the chaperoning properties of the human PrP reside within the N-terminal region of the protein (Figures 1–4).

Interestingly this N-terminal region possesses the characteristic features of a chaperone (54,55) since it contains aromatic residues and clusters of basic amino acids in an intrinsically unstructured environment (52,56,57). Despite the absence (or relaxation) of structural constraints on mutations in the N-terminal domain of PrP, the sequence and distribution of basic amino acids is highly conserved between various mammalian PrPs (data not shown).

As a consequence of its chaperone activity, the N-terminal region of huPrP was found to activate by two orders of magnitude the kinetics of the cTAR/dTAR annealing reaction, probably through a reaction mechanism similar to that described for the HCV core protein (37). In this mechanism, the fast and slow pathways were found to be nucleated through the cTAR ends, but differ by the number of base pairs that should be premelted in the original cTAR secondary structure to nucleate the IC formation. In its free form, as a consequence of the thermal fraying that occurs spontaneously at room temperature, the closed cTAR species was shown to be in equilibrium with partially melted cTAR species where either the terminal or both the terminal and penultimate double-stranded segments of the stem are melted (47–49). In this respect, the 8.3 kcal/mol enthalpy energy associated to the fast pathway is consistent with the melting of the terminal double-stranded 3-bp segment, which is poorly stable due to its position at the end of the stem and the presence of the neighbouring G52 bulge. On the other hand, the 16.5 kcal/mol enthalpy energy associated with the slow pathway is consistent with the energy needed to melt both the terminal and penultimate double-stranded segments of the cTAR stem (Figure 7). As a consequence of this thermal fraying, the

**Figure 6.** Inhibition of huPrP(23–110)-promoted cTAR/dTAR annealing. (A) Comparative effects of GA-12 and mODN-11. A 10 nM of doubly labelled cTAR was mixed with 300 nM of non-labelled dTAR at a peptide/oligonucleotide molar ratio of 1:1 in the absence (blue) and presence of GA-12 (black) and mODN-11 (red) sequences, added at five times molar excess, as compared to cTAR and dTAR. Excitation and emission wavelengths were 520 and 550 nm, respectively. The gray lines correspond to the fits of the kinetic traces to Equation (1) and the fitting parameters given in Supplementary Table S1B. (B) Concentration dependence of the effect of GA-12 on the annealing kinetics. Kinetic traces were recorded with 10 nM doubly labelled TMR-cTAR-Fl and 300 nM non-labelled dTAR in the absence (black) and the presence of GA-12, added at 0.25× (green), 1× (cyan), 5× (magenta) and 10× (yellow) molar ratio as compared to cTAR and dTAR concentrations. HuPrP(23–110) was added at a peptide/oligonucleotide ratio of 1:1 Excitation and emission wavelengths were 480 and 520 nm, respectively. All experiments were performed in 25 mM Tris–HCl (pH 7.5), 30 mM NaCl, 0.2 mM MgCl2 at 20 °C. The gray lines correspond to the fits of the kinetic traces to Equation (1) and the fitting parameters given in Supplementary Table S1B.
PrP-bound cTAR₁ species in the fast pathway is thought to form IC₁, stabilized by the seven intermolecular base pairs resulting from the annealing of both the 3' and 5' terminal strands of cTAR with the complementary terminal strands of dTAR. Since the $K_M$ values of IC₁ and IC₂ are similar, we suggest that the PrP-bound cTAR₂ species in the slow pathway anneals only through one of its frayed strands with the complementary dTAR sequence to form IC₂, also stabilized by 7bp. Further conversion of both ICs into the ED most probably relies on the conformational rearrangement and melting of the stable upper part of both TAR species (58–61). As for the core protein of the hepatitis C virus (HCV) (37,62,63), the interconversion rate of IC₁ into ED was found to be much larger than for IC₂ (Table 1), probably as a consequence of the more

|          | $K_{M1}$ (M⁻¹) ($\times 10^{-6}$) | $K_{M2}$ (M⁻¹) ($\times 10^{-6}$) | $k_{f1}$ (s⁻¹) ($\times 10^2$) | $k_{f2}$ (s⁻¹) ($\times 10^2$) |
|----------|---------------------------------|---------------------------------|-----------------------------|-----------------------------|
| HuPrP    | 2.2 (±0.8)                      | 1.5 (±1)                        | 1.1 (±0.1)                  | 0.15 (±0.03)                |
| HCV Coreb | 4 (±2)                          | 1.4 (±0.7)                      | 4.5 (±0.9)                  | 1.6 (±0.2)                  |

Table 1. Comparison of the kinetic parameters of the huPrP(23–110)- and HCV core-promoted cTAR/dTAR annealing

aThe equilibrium and kinetic constants for HuPrP-promoted cTAR/dTAR annealing reaction are calculated, as described in Supplementary Figure S1B.

bData from (37) in the same buffer, with a core/oligonucleotide molar ratio of 1.4.
favorable zippering mechanism adopted by IC1 as compared to the invasion mechanism adopted by IC2 (Figure 7).

With respect to NCp7, the natural partner of HIV-1 cTAR and dTAR sequences, data revealed that the equilibrium constant $K_M$ for IC formation was two orders of magnitude lower for the N-terminal region of huPrP than for NCp7 added at a ratio of 10 peptides per oligonucleotide ($K = 10^8 M^{-1}$) (64). This difference may in part be explained by the incomplete coating of the oligonucleotides by huPrP(23–110), since the $K_M$ value of the IC was shown to be strongly dependent on the level of protein coating in the case of NCp7 (with about three orders of magnitude difference between low and full coating). Interestingly, the interconversion rate value of the fast pathway for the N-terminal region of huPrP was close to the value (0.03 s$^{-1}$) of the corresponding parameter obtained with NCp7. The comparison of this parameter for the two proteins is more straightforward, since this parameter was found to be only poorly dependent on the level of NCp7 coating (64). Thus, huPrP(23–110) appears to promote the conversion of IC1 to the final ED with the same efficiency as NCp7. Moreover, as for NCp7 (35,64,65), huPrP(23–110) was found to nucleate the cTAR/dTAR annealing reaction through the stems of cTAR and dTAR but without destabilization of the termini (Figure 1, inset). As for the HCV core protein, this is a likely consequence of the stronger ‘nucleic acid aggregating’ properties of the huPrP peptide that compensate for the absence of its destabilizing activity. This propensity of the huPrP N-terminal peptide to efficiently neutralize the negatively charged oligonucleotides and promote their aggregation is probably related to the more flexible nature of this peptide, as compared to NCp7 with the structured zinc fingers (66,67).

It is hypothesized that such a disorder status of the N-terminal domain of PrP allows for the recognition of a rather large number of cellular and viral RNA sequences, as well as protein partners, as amply shown for the cellular protein FMRP and the retroviral NC proteins [reviewed in (54)]. Recognition of many partners by nucleic acid chaperones favours the notion that these ubiquitous proteins can achieve several functions as exemplified by retroviral NC proteins in virus structure, genome replication and virus assembly [reviewed in (68,69)] and the cellular p53 and p50 proteins in DNA maintenance and mRNA translation, respectively (40,70).

What is the function of PrP and what could be its role as an RNA chaperone? For a long time the physiological function of PrP$^{C}$ remained a matter of speculation and debate. Recent data demonstrated that PrP is involved in cell–cell adhesion whereby it controls cell movements in the early gastrula of zebrafish embryos [reviewed in (71)]. In addition, PrP appears to trigger cellular signalling via Src-related kinases especially in neurons (18). These cellular functions of PrP could well be linked to the fact that PrP is, at least in part, a membrane associated protein through its GPI anchor or its transmembrane domain [reviewed in (72)]. Regarding the RNA chaperoning activity, PrP could act at the level of mRNA translation as shown for some fungal PrPs (73) and for the prion-like neuronal protein CPEB (cytoplasmic polyadenylation element binding protein) (74). In agreement with this notion, we have recently found that a fraction of huPrP$^{C}$ is associated with translating ribosomes in human cells, impacting on mRNA translation notably in cells in the G2M phase (to be published elsewhere). Thus PrP$^{C}$ would be another chaperone protein associated with the cellular translation machinery (Supplementary Figure S2), thereby regulating the level of mRNA translation in a manner similar to other essential RNA chaperones such as hnRNP A1 in every cell type and FMRP in neurons (to be published elsewhere). Indeed, formation of PrP$^{C}$ aggregates and fibrils upon binding to small nucleic acid molecules would have a negative impact on translation and thus on the cell physiology (75,76), and might also be involved in the pathological conversion of PrP$^{C}$ to the infectious form, ultimately facilitating prion propagation [(77,78), reviewed in (75,79)].

We also report that a small thioaptamer (GA-12) is capable of inhibiting the PrP chaperoning activity in vitro but not that of HIV-1 NC (data not shown) At the same time a small methylated oligoribonucleotide (mODN-11), previously found to be a potent inhibitor of HIV-1 NC in vitro and of HIV-1 replication in cells (31), had little inhibitory potential against PrP. Kinetic data suggest that the small thioaptamer functions by tightly binding to the N-terminal domain of huPrP. This in turn may cause the formation of a complex where the N-terminal disordered domain adopts a stable ordered conformation. Such thioaptamer derivatives were found to delay the onset of prion disease in the mouse model (80), most probably by binding to PrP$^{C}$, in turn preventing its recruitment into aggregates of PrP$^{Sc}$. These results and those recently published on HIV-1 NC pave the way for the future development of highly modified oligonucleotides to treat diseases caused by RNA viruses as well as by a non-conventional agent.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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