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A quantitative characterization of interaction between prion protein with nucleic acids

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ABSTRACT

Binding of recombinant prion protein with small highly structured RNAs, prokaryotic and eukaryotic prion protein mRNA pseudoknots, tRNA and polyA has been studied by the change in fluorescence anisotropy of the intrinsic tryptophan groups of the protein. The affinities of these RNAs to the prion protein and the number of sites where the protein binds to the nucleic acids do not vary appreciably although the RNAs have very different compositions and structures. The binding parameters do not depend upon pH of the solution and show a poor co-operativity. The reactants form larger nucleoprotein complexes at pH 5 compared to that at neutral pH. The electrostatic force between the protein and nucleic acids dominates the binding interaction at neutral pH. In contrast, nucleic acid interaction with the incipient nonpolar groups exposed from the structured region of the prion protein dominates the reaction at pH 5. Prion protein of a particular species forms larger complexes with prion protein mRNA pseudoknots of the same species. The structure of the pseudoknots and not their base sequences probably dominates their interaction with prion protein. Possibilities of the conversion of the prion protein to its infectious form in the cytoplasm by nucleic acids have been discussed.

1. Introduction

Cellular prion protein, PrP^{C}, is a soluble α-helix rich glycoprotein attached to the outer cell surface by a glycophosphatidyl inositol linkage [1,2]. The biological role of PrP^{C} remains mostly unknown but the protein has been suggested to play different roles including maintain the cellular copper concentration, different signal transduction, RNA binding, and DNA metabolism [3–6]. The protein is non-infectious but its β-sheet rich isoform, PrP^{Sc}, is considered as the major infectious component for the genetic, sporadic as well as transmissible fatal neurodegenerative prion diseases [1,2]. It has been demonstrated that structural conversion of the cellular prion protein to its scrapie isoform PrP^{Sc} takes place in acidic pH5 in the endosomes and lysosomes [7–11]. PrP^{Sc} can exist as oligomers or insoluble amyloid polymers and is resistant to Proteinase K (PK) digestion whereas PrP^{C} is digested by the PK enzyme [1,2].

Unlike bacterial and viral diseases where nucleic acid transmits the infection, prion disease has been considered to propagate by the conversion of PrP^{C} to PrP^{Sc}, which can occur either by a template or a nucleation mechanism [1,2]. 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 [12–15]. The propagation of non-neuronal PrP^{Sc} in the experimental mice has been found to be non-pathogenic and arresting the conversion of PrP^{C} to PrP^{Sc} within neurons during prion infection has been found to prevent prion neurotoxicity [16]. The fibrils formed from in vivo isolated hamster PrP 27–30 amyloid or fibrils obtained by converting cellular hamster PrP^{C} have been found to be noninfectious in transgenic mice over-expressing full-length Syrian prion protein [17]. However, the amyloid formed from the truncated 90–231 fragment of mouse recombinant prion protein (23–231 amino acid) is found to be infectious in the experimental mice over-expressing this protein fragment and also shows strain characteristics of the prion disease [18,19]. Inoculation of wild-type hamsters with in vitro-generated PK-resistant prion protein formed by protein misfolding cyclic amplification has been found to be infectious [20]. By partially disaggregating PK-resistant amyloid isolated from scrapie infected hamster brain, it has been shown that the maximum prion infectivity is associated with prion particles having 17–27 nm diameter (300–600 kDa) whereas the large fibrils show lower prion infectivity [21].

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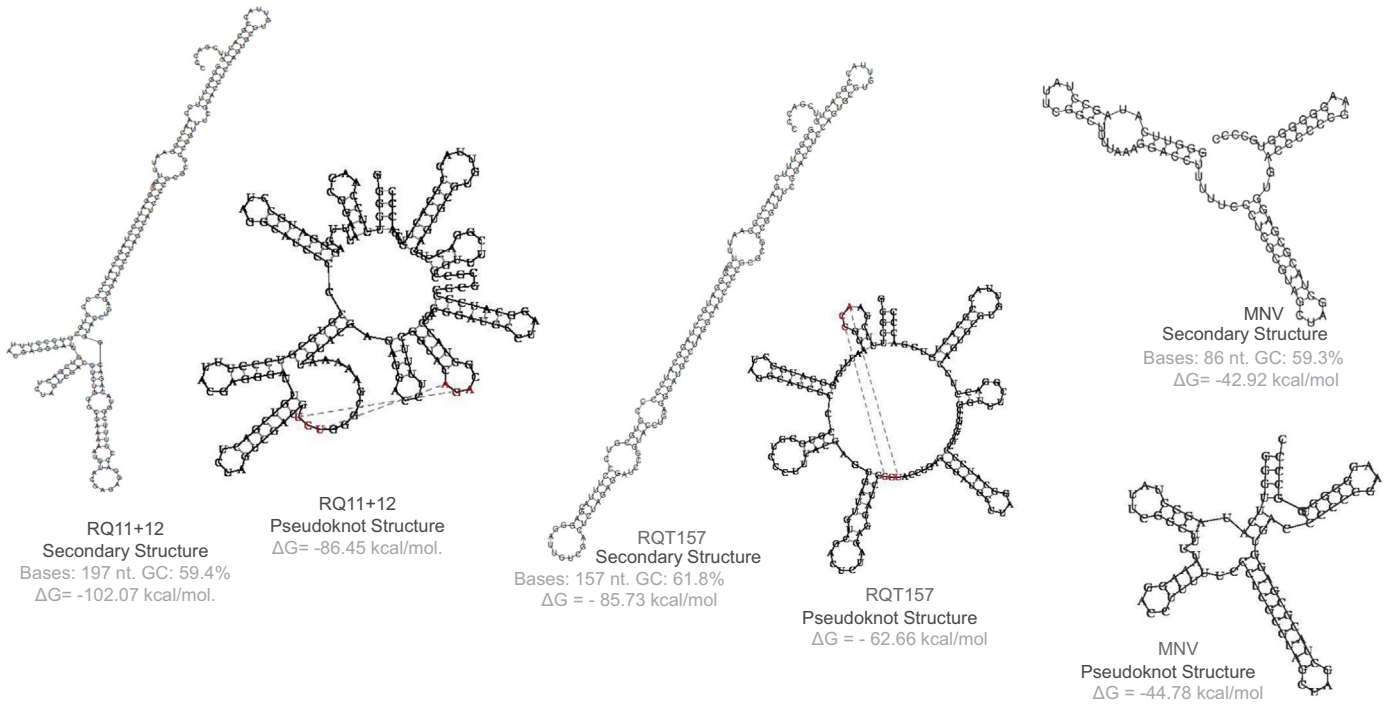

Fig. 1. Structures of different small highly structured RNAs (shsRNAs) used in this study. The shsRNAs RQ11 + 12, RQT 157 and MNV contain 197, 157 and 86 nucleotides respectively. The sequence and other details are described in Table 1. The free-energy of formation and the secondary structures are made through *RNAstructure* website. The RNA pseudoknot structures are also projected through Heuristic Modeling *vsfold5*.

A number of different molecules have been found to facilitate conversion of prion protein to insoluble aggregates [22–24]. Our previous studies also indicated that the osmolyte trimethylamine N-oxide converts recombinant prion protein to its soluble beta-structured form at high temperature [25]. Besides, the synthetic nucleic acids, both in solution and in vitro, can catalyze conversion of recombinant and cellular PrP^{C} to PrP^{Sc} as evidenced from secondary structural studies of the protein and PK resistance properties [26–30]. The highly structured small RNA (shsRNAs) binds to PrP^{C} at neutral pH which yields Proteinase K resistant component in the presence of other cellular cofactors [28]. Another study based on using brain tissues have shown that an endogenous 300 nucleotide long RNA (100 kDa) can convert PrP^{C} to Protienase K resistant form in vitro [29]. These results indicate that nucleic acid can act as a cofactor for the conversion of PrP^{C} to PrP^{Sc} and can be the TSE mediator. In addition, multiple studies also indicated that the interaction between recombinant PrP^{C} and nucleic acids simultaneously produces a mixture of condensed and functionally active nucleoprotein complex, as well as PrP^{Sc} like oligomers and linear and spherical amyloids [6,27,31,32]. To date, a specific nucleic acid as a cofactor for the propagation of prion infection has not been identified [33]. A recent study indicated that the lipid and RNA act as a cofactor for the recombinant prion protein to form PrP^{Sc} -like signature but lacks in vivo infectivity [34]. PrP^{C} is a cell surface protein, and nucleic acids in extra-cellular circulation can interact with it [6]. However, it has been considered that the relevant nucleic acid mediated PrP^{C} conversion towards its pathogenic form would be of cytoplasmic origin [6,26,27,29,31]. The presence of prion protein in cytoplasm of cells including neurons has been shown, and the exact biological role of prion protein-nucleic acid interaction is not known at present. However, it is hypothesized that the structural conversion of PrP^{C} to PrP^{Sc} can be catalyzed by cytoplasmic nucleic acids that can play a role in the prion diseases [35–38]. Anti-prion activity of RNA aptamer reported as the RNA aptamers having preferential affinity to the PrP^{Sc} form [39]. These RNA aptamers also inhibit the conversion of PrP^{C} to the infectious form [40]. Besides, the small RNA drug is also suggested for prion disease [41].

At present no detailed study is available on the quantitative aspects of binding of the prion protein and nucleic acids, particularly RNAs. As mentioned above, small highly structured RNAs (shsRNA) bind to human recombinant prion protein with high affinity and specificity under physiological conditions demonstrated from gel electrophoresis studies [28]. These RNAs also can form highly stable nucleoprotein complexes with recombinant and cellular human prion protein (α-PrP) from various cell extracts and mammalian brain homogenates [28,42].

The human prion protein gene contains five copies of a 24 nt repeat that is highly conserved among species [43–45]. Thermodynamic analyses of the repeat region suggest the presence of several hairpin loop structures and the presence of an RNA pseudoknot in human prion mRNA [43]. Computer generated three-dimensional structures of the human prion pseudoknot indicate prion protein and RNA interaction domains and the possible involvement in prion protein (PrP^{C}) translation [43–45]. In the present investigation, we have studied the binding of shsRNAs and prion protein mRNA pseudoknots with human recombinant full-length prion protein and compared with binding properties of tRNA and poly A to the protein. A couple of studies have also been carried out with full-length mouse recombinant prion protein.

2. Materials and methods

2.1. Prion protein

The full length human and mouse recombinant prion proteins were isolated following the standard procedures [46,47]. The human prion protein was a kind gift from Dr. H Rezaie (INRA, Jouy-en-Josas, France) [45]. The mouse prion protein expression plasmid was a kind gift from Dr. R. Glockshuber [48]. The purity of both the proteins was over 95% as evidenced by polyacrylamide gel electrophoresis (PAGE) and mass spectrometry. Human prion protein concentration was calculated from the measured optical density at 280 nm applying an extinction coefficient value of 56795 M^{-1} Cm^{-1}. Similarly, mouse prion protein concentration was calculated considering specific absorbance of 2.70 for 1 mg/ml at 280 nm.