Dominant-negative effects in prion diseases: insights from molecular dynamics simulations on mouse prion protein chimeras

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Mutations in the prion protein (PrP) can cause spontaneous prion diseases in humans (Hu) and animals. In transgenic mice, mutations can determine the susceptibility to the infection of different prion strains. Some of these mutations also show a dominant-negative effect, thus halting the replication process by which wild type mouse (Mo) PrP is converted into Mo scrapie. Using all-atom molecular dynamics (MD) simulations, here we studied the structure of HuPrP, MoPrP, 10 Hu/MoPrP chimeras, and 1 Mo/sheepPrP chimera in explicit solvent. Overall, ~2 μs of MD were collected. Our findings suggest that the interactions between α1 helix and N-terminal of α3 helix are critical in prion propagation, whereas the β2–α2 loop conformation plays a role in the dominant-negative effect.

An animated Interactive 3D Complement (I3DC) is available in Proteopedia at http://proteopedia.org/w/Journal:JBSD:4.

Keywords: prion protein; dominant-negative; molecular dynamics

1. Introduction

The key event in prion diseases, or transmissible spongiform encephalopathies, is the conversion of the cellular prion protein (PrP*) to its pathogenic scrapie form denoted PrPSc or prion (Brandner et al., 1996; Bueler et al., 1993). The conversion is the result of a posttranslational process whereby most α-helical motifs are replaced by β-sheet secondary structures without any covalent modifications. PrPSc catalyzes the conversion of PrP* to nascent PrPSc and triggers prion propagation. Structurally, PrP* is a glycosphatidylinositol-anchored glycoprotein composed of 209 amino acids (in human [Hu] numbering) including an N-terminal (N-) unstructured domain (residues 23–127) and a C-terminal (C-) globular domain (GD) of three α-helices and a short, two-stranded, antiparallel β-sheet (residues 128–231) (Zahn et al., 2000). In contrast to PrP*, PrPSc structure has significant β-sheet content and exhibits distinct biophysical and biochemical properties (Pan et al., 1993), albeit no atomistic-resolution structural information is available.

Although PrP* influences several processes in the central and peripheral nervous systems, its function has not been established with certainty (Linden et al., 2008). Key evidence exists supporting the link between mutations in prion protein (PrP) gene (PRNP in Hu or Prnp in other mammalian species) and the spontaneous generation of prion diseases (Dossena et al., 2008; Friedman-Levi et al., 2011; Jackson et al., 2009). Several insertion and point mutations linked to genetic forms of Hu prion diseases have been identified (Mastrianni, 2010). Polymorphisms in PrP may influence the etiology and neuropathology of the disease both in humans (Bishop, Pennington, Heath,...
...from being converted to PrPSc. The protective effects of
"ko et al., 1997). Some of these CMPrP also acted as
susceptibility or resistance to Mo scrapie infection (Kaneko
infected Mo neuroblastoma (ScN2a) cells showed diverse
Interestingly, a variety of CMPrP investigated in scrapie
uous conversion into prions (Sigurdson et al., 2009, 2011).
been reported to exhibit both transmission barriers against
(1994). Recently, Tg mice expressing MoPrP chimeras
1994). Transgenic (Tg) mice expressing both mouse (Mo) PrP and HuPrP did not exhi-
sequence was found highly susceptible to Hu prions and
bit abbreviated incubation times when infected with Hu
is listed in Table 1. This study may explain the transmission
barrier between Hu and Mo prions, as well as the domi-
nant-negative effects of certain polymorphisms.

2. Materials and methods

2.1. Preparation of the simulation systems

The structural models of MoPrP and the CMPrP GD are
based on the NMR structure of MoPrP (PDB entry 1XYX)
(Gossert, Bonjour, Lysek, Fiorito, & Wuthrich, 2005)
resolved at pH 4.5, the only solution structure available so
far for MoPrP. Note that 1XYX is the refined structure of
an earlier PDB entry 1AG2 (Riek et al., 1996) resolved at
the same pH. The best representative conformation (model 1)
of the 1XYX ensemble (Gossert et al., 2005) was used.
Following the procedure validated in references Rossetti, Gia-
chin, Legname, and Carloni (2010) and Rossetti, Cong,
Calandra, Legname, and Carloni (2011), the mutations in
CMPrP were introduced into WT MoPrP using Swiss-Pdb-
Viewer (DeepView 4.0) (Guet & Peitsch, 1997). The
model of HuPrP is based on the NMR structure of HuPrP
GD (PDB entry 1HJN) (Calzolai & Zahn, 2003). 1HJN is
the only solution structure of HuPrP resolved at pH 7,
which corresponds to the pH of our MD simulations. The
best representative conformer of 1HJN is not indicated,
whilst the minimized average structure 1HJM.pdb may not
represent well the structure ensemble (Vuctilfe, 1993).
Therefore, a cluster analysis (Micheletti, Seno, & Maritan,
2000) was performed on the 20 models in 1HJN to identify
a representative model as the initial structure for the MD
simulations. The same procedure has been successfully
applied in references Rossetti et al. (2010, 2011).

Test simulations were carried out to determine the
protonation states of histidine (H) residues in these simu-
lation systems. At pH 7, D, E, K, and R residues were
considered in their ionized form but H residues may be
neutral or, to a smaller extent, positively charged. We
observed in reference Rossetti et al. (2010, 2011) that in
HuPrP and its naturally occurring mutants, H140 and
H177 are solvent exposed while H187 forms a single
intramolecular hydrogen bond (HB) that involves
H187 Nε and R156 backbone carbonyl. When all four
His (H140, H155, H177, and H187) were monoprotonat-
ed at the Nε atom, our calculations reproduced accurately
the structural determinants of the available NMR struc-
tures. In the GD of MoPrP and the CMPrP, only H140,
H177, and H187 are present. They have the same local
environment as those in HuPrP. Our test calculations
(see “Supplementary material” for details) suggest that
these H residues take the same Nε-protonation neutral
state as that in HuPrP. Other protonation states introduce
instability to the protein structure.

2.2. MD simulations

The Gromacs 4.5 package (Hess, Kutzner, van der Spoel, &
Lindahl, 2008) was used to perform MD simulations.
Table 1. Experimental observations of the CMPPrP studied by MD simulations.

| Mutation name in Hu numbering | Experimental system¹ (Ref.) | Resistance to Mo scrapie replication² | Dominant-negative effect³ | Origin of the mutation |
|-------------------------------|-----------------------------|--------------------------------------|---------------------------|-----------------------|
| Q168E                         | ScN2a cells (Kaneko et al., 1997) | #                                   | –                         | Hu codon              |
| N171S                         | ScN2a cells (Kaneko et al., 1997) | –                                   | –                         | Hu mutation linked to prion-like disorders (Fink, Peacock, Warren, Roses, & Prusiner, 1994) |
| Q172R                         | ScN2a cells (Kaneko et al., 1997) | +                                   | +                         | Sheep protective polymorphism (Goldmann et al., 1994; Westaway et al., 1994) |
| V215I                         | ScN2a cells (Kaneko et al., 1997) | #                                   | +                         | Hu codon              |
| V215I_Q219E                   | ScN2a cells (Kaneko et al., 1997) | +                                   | –                         | Hu codons             |
| Q217R                         | ScN2a cells (Kaneko et al., 1997) | +                                   | +                         | Hu GSS-linked mutation (van der Kamp & Daggett, 2009) |
| Q217R_Q219E                   | ScN2a cells (Kaneko et al., 1997) | +                                   | –                         | R217: Hu GSS-linked mutation (van der Kamp & Daggett, 2009) E219: Hu codon |
| Q219K                         | ScN2a cells (Kaneko et al., 1997), Tg mice (Perrier et al., 2002) | +                                   | +                         | Hu protective polymorphism (Mead et al., 2003) |
| Q219E                         | ScN2a cells (Kaneko et al., 1997) | +                                   | –                         | Hu codon              |
| K220R                         | ScN2a cells (Kaneko et al., 1997) | –                                   | –                         | Hu codon              |
| K220R_Q219E                   | ScN2a cells (Kaneko et al., 1997) | +                                   | –                         | Hu codons             |

¹All the experiments were performed in vitro except for Q219K where the same findings have been reported also in Tg mice.
²“+”: CMPPrP is NOT converted to PrPSc;
³“−”: CMPPrP is less efficiently converted than the control (MoPrP);
⁴“#”: CMPPrP shows no resistance to Mo scrapie;
⁵“#−”: CMPPrP inhibits the co-expressed MoPrP from being converted to PrPSc;
⁶“#−”: CMPPrP shows no dominant-negative effect.
Following the protocol of references Rossetti et al. (2010, 2011), the protein models were solvated in a box of explicit water, ensuring that the solvent shell extended for at least 16 Å around them. Each system contained of explicit water, ensuring that the solvent shell extended (2010, 2011), the protein models were solvated in a box Following the protocol of references Rossetti et al. (Table 3). All the systems appeared to reach equilibrium within 10 ns, as indicated by the Cu root-mean-square deviation (RMSD) as a function of simulation time (Figure S1 in “Supplementary material”). Fifty nanoseconds of equilibrated trajectory were taken from each MD run for analysis. Overall, 1.95 μs of MD trajectories were collected.

All CMPrP maintained the 3D fold in GD similar to MoPrP and HuPrP during the simulations. Large fluctuations were observed in β2–α2 loop (residues 167–171) and C- of α3 helix (residues 219–231) (Figure 1). These two fragments were poorly defined in MoPrP NMR structure, indicating structural disorder or increased mobility (Riek et al., 1998). Excluding the flexible terminal residues (125–127 and 220–231), the GY of CMPrP during our simulations showed Cu RMSD not more than 2.7 ± .4 Å from the MoPrP NMR structure (Riek et al., 1998) or 2.8 ± .7 Å from the HuPrP NMR structure (Zahn et al., 2000) (Table 2). Consistently, the Ca radius of gyration averaged along the x, y, and z axes ranged from 13.3 ± .1 to 13.6 ± 2 Å for residues 128–219 of each CMPrP (Table 2). All CMPrP except K220R showed smaller Ca root-mean-square fluctuations (RMSF) than MoPrP. These fluctuations were similar to those of HuPrP (Figure 1). K220R exhibited large fluctuations in residues 133–157, which contain the β1–α1 loop and the α1 helix, as well as in residues 190–199 which correspond to N-α3 (Figure 1(D)). These fluctuations are due to a loss of intramolecular interactions between α1 and N-α3, which are reported in detail in the following section.

We analyzed the interactions between α1 and N-α3 in all simulated systems. Strikingly, eight CMPrP (Q168E, Q172R, V215I_Q219E, Q217R, Q217R_Q219E, Q219 K, Q219E, and K220R_Q219E) exhibited stable HB interactions between residues Y149 and D202, Y157 and D202 (Table 2, Figure 2(A)). These HBs appeared less stable in MoPrP and HuPrP (Table 3, Figure 2(B)). These HB and SB interactions keep the α1 helix in close contact with N-α3, conserving the hydrophobic interactions among residues F141, Y149, Y157, M205, and M206 at this interface. The same interactions were observed also in V215I, although less pronounced. In contrast, CMPrP N171S, and K220R lacked these HB and SB interactions for most of the simulated time (Table 3, Figure 2(C) and (D)). Without them, the hydrophobic interactions were weakened and the α1 helix shifted apart from N-α3 (Figure 2(E)). Correspondingly, the average Ca-distance between residue 144 (at C-α1) and residue 202 (at N-α3) increased significantly in N171S and K220R (17.7±1.0 and 17.5 ±1.5 Å, respectively). In sharp contrast, this distance is below 16.0 Å in seven CMPrP (Q172R, V215I_Q219E, Q217R, Q217R_Q219E, Q219 K, Q219E, and
K220R_Q219E). In Q168E, V215I, MoPrP, and HuPrP this distance is between 16.0 and 17.0 Å (Table 3).

Next, we examined the β2–α2 loop where all CMPrP and HuPrP exhibited smaller RMSF than MoPrP. We analyzed the secondary structure elements of this loop.

**Figure 1.** (A–D) Ca RMSF of GD residues averaged over three independent 50 ns MD trajectories of each CMPrP with respect to those of MoPrP and HuPrP. (E) The final MD snapshots of MoPrP, of HuPrP, and of all the CMPrP are superimposed on HuPrP NMR structure (Calzolai & Zahn, 2003). The β2–α2 loop (residues 167–171, blue) and C-α3 (residues 219–231, orange) that experience the largest fluctuations are labeled.

**Table 2.** Ca RMSD (Å) and radius (Å) of gyrations of residues 128–219 of MoPrP, HuPrP, and all the CMPrP during MD. The RMSD is calculated relative to the NMR structures of MoPrP (PDB code: 1XYX) and HuPrP (PDB code: 1HJN), respectively.

| Protein name         | MD runs | Ref. 1XYX | Ref. 1HJN | Radius of gyration | Protein name         | MD runs | Ref. 1XYX | Ref. 1HJN | Radius of gyration |
|----------------------|---------|-----------|-----------|--------------------|----------------------|---------|-----------|-----------|--------------------|
| MoPrP                | 1       | 1.8 ± .4  | 2.4 ± .2  | 13.6 ± .2          | Q217R               | 1       | 1.7 ± .2  | 2.2 ± .2  | 13.3 ± .1          |
|                      | 2       | 1.8 ± .2  | 2.1 ± .2  | 13.3 ± .1          |                      | 2       | 1.3 ± .1  | 2.2 ± .2  | 13.3 ± .1          |
|                      | 3       | 1.8 ± .2  | 1.9 ± .1  | 13.3 ± .1          |                      | 3       | 2.4 ± .2  | 2.1 ± .2  | 13.6 ± .1          |
| HuPrP                | 1       | 1.9 ± .2  | 1.7 ± .2  | 13.5 ± .2          | Q217R_Q219E          | 1       | 1.8 ± .6  | 2.0 ± .2  | 13.3 ± .1          |
|                      | 2       | 1.7 ± .2  | 2.2 ± .2  | 13.2 ± .1          |                      | 2       | 1.7 ± .2  | 2.1 ± .6  | 13.5 ± .1          |
|                      | 3       | 1.8 ± .2  | 2.0 ± .2  | 13.3 ± .1          |                      | 3       | 1.7 ± .2  | 1.8 ± .2  | 13.5 ± .1          |
| Q168E                | 1       | 1.8 ± .2  | 2.0 ± .2  | 13.5 ± .1          | Q219K                | 1       | 1.7 ± .3  | 2.0 ± .2  | 13.6 ± .2          |
|                      | 2       | 2.4 ± .2  | 2.1 ± .2  | 13.7 ± .1          |                      | 2       | 1.4 ± .2  | 2.3 ± .2  | 13.3 ± .1          |
|                      | 3       | 1.7 ± .2  | 2.0 ± .2  | 13.3 ± .1          |                      | 3       | 1.6 ± .2  | 2.1 ± .2  | 13.4 ± .1          |
| N171S                | 1       | 1.8 ± .2  | 2.0 ± .2  | 13.5 ± .1          | Q219E                | 1       | 1.9 ± .2  | 2.2 ± .2  | 13.6 ± .2          |
|                      | 2       | 2.1 ± .2  | 2.3 ± .2  | 13.5 ± .1          |                      | 2       | 1.6 ± .2  | 2.0 ± .2  | 13.4 ± .1          |
|                      | 3       | 2.2 ± .2  | 2.1 ± .2  | 13.3 ± .1          |                      | 3       | 1.8 ± .2  | 2.1 ± .3  | 13.4 ± .2          |
| Q172R                | 1       | 1.4 ± .2  | 2.2 ± .2  | 13.4 ± .1          | K220R                | 1       | 2.6 ± .4  | 2.8 ± .6  | 13.5 ± .2          |
|                      | 2       | 1.5 ± .2  | 1.9 ± .2  | 13.4 ± .1          |                      | 2       | 1.8 ± .2  | 2.4 ± .2  | 13.3 ± .1          |
|                      | 3       | 1.6 ± .2  | 2.6 ± .2  | 13.2 ± .1          |                      | 3       | 1.9 ± .3  | 1.9 ± .2  | 13.5 ± .1          |
| V215I                | 1       | 1.6 ± .2  | 2.0 ± .2  | 13.4 ± .1          | K220R_Q219E          | 1       | 1.4 ± .2  | 2.1 ± .2  | 13.3 ± .1          |
|                      | 2       | 1.7 ± .2  | 2.0 ± .2  | 13.5 ± .1          |                      | 2       | 1.5 ± .2  | 2.1 ± .2  | 13.3 ± .1          |
|                      | 3       | 1.7 ± .2  | 2.1 ± .2  | 13.3 ± .1          |                      | 3       | 1.6 ± .2  | 2.3 ± .2  | 13.3 ± .1          |
| V215I_Q219E          | 1       | 1.7 ± .1  | 2.0 ± .2  | 13.4 ± .1          |                      | 1       | 1.7 ± .2  | 2.2 ± .2  | 13.4 ± .1          |
|                      | 2       | 1.9 ± .2  | 2.1 ± .2  | 13.4 ± .1          |                      | 2       | 1.6 ± .2  | 2.2 ± .2  | 13.4 ± .1          |
|                      | 3       | 1.6 ± .2  | 2.2 ± .2  | 13.4 ± .1          |                      | 3       | 1.6 ± .2  | 2.2 ± .2  | 13.4 ± .1          |
during each simulation. Residues 169–171 showed a conformational exchange between coil and bend in all the systems. Residues 166–168, however, exhibited different exchange patterns in distinct PrP. In MoPrP, residues 166–168 revealed an exchange pattern mainly between $\alpha$-helix (68.9%) and turn (29.6%), whereas in HuPrP, an exchange pattern among bend (48.2%), turn (35.5%), and coil (16.2%) was observed. Out of the 11 CMPrP studied here, seven (N171S, Q172R, V215I, Q217R, Q219 K, K220R, and K220R_Q219E) showed the "$\alpha$-helix/turn"-exchange pattern at residues 166–168, similar to that of MoPrP (Table 4). In such a Mo-like pattern, $\alpha$-helix covered over 45% of the simulation time while coil occurred in less than 3%.

Table 3. Selected intramolecular interactions between $\alpha_1$ and N-$\alpha_3$ of MoPrP, HuPrP, and of all the CMPrP during MD. Related experimental findings are also reported.

| Protein name | MD runs/NMR structures | HB Y149-D202 (average number per frame$^*$) | HB Y157-D202 (average number per frame) | SB R156-E196 (average number per frame) | D144-D202 C distance averaged over 3 runs (Å) | Resistance to mouse scrapie propagation$^2$ | Dominant negative inhibition$^3$ |
|--------------|------------------------|---------------------------------------------|------------------------------------------|------------------------------------------|-----------------------------------------------|-------------------------------------------|-------------------------------|
| MoPrP 1XYX (NMR) | 16 out of 20 frames | 7 out of 20 frames | 1 out of 20 frames | 14.8 ± 1.1 | – | – |
| 1 | .3 | .3 | .0 | 16.7 ± 1.1 | | |
| 2 | .8 | .6 | .3 | | | |
| 3 | .4 | .8 | .7 | | | |
| HuPrP 1HJN (NMR) | 5 out of 20 frames | 9 out of 20 frames | 2 out of 20 frames | 15.0 ± 1.0 | N/A | N/A |
| 1 | .9 | .8 | .7 | 16.5 ± 1.0 | | |
| 2 | .5 | .3 | .9 | | | |
| 3 | 1.0 | 1.0 | .5 | | | |
| Q168E | 1 | 1.0 | .9 | .6 | 17.0 ± 1.5 | # | – |
| 2 | 1.1 | 1.0 | .7 | | | |
| 3 | .9 | 1.0 | .9 | | | |
| N171S | 1 | 1.0 | .0 | .0 | 17.7 ± 1.0 | – | – |
| 2 | 0.0 | .0 | .1 | | | |
| 3 | 1.0 | 1.0 | .0 | | | |
| Q172R | 1 | 1.0 | 1.0 | .7 | 15.8 ± 1.3 | + | |
| 2 | 1.0 | 1.0 | 1.0 | | | |
| 3 | 1.0 | .9 | 1.0 | | | |
| V215I | 1 | 1.1 | 1.0 | .5 | 16.5 ± 1.0 | # | + |
| 2 | .7 | .5 | .0 | | | |
| 3 | .5 | .4 | .2 | | | |
| V215I_Q219E | 1 | 1.0 | 1.0 | .8 | 15.7 ± 1.3 | + | – |
| 2 | 1.1 | 1.0 | .6 | | | |
| 3 | 1.0 | .9 | 1.0 | | | |
| Q217R | 1 | 1.0 | 1.0 | .7 | 15.6 ± 1.4 | + | + |
| 2 | 1.0 | 1.0 | .9 | | | |
| 3 | 1.1 | 1.0 | .4 | | | |
| Q217R_Q219E | 1 | 1.1 | .9 | .4 | 15.8 ± 1.4 | + | – |
| 2 | 1.1 | 1.0 | .5 | | | |
| 3 | 1.0 | 1.0 | .3 | | | |
| Q219 K | 1 | 1.1 | 1.0 | .5 | 15.9 ± 1.4 | + | + |
| 2 | 1.1 | 1.0 | .5 | | | |
| 3 | 1.1 | 1.0 | .5 | | | |
| Q219E | 1 | 1.0 | .9 | .9 | 15.9 ± 1.4 | + | – |
| 2 | 1.1 | 1.0 | .5 | | | |
| 3 | 1.0 | 1.0 | .9 | | | |
| K220R | 1 | .0 | .0 | .0 | 17.5 ± 1.5 | – | – |
| 2 | .8 | .3 | .0 | | | |
| 3 | .1 | .1 | .4 | | | |
| K220R_Q219E | 1 | 1.0 | 1.0 | .7 | 15.9 ± 1.4 | + | N/A |
| 2 | 1.1 | .9 | .7 | | | |
| 3 | 1.0 | .9 | .3 | | | |

$^*$This number can be slightly >1 because in a small number of frames, D202 has the chance to form two HBs with Y149.
$3_{10}$-helix conformation is stabilized by two HBs: one is formed between the backbone of Q168 N atom and P165 O atom, the other is between the backbone of Y169 N atom and V166 O atom. The other four CMPrP (Q168E, V215I_Q219E, Q217R_Q219E, and Q219E) manifested a more Hu-like pattern: the “bend/turn”-exchange dominates the loop conformation, while $3_{10}$-helix covered less than 20% whereas coil accounted for over 10% (Table 4). A HB formed between D167 backbone N atom and S170 sidechain O atom stabilizes the bend conformation. Alternatively, a HB between the E168 backbone N atom and M166 backbone O atom contributes to the turn.

Table 4. Content of secondary structure elements of residues 166–168 of MoPrP, of HuPrP, and of all the CMPrP during the MD. This analysis was carried out using DSSP software (Kabsch & Sander, 1983).

| Protein name | $3_{10}$-helix (%) | Turn (%) | Bend (%) | Coil (%) |
|--------------|-------------------|----------|----------|----------|
| MoPrP        | 68.9              | 29.6     | 4.2      | 2.9      |
| HuPrP        | 0                 | 35.5     | 48.2     | 16.2     |
| Q168E        | 14.4              | 54.9     | 16.8     | 13.2     |
| N171S        | 49.0              | 37.5     | 10.4     | 1.4      |
| Q172R        | 62.2              | 30.0     | 6.3      | 1.5      |
| V215I        | 45.6              | 42.9     | 8.9      | 2.1      |
| V215I_Q219E  | 8.9               | 21.1     | 42.3     | 27.7     |
| Q217R        | 57.0              | 27.2     | 10.8     | 5.1      |
| Q217R_Q219E  | 14.1              | 31.2     | 29.4     | 25.3     |
| Q219 K       | 62.8              | 24.3     | 5.7      | 1.5      |
| Q219E        | 20.0              | 38.5     | 18.6     | 22.2     |
| K220R        | 45.1              | 42.5     | 11.2     | 1.1      |
| K220R_Q219E  | 67.2              | 25.4     | 5.9      | 1.6      |
conformation. Our analyses did not identify any salt bridge or hydrophobic interactions that particularly contribute to these specific loop conformations.

4. Discussion

Our simulations highlighted shorter intramolecular contacts between α1 helix and N-α3 in seven CMPrP (Q172R, V215I_Q219E, Q217R, Q217R_Q219E, Q219K, Q219E, and K220R_Q219E) than in MoPrP and HuPrP. Indeed, the average distance between C-α1 and N-α3 appeared shorter in these seven CMPrP than in MoPrP and HuPrP. Notably, all these seven CMPrP were reported to highly resist the Mo scrapie replication in ScN2a cells (Kaneko et al., 1997) (Table 1). Although less pronounced, these features were also observed in Q168E and V215I. The latter two CMPrP were reported with only mild resistance in ScN2a cells (Kaneko et al., 1997). In contrast, N171S and K220R lacked these interac-tions. They exhibited a longer distance between C-α1 and N-α3. Accordingly, these two CMPrP are both susceptible to Mo scrapie. These data indicate that the resistance to Mo scrapie is related to stronger intramolecular interactions between α1 helix and N-α3. A previous study from us (Rossetti et al., 2011) suggested that a loss of SB interactions between α1 and the N-α3 plays a role in the spontaneous conversion of Hu pathogenic mutants of SB interactions between study from us (Rossetti et al., 2011) suggested that a loss astrain to Mo scrapie is related to stronger intramolecular actions. They exhibited a longer distance between C-α1 and N-α3. These data indicate that the resistance to Mo scrapie is related to stronger intramolecular interactions between α1 helix and N-α3. A previous study from us (Rossetti et al., 2011) suggested that a loss of SB interactions between α1 and the N-α3 plays a role in the spontaneous conversion of Hu pathogenic mutants of PrPSc. Recently solution NMR study on Hu protective polymorphism E219K discovered stronger interactions between N-α3 and α1 as well as a few residues proceeding α1 (Biljan et al., 2012). Interestingly, a number of MD studies suggest that the region from C-α2 to N-α3 is more flexible in HuPrP pathogenic mutants than in WT HuPrP (Alonso, DeArmond, Cohen, & Daggett, 2001; Bamdad & Naderi-Manesh, 2007; Capellari, Strammiello, Saverioni, Kretzschmar, & Parchi, 2011; Chebaro & Derreumaux, 2009; Rossetti et al., 2011; Santini, Claude, Audic, & Derreumaux, 2003; Santini & Derreumaux, 2004; Sekijima, Motono, Yamasaki, Kaneko, & Akiyama, 2003; van der Kamp & Daggett, 2010). Many other studies have pointed out that the α2–α3 region is per se highly fibrilogenic (Adrover et al., 2010; Chakrour et al., 2010; Kallberg, Gustafsson, Persson, Thyberg, & Johansson, 2001; Kuwata et al., 2002; Kuwata, Kamatari, Akasaka, & James, 2004; Lu, Wintrode, & Surewicz, 2007). Hence, the α2–α3 region is likely to be stabilized by interactions with α1 and its flanking residues. This hypothesis is in line with several investigations on the hydrophobic core of PrP amyloid fibrils. Hydrogen exchange, site-directed spin labeling, electron paramagnetic resonance spectroscopy, and solid-state NMR studies have suggested that the core of PrP amyloid fibrils is composed of the region spanning the α2 helix, the α2–α3 loop, and a major part of α3 helix of PrPSc (Cobb, Sonnichsen, McHaourab, & Surewicz, 2007; Lu et al., 2007; Tycko, Savchenko, Ostapchenko, Makarava, & Baskakov, 2010). These studies indicate a contribution of the α2–α3 region from the rest of PrP during the conversion. Therefore, enhanced interactions between α1 and N-α3 might inhibit the dissociation and the conversion, as seen in our simulations on the scrapie-resistant CMPrP. However, some other studies suggest that amyloid fibrils can be formed by the structured regions preceding the α1 helix, likely via an extended β-structure with residues 102–124. This hypothesis is supported by the extensive studies (Damo et al., 2010; Helmus, Surewicz, Nadaud, Surewicz, & Jaroniec, 2008; Lim et al., 2006) on the pathogenic HuPrP mutation Y145stop which contains only residues 23–144. These studies indicate that in the absence of the C-terminal residues 145–230, the fibril core is composed mostly of residues 102–139. While the misfolding mechanism of PrP remains obscure, a growing body of evidence suggests that distinct aggregate structures can be formed through multiple pathways by different prion strains (Cohen & Kelly, 2003; Legname et al., 2006; Nekooki-Machida et al., 2009; Prusiner, 2001; Tessier & Lindquist, 2007). This may explain the discrepancy of our findings and some others derived from different PrP variants or prion strains.

The β2–α2 loop of PrP conformation has been deeply investigated in different mammalian PrPs (Christen, Perez, Hornemann, & Wuthrich, 2008; Gossert, Bonjour, Lysek, Fiorito & Wuthrich, 2005; Perez, Damberger, & Wuthrich, 2010). It has been suggested that the loop plasticity is related to the transmissable barrier among prion strains (Agrimi et al., 2008; Christen et al., 2008; Christen, Hornemann, Damberger, & Wuthrich, 2009; Gossert et al., 2005; James et al., 1997; Lysek et al., 2005; Perez et al., 2010; Wen et al., 2010). Solution NMR studies on MoPrP and HuPrP structures found that at room temperature (293–298 K) a line broadening of NMR signals was reported for the β2–α2 loop in all the Mo and Hu PrP constructs due to local conformational exchanges (Calzolai & Zahn, 2003; Damberger, Christen, Perez, Hornemann, & Wuthrich, 2011; Gossert et al., 2005; Zahn et al., 2000). A recent solution NMR study discovered that at 310 K, the β2–α2 loop of MoPrP is characterized by 310-helix, while Y169A/G mutations result in β-turn conformation (Damberger, Christen, Perez, Hornemann, & Wuthrich, 2011). This study suggests that at room temperature the β2–α2 loop of MoPrP is disordered, likely due to conformational exchange between 310-helix and another conformation. These studies imply that the loop conformation may play an essential role by binding to an unknown factor required for prion propagation. Consistently, previous studies on pathogenic HuPrP mutants (Meli, Gasset, & Colombo 2011; Rossetti et al., 2010, 2011) indicated that the
conformation of the β2–α2 loop was related to the spontaneous human familial prion diseases. In this study, we identified a “310-helix/turn” conformational exchange pattern of the β2–α2 loop in MoPrP and a “bend/turn/coil” pattern in HuPrP. The intramolecular interactions that contribute to the 310-helix turned out to be the same HBs as those observed in the MoPrP NMR structure at 310 K, which involve residues P165, V166, Q168, and Y169 (Damberger et al., 2011). Another two HBs were identified to control the bend and turn conformations, which involve residues M166, D167, E168, and S170. These specific interactions in the β2–α2 loop may be the determinants of the loop conformation. Seven CMPrP (N171S, Q172R, V215I, Q217R, Q219K, K220R, and K220R_Q219E) exhibited the Mo-like pattern in MD. Among these, two of them (N171S and K220R) turned out to be susceptible to Mo scrapie in ScN2a cells, whereas the other four (Q172R, V215I, Q217R, and Q219K) showed the dominant-negative effect (Kaneko et al., 1997). K220R_Q219E was resistant to Mo scrapie (Kaneko et al., 1997) but no data have been reported about the dominant-negative effect. The other four CMPrP studied here (Q168E, V215I Q219E, Q217R_Q219E, and Q219E) showed a more Hu-like pattern. They turned out to be resistant to Mo scrapie but not to have dominant-negative effect (Kaneko et al., 1997). The data indicate that the Mo-like conformational exchange pattern is necessary for the dominant-negative effect if the CMPrP is also scrapie-resistant.

Based on these findings, we propose the following mechanism for the dominant-negative effect: the Mo-like conformation at the β2–α2 loop can bind to an unknown factor that facilitates prion propagation. When the scrapie-resistant CMPrP have the Mo-like β2–α2 loop conformation, they can bind to this factor but cannot be converted to PrPSc. They stay bound with the factor and keep its binding site inaccessible for the WT MoPrP. Therefore, WT MoPrP is not converted to PrPSc. In the case of nonresistant CMPrP such as N171S and K220R, they can bind to the factor but are easily converted to PrPSc and released from it. The binding site is then free again to bind new CMPrP or WT MoPrP. Therefore, WT MoPrP is also converted to PrPSc and no dominant-negative effect is observed.

Evidence exists that prion propagation requires an accessory cofactor (Deleault et al., 2012a; Deleault, Lucassen, & Supattapone, 2003; Telling et al., 1995). Recently, Deleault et al. demonstrated that a small molecule cofactor can regulate the conformation, strains and infectivity of PrPSc in vivo (Deleault et al., 2012b). However, some other studies argue against the intervention of any ancillary components (Geoghegan, Miller, Kwak, Harris, & Supattapone, 2009) and suggest that the dominant-negative effect is only due to the heterozygosis of PRNP or Prnp genes (Geoghegan et al., 2009; Hizume et al., 2009; Lee, Yang, Perrier, & Baskakov, 2007). This alternative model, denoted as “stone fence model,” suggests that Hu individuals heterozygous for E/K at codon 219 are protected by sporadic CJD because the PrPs deriving from two allelic variants interfere with each other in the conversion process due to their incompatible structures. The in vivo and in vitro evidences supporting this model are reviewed in (Kobayashi, Hizume, Teruya, Mohri, & Kitamoto, 2009). However, an ultimate structural explanation of the dominant-negative effect is still missing in prion biology.

Considering the previous findings on CMPrP (Kaneko et al., 1997; Telling et al., 1994), which have inspired this work, we propose the above possible mechanism of the dominant-negative effect. Nevertheless, further investigations are needed to verify this hypothesis. In particular, the alternative hypothesis that the dominant-negative effects are due to PRNP or Prnp heterozygosis may suggest novel rationally designed MD experiments aimed to investigate the interaction between two different allelic variants to mimic the heterozygosis condition.

Supplementary material
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Abbreviations
PrP – prion protein
Mo – mouse
Hu – human
GD – globular domain
CMPrP – PrP chimera
PrPSc – scrapie form of PrP
Tg – transgenic
ScN2a – scrapie infected Mo neuroblastoma cells
MD – molecular dynamics
C- – C-terminal
N- – N-terminal
PME – particle mesh Ewald
RMSD – root-mean-square deviation
RMSF – root-mean-square fluctuation
HB – hydrogen bond
SB – salt bridge
WT – wild type

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