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Molecular dynamics studies of dog prion protein wild-type and its D159N mutant

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ABSTRACT

Prion diseases (e.g. 'mad cow' disease in cattle, chronic wasting disease in deer and elk, Creutzfeldt-Jakob disease in humans) have been a major public health concern affecting humans and almost all animals. However, dogs are strongly resistant to prion diseases. Recently, through transgenic techniques, it was reported that the single (surface) residue D159 is sufficient to confer protection against protein conformational change and pathogenesis, thus provides conformational stability for dog prion protein. This made a big breakthrough in dog prion protein research field. For dog prion protein, another advancement is the produce of its NMR structure in 2005. However, all these breakthroughs are still short of enough structural informatics of dog prion protein. This paper studies dog prion protein wild-type and D159N mutant through molecular dynamics (MD) techniques. Our MD results reveal sufficient structural informatics on the residue at position 159 to understand the mechanism underlying the resistance to prion diseases of dogs. The structural informatics of this paper should be very useful for the medicinal treatment of prion diseases.

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Prion diseases; immunity of dogs; key residue D159; wild-type and mutant; molecular dynamics

Introduction

In Prion research field, rabbits, dogs, and horses are the most famous and well-known species with immunity (or low susceptibility) to prion diseases. For rabbit prion protein (PrP), multiple amino acid residues G99, M108, S173, I214, together inhibit formation of its abnormal isoform (Vorberg et al., 2003). For dog PrP, a single amino acid D159 is sufficient to prevent PrP conformational change and pathogenesis (Sanchez-Garcia et al., 2016). These are the laboratory results from mice, rabbits and dogs. However, structural bioinformatics are not enough to understand the mechanism underlying the resistance to prion diseases. This paper is dealing with this problem to reveal sufficient secrets of immunity of dogs to prion diseases from the protein structure and structural dynamics point of view. This paper will also do a complete review/survey on previous literatures about dog PrP. Thus, this paper should play great important roles in the research topic of dog PrP.

Unlike bacteria and viruses, which are based on DNA and RNA, prions are unique as disease-causing agents since they are misfolded proteins. Prions propagate by deforming harmless, correctly folded proteins into copies of themselves. The misfolding is irreversible. Prions attack the nervous system of the organism, causing an incurable, fatal deterioration of the brain and nervous system until death occurs. Some examples of these diseases are 'mad cow' disease (BSE) in cattle,

chronic wasting disease (CWD) in deer and elk, and Creutzfeldt-Jakob disease (CJD) in humans.

Not every species is affected by prion disease. Rabbits, water buffalo, horses, and dogs are resistant to prion diseases (Zhang, 2015, 2018). The research question arises: What are reasons allowing the protein of a resistant species to retain its folding? For rabbit normal cellular prion protein (PrP^C, where the structural region of a PrP^C consists of β -strand 1 (β 1), α -helix 1 (α 1 or H1), β -strand 2 (β 2), α -helix 2 (α 2 or H2), α -helix 3 (α 3 or H3), and the loops linking them each other), multiple amino acid residues G99, M108, S173, I214, together inhibit formation of its abnormal isoform (Kanata et al., 2016; Vorberg et al., 2003). For dog PrP, D159 is the key protective residue that provides conformational stability and confers protection against prions, suggesting that a single amino acid D159 is sufficient to prevent PrP conformational change and pathogenesis (Sanchez-Garcia et al., 2016). This paper will specially study the residue D159 of dog PrP from the protein structural dynamics point of view.

First, let us review some known literatures on dog PrP (and we particularly focus on its structural bioinformatics). There are a lot of literatures reporting dogs (and other canines) are rare animals being resistant to prion diseases, for examples (Fernandez-Funez et al., 2011; Hasegawa et al., 2013; Khan et al., 2010; Kirkwood & Cunningham, 1994; Nystromh and Hammarstrom, 2015; Polymenidou et al., 2008; Sanchez-Garcia & Fernandez-Funez, 2018; Qing et al., 2014;

Vidal et al., 2013; Won et al., 2019; Wopfner et al., 1999; Zhang et al., 2013; Fernández-Borges et al., 2017; Fernández-Borges et al., 2018; Sanchez-Garcia & Fernandez-Funez, 2018; Zhang & Liu, 2011).

- Early in 1994, it was found that not a single case of prion disease has been described among dogs through the exposure of dogs to prions (being fed prion-infected pet food) (Kirkwood & Cunningham, 1994).
- In 1999, it was reported that two differences between feline and canine PrP sequences, at codons 187 and 229, both involve substitutions to Arg residues which, together with the His-Arg substitution at codon 177 common to cat and dog, would increase the total positive surface charge on the molecule - this might in turn affect the potential intermolecular interactions critical for cross-species transmission of prion disease (Wopfner et al., 1999).
- In 2004, it was reported that the three substitutions in positions 108, 164, and 182 are unique to the canine species and are thus candidates for causing a substantial species barrier, and dogs are among the few mammals that neither contain Asn at position 164 (or 159) nor His at position 182 (or 177) (Lysek, 2003; Lysek et al., 2004).
- In 2005, the NMR structure of dog PrP was released (PDB entry 1XYK) and it was reported that the residues at positions 159 and 177 have unique charge distribution patterns on the front as well as the back side of dog PrP^C (Lysek et al., 2005). The residue D159 (less defined by NMR) is proposed to change the surface charge (Lysek et al., 2005) due to its sticking out acidic side chain.
- In 2006, the open reading frame of the prion protein (Prnp) gene from 16 Pekingese dogs was cloned and screened for polymorphisms (Wu et al., 2006). One nucleotide polymorphism (G489C) was found; the G to C nucleotide substitution results in a glutamic to aspartic acid change at codon 163; E/D163 and asparagine 107 in canine prion protein genes were replaced by asparagine and serine, respectively, in all the prion protein genes examined (Wu et al., 2006).
- In 2008, transmission experiments in Madin Darby canine kidney (MDCK) cells showed they do not replicate human CJD prions and mouse (infected with scrapie) prions (Polymenidou et al., 2008), supporting the resistance of dogs to prions. Human PrP^C is selectively targeted to the apical side of the MDCK (De Keukeleire et al., 2007).
- In 2009, Onizuka (2009) reported the substitutions N104G and S107N have the biggest impact to the conformational transition and stability of dog PrP (Onizuka, 2009). In 2009, it was reported in (Wan et al., 2009) that the three substitutions in positions 107, 163, and 181 are unique to the Arctic fox and dog, and these substitutes might be associated with susceptibility and species barriers in prion diseases.
- In 2010, Khan et al. (2010) found that at pH 4.0 dogs have the lowest concentration of β -intermediate state compared with hamsters, mice, rabbits and horses (Khan et al., 2010; Qing et al., 2014) - this can be adopted in evaluating the prion disease susceptibility of each species

(hamster > mouse > rabbit > horse > dog) (Fernandez-Funez et al., 2011; Khan et al., 2010; Qing et al., 2014).

- In 2013, Hasegawa et al. (2013) reported that there are large differences in local structural stabilities between canine and bovine PrP, and this appearance might link diversity in susceptibility to BSE prion infection (Hasegawa et al., 2013).
- In 2015, a survey of prnp genes implied that the prion disease resistant canines harbor amino acids DRK in positions 159, 177, and 185 (Nystromh and Hammarstrom, 2015). Arg177 in H2 of dog PrP also causes unique charge distribution patterns on the front and the back side of dog PrP^C (Lysek et al., 2005), but Arg177 are positively charged residue that should have minor effects on PrP structure and surface charge (Sanchez-Garcia et al., 2016), because the sequence of the rigid β 2- α 2 loop in the dog is identical to mouse PrP, indicating that this region does not contribute to the conformational stability of dog PrP (Fernandez-Funez et al., 2011).
- In 2017, 2018 and 2020, it has been reported that dog D163 amino acid is primarily (if not totally) responsible for the prion resistance of canids (Fernandez-funez et al., 2017; Fernandez-funez et al., 2018; Vidal et al., 2020).

In 2016, Sanchez-Garcia et al. (2016), Sanchez-Garcia and Fernandez-Funez (2018) reported that a single amino acid (D159) from the dog PrP suppresses the toxicity of the mouse PrP in *Drosophila* (Sanchez-Garcia et al., 2016;; Sanchez-Garcia & Fernandez-Funez, 2018) and their laboratory experience suggested that a single D159 substitution is sufficient to prevent PrP conformational change and pathogenesis (Sanchez-Garcia et al., 2016). D159 is a unique amino acid found in PrP from dogs and other canines that was shown to alter surface charge. The acidic amino acid D159 is on the α 1- β 2 loop and exposed on the surface of dog PrP, resulting in increased negative charge (Sanchez-Garcia et al., 2016). The β 1- α 1 and α 1- β 2 loops interact more closely in dog PrP than in susceptible animals thus the subtle changes in the orientation of the side chains and the closer loops may affecting the stability of the β -sheet (Hasegawa et al., 2013; Lysek et al., 2005) - this might explain the poor NMR resolution of residue D159. The mutation N159 will create a neutral surface that extends over the surface of the two loops (i.e. the β 1- α 1 loop and the α 1- β 2 loop) and H2 (Sanchez-Garcia et al., 2016). In the D159 region there only harbors one nonsense pathogenic mutation Q160X, however, this critical domain should be investigated in more details, because the identification of α 1- β 2 loop-binding proteins are expected to reveal clues about the molecular mechanisms and the extrinsic factors mediating PrP conversion from soluble normal prion protein PrP^C (predominant in α -helices) to insoluble diseased infectious prions PrP^{Sc} (rich in β -sheets) (Sanchez-Garcia et al., 2016). It has been proposed that this change in surface charge will result in altered interactions with other proteins, possibly proteins that contribute to PrP conversion (Sanchez-Garcia et al., 2016). The α 1- β 2 loop is highly conserved among mammals but only dog PrP

Figure 1. Secondary Structure graph for dog PrP D159N mutant and dog PrP wild-type (from up to down) at 300 K under neutral pH environment (x-axis: time (0–30 ns), y-axis: residue number; red: α -helix (H), blue: β -sheet (E), where H = α -helix, B = residue in isolated β -bridge, E = extended strand, participates in β -ladder, G = 3-helix (3_{10} helix), I = 5 helix (π -helix), T = hydrogen bonded turn, and S = bend).

possesses the unique acidic residue D159 suggesting that D159 plays a role in providing *global* stability to dog PrP (Fernandez-Funez et al., 2011). We also once reported that the residue at position 159 is unique in dog PrP^C (Zhang, 2012; 2015; Zhang et al., 2011). This paper will continue our research on the molecular dynamics (MD) studies of dog PrP, especially on the MD studies of its D159N mutant and their comparisons.

The rest of this paper is organized as follows. In the section of *Materials and Methods* we will give the MD simulation materials and methods. Section *Results and Discussions* will present the MD results and their analyses (where surface charge distributions are specially focused to analyse the MD trajectories). After the *Results and Discussions* section, some *Concluding Remarks* (revealed from the MD to understand the mechanism underlying the resistance to prion diseases of dogs) will be given in the last section of this paper.

Materials and methods

The MD simulation materials and methods are completely as the ones of (Zhang, 2012; 2015; Zhang et al., 2011). The D159N mutant model used in this study was constructed by one mutation D159N at position 159 using the NMR structure 1XYK.pdb of dog PrP (121–231), where the NMR experimental temperature is 293 K (i.e. the room temperature), pH value is 4.5, and pressure is AMBIENT. To neutralize the MD systems by sodium ions, 2 Na⁺ ions were added to the wild-type, and 1 Na⁺ ion was added to the D159N mutant (because the residue Asn is without charge). 1XYK.pdb has 8 Arg⁺ residues and 4 Lys⁺ residues (and 2 His⁺ residues), 6 Asp⁻ residues and 8 Glu⁻ residues, two salt bridges D144.OD1-R + 148.NH1/2, D178.OD2-R + 164.NE/NH2 and one cation- π interaction R164.CD-Y128 detected by FirstGlance in Jmol (bioinformatics.org/firstglance/fgij/). This paper uses Maestro 9.7 2014-1 (Academic use only) free package to

Salt bridge ASP178-ARG164 of dog D159N mutant

at 300 K, neutral pH

Salt bridge ASP178-ARG164 of dog PrP wild-type

at 300 K, neutral pH

Figure 2. The SB D178-R164 of the D159N mutant (with occupied rate 9.25% during the whole 30 ns of MD) and of the wild-type (with occupied rate 49.43% during the whole 30 ns of MD) at 300 K under neutral pH condition.

draw the Poisson–Boltzmann electrostatic potential surface charges of the external polarization: we choose $\epsilon_{\text{in}} = 1.0$ as the solute dielectric constant, $\epsilon_{\text{out}} = 80.0$ as the solvent dielectric constant, $12 \text{ \AA}$ as the solvent radius, and 300 K as the Temperature: EPS mapped on molecular surface is chosen.

Electrostatic potential surfaces are valuable in structure-based/computer-aided drug design because they help in optimization of electrostatic interactions between the protein and the ligand. These surfaces can be used to compare different inhibitors with substrates or transition states of the reaction. Active sites and binding surfaces can be found by

Table 1. All SBs of the D159N mutant and of the wild-type at 300 K, neutral pH value during the whole 30 ns' MD simulations.

Salt Bridges (SBs)	D159N mutant (% occupied rate)		Wild-type (% occupied rate)	In PrP, where?
D147-R + 148	100.00		100.00	in H1
D178-R + 177	99.82		99.57	in H2
E223-K + 220	98.56		41.85	in H3
E207-K + 204	98.43		96.13	in H3
E211-R + 208	97.71	*	99.13	in H3
D144-R + 148	93.72		53.47	in H1
D147-H + 140	82.21		72.37	H1 - β 1- α 1-loop
E207-R + 208	76.43		44.87	in H3
E196-K + 194	75.98		59.57	in α 2- α 3-loop
E152-R + 148	53.16	<	53.52	in H1
E152-R + 151	51.11		43.63	in H1
H187-R + 156	42.75	<	44.74	H2 - α 1- β 2-loop
E211-R + 177	30.02	<	45.91	H3 - H2
E221-K + 220	14.07	*	53.71	in H3
D147-R + 151	11.41	<	25.68	in H1
D202-H + 187	9.25		1.19	H3 - H2
D178-R + 164	7.82	<	49.43	H2 - β 2- α 2-loop
D167-R + 228	4.22			β 2- α 2-loop - H3
E146-K + 204	2.96	<	24.73	H1 - H3
H187-K + 185	2.31		0.46	in H2
E196-R + 156	0.43	<	16.20	α 2- α 3-loop - α 1- β 2-loop
H140-R + 151	0.20	*	0.29	β 1- α 1-loop - H1
E200-K + 204	0.03	*	0.21	in H3
E196-H + 187	0.03	*	0.09	α 2- α 3-loop - H2
E221-R + 228	0.02			in H3
D159-R + 136		<	92.48	α 1- β 2-loop - β 1- α 1-loop
D202-R + 156		<	8.20	In H3 - α 1- β 2-loop
E223-R + 228		*	0.15	in H3
D144-H + 140		*	0.05	in β 1- α 1-loop
H140-R + 228		*	0.01	β 1- α 1-loop - H3

Figure 3. Surface charge distributions of the 30 ns' average structure. Left: The N159 mutant of dog PrP; right: The wild-type of dog PrP. The circles indicate charge differences originating at position 159. Red, blue, and white indicate the negative, positive charges and uncharged respectively.

colouring the surface by the electrostatic potential. To study the surface charge of a residue and its local and global impacts should firstly consider the salt bridges (SBs) it linked with (McCoy et al., 1997; Zhang et al., 2015). SBs are calculated by oppositely charged atoms that are within 6.5 Å and are not directly hydrogen-bonded. It should be the average charge calculated per residue or the specific atom charge of the residue. The donor residues involved are Asp⁻, Glu⁻, and the acceptor residues involved are Lys⁺, Arg⁺, His⁺, and the real computed distance is within 6.5 Å in Amber package.

Results and discussions

First, we see the secondary structure changes of the D159N mutant and of the wild-type during the whole 30 ns' molecular movement. By Fig. 1, we may see what we want: for β 1 and β 2, the wild-type has the clear extended β -strand (participates in β -ladder) structure (with the occupied rate 3.66%

during the whole 30 ns), but the D159N mutant has changed into β -bridge structures (occupied rate 0.34% during 30 ns). This performance implied to us the mutation D159N has clearly changed the PrP structure in domains of β 1, the β 1- α 1 loop, H1, the α 1- β 2 loop, β 2, and the β 2- α 2 loop. The mutation made the stable wild-type structure (before H2) become unstable.

Second, we see the SBs of the D159N mutant and of the wild-type during the 30 ns' MD simulations. As we previously reported (Zhang, 2012; 2015; Zhang et al., 2011) the SB D178-R164 like a 'taut bow string' of the β 2- α 2 loop was broken by the D159N mutation (Fig. 2). This implies to us that residue R164 spans only 4 residues from residue D159, and residue D159 has a rather impact on the local structure. The SB D159-R136 at residue D159, with occupied rate 92.48% during 30 ns, was broken in the D159N mutant; this SB keeps the β 1- α 1 loop apart from the α 1- β 2 loop. This shows the stabilizing effect of D159 in dog PrP to affect the both locally

Table 2. All HBs of the D159N mutant and of the wild-type at 300 K, under neutral pH environment during the whole 30 ns' MD simulations.

Hydrogen bonds (HBs)	D159N mutant (% occupied rate)	Wild-type (% occupied rate)
T188@O-T192@HG1	89.69	90.84
C179@O-Y183@HG1	86.21	89.55
D178@OD1-R + 164@HE	13.13	54.05
D178@OD2-R + 164@HH21	9.18	52.07
D178@OD1-R + 164@HH21	6.95	38.84
D202@OD2-R + 156@HH21		46.46
D202@OD1-R + 156@HH21		38.87
D202@OD1-R + 156@HE		37.73
D202@OD2-R + 156@HE		17.25
D202@OD1-Y157@HH	9.74	53.98
D202@OD2-T199@HG1		41.63
D202@OD1-T199@HG1		13.73
D202@OD2-Y149@HH		24.72
D202@OD1-Y149@HH		17.85
H + 187@O-T191@HG1	71.25	84.35
Y149@O-N153@HD21		17.46
Q212@O-T216@HG1	48.43	81.55
Q227@OE1-Q212@HE21		13.29
Q212@OE1-Q227@HE21		9.74
D147@OD1-R + 151@HH12	7.00	26.35
D147@OD1-R + 151@HH11	6.75	24.47
D147@OD2-H + 140@HD1	16.73	26.46
D144@OD1-R + 151@HH12		11.39
V189@O-T193@HG1	21.28	32.71
E211@OE2-R + 177@HH21	11.91	15.17
E211@OE1-R + 177@HH21	8.41	18.94
E211@OE2-R + 177@HE		29.98
E211@OE1-R + 177@HE		26.05
E211@OE1-R + 177@HH12		8.71
E207@OE2-R + 177@HH22	6.55	10.69
E207@OE1-R + 177@HH22	5.21	14.15
E207@OE1-R + 177@HH12		6.57
E223@O-R + 228@HH21		10.76
E223@O-R + 228@HE		11.24
E223@O-R + 228@HH11		7.45
E223@O-Q219@HE22		13.09
E146@OE2-K + 204@HZ2	6.34	18.46
E146@OE2-K + 204@HZ1	5.96	16.90
E146@OE1-K + 204@HZ2	5.94	17.65
E146@OE2-K + 204@HZ3	5.69	15.90
E146@OE1-K + 204@HZ3	5.54	15.75
E146@OE1-K + 204@HZ1	5.31	16.76
Q219@OE1-Q227@HE22		24.37
Q227@OE1-Q219@HE22		5.71
T216@OG1-Q227@HE21		6.78
S231@OG-N171@HD22		9.12
G229@O-N171@HD21		28.10
H + 140@O-R + 208@HH21		22.37
H + 140@O-R + 208@HE		11.59
P158@O-R + 136@HH12		15.63
V176@O-Y218@HH		14.65
D178@OD1-Y128@HH		8.95
Q186@OE1-Y128@HH		8.62
S132@OG-Q217@HE21		7.61
N174@OD1-Q172@HE22		7.36
A224@O-R + 228@HH11		6.95
Y225@O-R + 228@HE		6.63
N197@O-R + 156@HH22		6.63
E196@OE2-R + 156@HH12		5.33

and globally unusual charge distribution of NMR structure of dog PrP.

Furthermore, we want to see and analyse all the SBs during the whole 30 ns (Table 1).

Seeing Table 1, we may know that "<" shows the local impact of the D159N mutation which made the weaker of the wild-type's SBs such as D159-R + 136, E211-R + 177, D147-R + 151, D178-R + 164, E146-K + 204, E196-R + 156, D202-R + 156, H + 187-R + 156, E152-R + 148, and "*" implies the global impact of the D159N mutation which made

weaker of the wild-type's SBs. To understand better the above SBs, we illuminate the surface charge distributions of the 30 ns' average structure of the D159N mutant and the wild-type respectively (Figure 3).

From Figure 3, we see that the D159N mutation made the negative charges and the positive charges redistributed around the residue at position 159; however, the negative charges covering H1 and the tail of H3 are not changed very much – this implies to us we had better not seek drug target(s) from H1 or the tail of H3.

Table 3. The pKa values of the 30 ns' average structure for the wild-type and the D159N mutant (values in the brackets) at 300 K, neutral pH value.

Residue Name	pKa	polar (charged)	polar (neutral)	de-solvation (charged)	de-solvation (neutral)
ARG136	12.01 (11.96)	−0.1922 (−0.5657)	−0.1970 (−0.6404)	0.0641 (0.0955)	0.0549 (0.0947)
ASP144	3.94 (3.42)	−0.0786 (−0.2849)	0.1048 (0.0450)	0.0608 (0.2173)	0.0216 (0.0826)
GLU146	3.46 (4.42)	−0.5985 (−0.3709)	−0.0677 (−0.0585)	0.1357 (0.0639)	0.0103 (0.0056)
ASP147	4.06 (3.76)	0.0213 (−0.4601)	0.1152 (−0.0431)	0.0617 (0.0929)	0.0339 (0.0160)
ARG148	12.00 (11.88)	0.1632 (0.2404)	0.1556 (0.2194)	0.0501 (0.1162)	0.0359 (0.1211)
ARG151	12.00 (11.98)	0.0590 (0.2061)	0.0619 (0.2096)	0.0409 (0.0693)	0.0307 (0.0687)
GLU152	4.47 (4.28)	0.0382 (−0.0142)	0.0029 (0.0200)	0.0283 (0.0359)	0.0018 (0.0026)
ARG156	11.95 (11.99)	0.1756 (0.0016)	0.2206 (−0.0155)	0.0862 (0.1320)	0.0823 (0.1334)
ASP159	3.72	−0.9875	−0.4723	0.1609	0.0259
ARG164	11.98 (12.00)	0.1547 (0.2866)	0.2103 (0.2739)	0.0872 (0.0274)	0.0836 (0.0121)
ASP167	4.48 (4.44)	0.0371 (−0.0336)	0.1008 (0.1266)	0.0522 (0.0796)	0.0128 (0.0125)
ARG177	11.94 (11.99)	0.0968 (−0.1107)	0.0860 (−0.1142)	0.0652 (0.0514)	0.0575 (0.0423)
ASP178	3.50 (3.98)	−0.0383 (−0.1739)	0.1621 (0.0485)	0.1111 (0.0747)	0.0798 (0.0294)
LYS185	10.06 (10.10)	−0.0347 (−0.0502)	−0.0134 (−0.0232)	0.0081 (0.0066)	0.0066 (0.0054)
LYS194	10.23 (10.26)	−0.1205 (−0.1328)	−0.0327 (−0.0457)	−0.0036 (−0.0102)	0.0061 (0.0064)
GLU196	4.49 (4.47)	0.0577 (0.0564)	0.0057 (0.0271)	0.0344 (0.0458)	0.0053 (0.0034)
GLU200	4.50 (4.52)	−0.3243 (−0.2253)	0.0430 (0.0415)	0.0158 (0.0093)	0.0008 (0.0026)
ASP202	3.14 (3.74)	−0.7954 (−0.1331)	−0.1489 (0.1493)	0.1667 (0.1651)	0.0174 (0.0685)
LYS204	10.99 (10.26)	0.2825 (0.0984)	0.0119 (0.0147)	0.0250 (0.0197)	0.0126 (0.0054)
GLU207	4.48 (4.50)	−0.1348 (−0.0280)	−0.0050 (0.0200)	0.0402 (0.0356)	0.0025 (0.0025)
ARG208	12.01 (11.99)	0.1597 (0.0437)	0.1383 (0.0362)	0.0533 (0.0727)	0.0376 (0.0602)
GLU211	3.81 (4.01)	−0.0695 (0.0342)	0.0043 (−0.0048)	0.0678 (0.0532)	0.0036 (0.0013)
LYS220	10.21 (10.73)	−0.1804 (−0.0400)	−0.0155 (0.0042)	0.0290 (0.0071)	0.0066 (0.0065)
GLU221	4.76 (4.06)	−0.1409 (−0.7819)	−0.0294 (−0.1045)	0.0216 (0.0793)	0.0028 (0.0032)
GLU223	4.77 (4.45)	0.1108 (0.0004)	0.0208 (0.0075)	0.0205 (0.0365)	0.0025 (0.0016)
ARG228	11.99 (12.05)	−0.4999 (−0.1665)	−0.5018 (−0.0616)	0.0900 (0.0695)	0.0748 (0.0572)

Thirdly, we will give a brief overview of the changes of some hydrogen bonds (HBs) and hydrophobic interactions (HYDs) made by the D159N mutation. In view of the number of HBs, we cannot say differences between the D159N mutant and the wild-type; however, the HBs listed in Table 2 contribute to the structural stability of wild-type dog PrP more than of the D159N mutant. We may see from Tables 1 and 2 that (i) D202-R + 156 and E223-R + 228 are two polar contacts for the wild-type, but the D159N mutant is without these polar contacts; (ii) D147-H + 140, E211-R + 177, D147-R + 151, D178-R + 164 are four strong polar contacts for the wild-type but weaker for the D159N mutant.

Regarding hydrophobic interactions (HYDs), throughout the whole 30 ns' MD simulations, the D159N mutation has a local impact – it made HYD M213-V161 become weaker than in the wild-type, and has global impacts – it made V215-M213 and V209-I205 become weaker than in the wild-type. Around the residue at position 159, we find there are one π - π stacking F141-Y150 and one π -cation Y163-R164 in the D159N mutant and the π - π stacking F141-Y150 in the wild-type PrP throughout the whole 30 ns' protein movement. We also noticed that GN8 (Hosokawa-Muto et al., 2012; Kuwata et al., 2007), an antiprion drug fixing the distance between N159 and E196 being 1.54 Å, was designed at the position 159 – this might show the importance of the PrP residue at position 159. We also used the “DelPhiPKa” protonation and pKa calculation web server (http://compbio.clemson.edu/pka_webserver) for the 30 ns' average structures to detect the difference between the wild-type and the D159N mutant (Table 3) – we cannot detect the significant difference between the wild-type and the D159N mutant.

The residue D163 spans four residues from D159 and has been reported playing an important role in dog PrP (Fernandez-Funez et al., 2017; 2018; Vidal et al., 2020; Wan

et al., 2009; Wu et al., 2006). In 1XYK.pdb of dog PrP, at 163 it is residue Tyr163, and between D159 and D167 the residues are Gln160, Val161, Tyr162, Tyr163, Arg + 164, Pro165, Val166. So, at position 163 the author will furthermore do MD studies on the homology modelling mutation of Tyr163 into Asp163 (D163) or Asn163 (N163) or Glu163 (E163), and furthermore investigate the residue at 163 in another paper.

Concluding remarks

The mutation D159N of dog PrP had profound effects on protein structure of dog PrP: (1) it altered the surface charge distribution, both locally and globally, (2) it reduced the stability of the tertiary structure of dog PrP, and (3) it increased the mobility of dog PrP structure before H2. The MD studies of this paper confirmed these three findings and emphasized the contribution of the single residue D159 in dictating the global (and local) charge distribution and structural stability of dog PrP. This paper presented detailed sufficient structural informatics on the residue at position 159 to understand the mechanism underlying the resistance to prion diseases of dogs; this may be useful for the medicinal treatment of prion diseases.

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