Solution Structure and Dynamics of the I214V Mutant of the Rabbit Prion Protein

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Abstract

Background: The conformational conversion of the host-derived cellular prion protein (PrPC) into the disease-associated scrapie isoform (PrPSc) is responsible for the pathogenesis of transmissible spongiform encephalopathies (TSEs). Various single-point mutations in PrPSc could cause structural changes and thereby distinctly influence the conformational conversion. Elucidation of the differences between the wild-type rabbit PrPSc (RaPrPSc) and various mutants would be of great help to understand the ability of RaPrPSc to be resistant to TSE agents.

Methodology/Principal Findings: We determined the solution structure of the I214V mutant of RaPrPSc(91–228) and detected the backbone dynamics of its structured C-terminal domain (121–228). The I214V mutant displays a visible shift of surface charge distribution that may have a potential effect on the binding specificity and affinity with other chaperones. The number of hydrogen bonds declines dramatically. Urea-induced transition experiments reveal an obvious decrease in the conformational stability. Furthermore, the NMR dynamics analysis discloses a significant increase in the backbone flexibility on the pico- to nanosecond time scale, indicative of lower energy barrier for structural rearrangement.

Conclusions/Significance: Our results suggest that both the surface charge distribution and the intrinsic backbone flexibility greatly contribute to species barriers for the transmission of TSEs, and thereby provide valuable hints for understanding the inability of the conformational conversion for RaPrPSc.

Introduction

The conformational conversion of the prion protein, from the normal cellular form (PrPc) to the abnormal scrapie isoform (PrPSc), is responsible for the pathogenesis of transmissible spongiform encephalopathies (TSEs) [1]. The “protein-only” hypothesis is supported by much compelling evidence presented recently [2–4]. To elucidate the detailed mechanism of the conformational change, understanding of the structural basis of the prion protein is required. The three-dimensional structures of PrPc among species have been extensively determined using NMR techniques so far [5–16]. Great effort has also been made to explore the architecture of PrPSc or PrPSc-like filaments [17–21].

Noticeably, all known forms of inherited human TSEs, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS) and fatal familial insomnia (FTI), are closely associated with dominant mutations in human PrPSc (hPrPSc) [22,23]. In addition to apparently disease-causing mutations in humans, polymorphisms in sheep appear to exert substantial effects on variations of PrPc in disease susceptibility [24–27]. Particularly, rabbits are one of the few mammalian animals reported to be resistant to TSE agents [28]. Multiple amino acid residues throughout the rabbit PrPc (RaPrPc) sequence contribute to the inability of RaPrPSc to form PrPSc. Several mouse PrPSc (mPrPSc) mutants, with one single residue substituted by the corresponding residue in RaPrPSc, are completely inhibited to undergo the conversion to the disease-related isoform [29]. All these facts indicate a significant influence on the property of the PrPSc structure as the result of one important residue mutation. Therefore, detailed comparisons of three-dimensional structures of wild-type PrPSc and its mutants could provide valuable insights into the underlying molecular mechanism of prion conversions.

In our previous work we have demonstrated that the S173N substitution leads to distinct structural changes for RaPrPSc [30]. However, whether similar changes could be observed in other single-point mutants remains to be addressed. Since mPrPSc with the V214I substitution is prohibited to convert to the abnormal form [29], we thereby expect that the I214V substitution would cause structural changes more or less for RaPrPSc. In this present work, we determined the solution structure of the I214V mutant of RaPrPSc(91–228) using multi-dimensional heteronuclear NMR
techniques. In addition, we performed ¹⁵N relaxation measurements to detect the backbone dynamics of its structured C-terminal domain (121–228). Furthermore, we investigated its structural stability using CD spectroscopy. Our results reveal significant structural changes caused by the single-residue mutation, which may be of benefit to understand the detailed molecular mechanism of the conformational conversion for prion proteins.

Results

Solution structure

A family of 200 structures is calculated and the structural statistics are presented in Table 1. The diagram representing 15 lowest-energy structures for the I214V mutant of RaPrP<sub>C</sub>(91–228) in solution is shown in Fig. 1A, together with a ribbon cartoon of average secondary structure elements displayed in Fig. 1B. The I214 mutant contains two short antiparallel β-sheets (S1: 128–130, S2: 160–162) and three α-helices (H1: 144–155, H2: 175–186, H3: 199–227), with a disulfide bond (C178–C213) stabilizing helices 2 and 3. The N-terminal loop 91–120 is highly disordered. Loop 165–172 is well defined owing to the long-range NOEs from residues at the end of helix 3. The overall structure of the I214V mutant appears to be identical to that of the wild-type (Fig. 1C).

Electrostatic potential

We evaluated the effect of the I214V substitution on the surface charge distribution of RaPrP<sub>C</sub>(91–228). Both the mutant and the wild-type carry neutral charge at the right substituted site 214 (Fig. 2A, B). The I214V substitution does not change the electrostatic potential at site 214 due to the similar non-charged feature of the two amino acids. Unexpectedly, significant changes are observed at many other unsubstituted sites. For example, the wild-type displays a neutral charge distribution at site 124, while the I214V mutant carries positive charge in the same position (Fig. 2C, D). Site 164 also alters electrostatic potential from positive to neutral after the mutation (Fig. 2C, D). The I214V substitution results in a prominent shift of the surface charge distribution.
Hydrogen bond

The hydrogen bond network usually makes a significant contribution to maintain secondary and tertiary structures of a protein. Stable hydrogen bonds are defined herein if they exist in at least 9 structures among the 15 lowest-energy structures. Totally, 41 hydrogen bonds are detected in the I214V mutant of RaPrPC(91–228), much less than the number 55 in the wild-type. In particular, only 4 hydrogen bonds participate in sustaining the tertiary structure of the mutant, compared with 8 in the wild-type (Table 2). As less hydrogen bonds are formed in the mutant, one could expect that the RaPrPC molecule is somehow readily to experience the conformational conversion after the mutation.

Conformational stability

To access the conformational stability of the I214V mutant of RaPrPC(121–228), we performed urea-induced unfolding transition experiments using far-UV CD spectroscopy. The I214V mutant is rich in α-helix in the absence of urea (Fig. 3A), indicating a well-folded state. This mutant loses its secondary structure entirely under the condition of 9 M urea (Fig. 3A), implying a completely unfolded state. The mean residue ellipticity at 222 nm versus the urea concentration is shown in Fig. 3B, with a solid line showing the theoretical curve on the basis of a two-state mechanism. The denaturation was not reversible in our experimental condition (Fig. 3B), thus we determined the apparent thermodynamic parameters for the equilibrium unfolding of the I214V mutant (Table 3). \( C_m \) is the concentration of urea required to denature 50% of a protein, and \( \Delta G^0_{N→U} \) presents the apparent estimated free energy of unfolding extrapolated to zero concentration of denaturant. The measured values of \( C_m \) and \( \Delta G^0_{N→U} \) for the mutant are less than those for the wild-type (Table 3), indicating reduced conformational stability of RaPrPC after the I214V substitution. The coefficient \( m \) is also different from that for the wild-type (Table 3).

Relaxation rates \( R_1, R_2 \) and heteronuclear NOEs

To analyze the backbone dynamics of the I214V mutant of RaPrPC(121–228), we performed \( ^{15} \)N relaxation measurements using NMR spectroscopy. As a whole, 103 assigned residues are used except 5 residues with unobservable resonance signals. We utilized the peak height for curve fitting so as to avoid the effect of partially overlapped resonance peaks. The relaxation rates \( R_1, R_2 \) and heteronuclear NOEs versus residue number are shown in Fig. 4. The \( R_1 \) values do not change much with the sequence, mostly between 1.1 and 1.5 s\(^{-1}\). Different from the \( R_1 \) distribution, the \( R_2 \) values are relatively variable with residue number, ranging from 3.0 to 18.1 s\(^{-1}\) approximately. The residue D166 shows the largest \( R_2 \) value over 18.1 s\(^{-1}\), and G130 also

Table 2. Hydrogen bonds maintaining the tertiary structures of RaPrPC(91–228) and the I214V mutant.

| Protein        | Hydrogen bonds                                                                 |
|----------------|-------------------------------------------------------------------------------|
| RaPrPC(91–228) | M128H-Y162O, G130H-Y160O, I138H-Y149O, R155H-H22-Y148O, H176-D1-E210-O2, H186-H1-R155O, S221-HG-D166-O1 |
| I214V          | G130H-Y160O, I138H-Y149O, Y162H-H1, M128O, H186-H1-R155O                      |

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Figure 2. Distributions of surface electrostatic potential. Site 214 of (A) the wild-type and (B) the I214V mutant. Sites 124 and 164 of (C) the wild-type and (D) the I214V mutant. Positive, neutral and negative charges are colored blue, white and red, respectively. The surface diagrams are generated using MolMol.
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displays distinctly large \( R_2 \) value as high as 16.0 s\(^{-1}\). Almost all residues exhibit positive NOE values except the first three residues. Typically, NOE values for the two antiparallel β-sheets and three α-helices are obviously higher than those for the loop fragments encompassing residues 121–127, 138–141 and 189–197. The secondary structure elements display NOE values larger than 0.7, indicative of more restricted dynamics in these regions.

### Reduced spectral density mapping

We adopted the spectral density function approach [31–33] to interpret the \(^{15}\)N relaxation data for the I214V mutant of RaPrP\(^{(121–228)}\). The calculated values of reduced spectral densities \( J(0) \), \( J(0.87\text{rad}) \) and \( J(0.87\text{rad}) \) versus residue number are shown in Fig. 5A. The middle frequency spectral densities \( J(\omega_0) \) exhibit relatively invariable values ranging from 0.25 to 0.38 ns\(^{-1}\), reflecting insensitivity to variations in backbone motion. Residues in helix 3 show a slight reduction in \( J(\omega_0) \) spectral densities compared to helices 1 and 2. It has been suggested that such a reduction reflects slightly anisotropic tumbling of PrP\(^{(121–228)}\) [34].

The low frequency spectral density \( J(0) \) is sensitive both to fast internal motions on the pico- to nanosecond (ps-ns) time scale and to slow motions \( (R_{\text{ns}}) \) on the micro- to millisecond (μs-ms) time scale. Rapid internal motions tend to reduce the \( J(0) \) value, while slow internal motions usually lead to anomalously large \( J(0) \) values [35]. The plot of \( J(0) \) versus residue number shows the trend of \( R_2 \) values change with the sequence since \( J(0) \) values are normally dominated by \( R_2 \) values. In contrast to \( J(\omega_0) \), the spectral densities \( J(0) \) cover a wider range of values. Residues with lower \( J(0) \) values are mainly observed in loop regions 121–127, 138–141 and 189–197, implying sub-nanosecond flexibility of the N-H bond vector. Dissimilarly, loop 165–172 is not as flexible as the other three loop fragments. Residues in secondary structure elements have relatively higher \( J(0) \) values. However, the residue H136 in helix 2 displays a fairly small \( J(0) \) value, implying significant internal motion on the ps-ns time scale. D166 exhibits the largest \( J(0) \) value around 7.0 ns\(^{-1}\), and G130 in β-sheet 1 also shows a remarkable \( J(0) \) value as large as 5.9 ns\(^{-1}\), both indicative of slow \( μs\text{-ms} \ R_{\text{ns}} \) motions. Furthermore, a few residues in helices 2 and 3 are likely to undergo slow \( R_{\text{ns}} \) motions as well.

The high frequency spectral density \( J(0.87\text{rad}) \) is sensitive only to fast internal motions on the sub-nanosecond time scale. Fast motions are reflected in relatively large values of \( J(0.87\text{rad}) \). Aside from residues in loop regions 121–127, 138–141 and 189–197, the C-terminal end of helix 3 and the particular residue H136 show large \( J(0.87\text{rad}) \) values, implying fast ps-ns motions. In addition, small \( J(0.87\text{rad}) \) values and large \( J(0) \) values are observed in most residues in secondary structure elements, indicative of restricted motions on the fast ps-ns time scale.

The overall distributions of the reduced spectral densities \( J(\omega_0) \), \( J(0) \) and \( J(0.87\text{rad}) \) for the I214V mutant are similar to those for the wild-type reported previously [30]. We compared the values of the spectral densities between the wild-type and the I214V mutant, and the differences versus residue number are shown in Fig. 5B. The most significant changes are observed in \( J(0) \). Almost all residues exhibit distinctly negative values in \( \Delta J(0) \). Generally, the smaller the value of \( J(0) \), the greater the sub-nanosecond flexibility of N-H bond vectors [36]. Thus the I214V mutation leads to increased internal dynamics.

### Order parameter \( S^2 \)

The modelfree formalism is usually used to analyze internal motions of a protein [37–39]. The \( D_3 \) in the rotational diffusion tensor for the I214V mutant of RaPrP\(^{(121–228)}\) was calculated to be 1.24 ± 0.01 using the r2r1_diffusion program (kindly provided by Prof. A.G. Palmer 3rd), suggesting that the axially symmetric model is suitable for the data fitting. The

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**Table 3.** Apparent thermodynamic parameters for the equilibrium unfolding of RaPrP\(^{(121–228)}\) and the I214V mutant at 25°C.

| Protein      | \( \Delta G_{\text{m}}^{H,D} \) (kJ mol\(^{-1}\)) | \( n \) (kJ mol\(^{-1}\) M\(^{-1}\)) | \( C_m \) (M) |
|--------------|-----------------------------------------------|-----------------------------------|--------------|
| RaPrP\(^{(121–228)}\) | 26.2 ± 2.7                                    | −3.88 ± 0.49                      | 6.49 ± 0.05  |
| I214V        | 16.1 ± 1.8                                    | −2.62 ± 0.38                      | 5.65 ± 0.06  |

Note: \( \Delta G_{\text{m}}^{H,D} \) is an estimate of the free energy in the absence of denaturant, the parameter \( n \) represents the cooperativity of the unfolding transition, and \( C_m \) is the concentration of urea at the midpoint of unfolding. The determined parameters for the wild-type [30] are listed here to facilitate comparison.
calculated order parameter $S^2$ versus residue number is plotted in Fig. 6A. This parameter describes the amplitude of the subnanosecond timescale motions ($0 \leq S^2 \leq 1$). Residues in loop 135–141 and 189–197 display the lowest $S^2$ values, which imply that these regions are highly disordered. In contrast to these two loops, loop 165–172 show higher $S^2$ values, indicating an ordered
Discussion

Single-point mutation may result in functional alteration owing to the global or local structural change in the protein. Several mPrPC mutants exhibit local structural changes and show distinctly different behavior from the wild-type [29,40]. Three single-residue mutants, including hPrPC(M166V), hPrPC(S170N) and hPrPC(R220K), show variations in the length and quality of definition of helix 3 [41]. In addition, loop 166–172, which is lack of backbone amide resonances in hPrPC and mPrPC, is well-defined in both hPrPC(S170N) and mPrPC(V166A) mutants [16,41].

In this present work, we have determined the solution structure of the I214V mutant of RaPrPC(91–228). The three-dimensional structure of the mutant is almost identical to that of the wild-type (Fig. 1), however, the altered hydrogen bond network and the changed surface charge distribution demonstrate that the I214V substitution could lead to marked structural changes (Fig. 2 and Table 2). Significant change in surface-restricted charges has also been observed in hPrPC(E200K) [42] and RaPrPC(S173N) mutants [30]. It is well known that electrostatic interaction could distinctly influence on both the binding specificity and affinity of a protein with substrates. Thus, the mutation-induced change of the I214V substitution significantly enhances the backbone flexibility of RaPrPC. The ΔS2 values are mapped onto the mean structure of the I214V mutant (Fig. 6C).

Materials and Methods

The I214V mutants of RaPrPC(91–228) and RaPrPC(121–228) were constructed using site-directed mutagenesis PCR. Plasmid construction, protein expression and purification, NMR spectroscopy, structure calculation, 15N relaxation measurements, dynamics analysis and CD experiments were conducted as described previously [30,60]. Nearly complete backbone and side-chain resonance assignments for the I214V mutant of RaPrPC(91–228)
have been obtained. The chemical shift data are available at the Biological Magnetic Resonance Data Bank with accession number 16616. The atomic coordinates have been deposited into the Protein Data Bank with PDB ID 2JM.

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Author Contributions

Conceived and designed the experiments: DL. Performed the experiments: YW, JL, MX, YP, WY, JH. Analyzed the data: YW, JL. Contributed reagents/materials/analysis tools: DL. Wrote the paper: YW, DL.

References

1. Prusiner SB (1998) Prions. Proc Natl Acad Sci U S A 95: 13363–13363.
2. Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, et al. (2004) Synthetic mammalian prions. Science 305: 673–676.
3. Castillo J, Saa P, Herz C, Soto C (2005) In vitro generation of infectious scrapie prions. Cell 121: 195–206.
4. Wang F, Wang X, Yuan CG, Ma J (2010) Generating a Prion with Bacterially Expressed Recombinant Prion Protein. Science 327: 1132–1135.
5. Riek R, Hornemann S, Wider G, Billiet M, Glockshuber R, et al. (1996) NMR structure of the mouse prion protein domain P(V121-231). Nature 382: 180–182.
6. Donne DG, Viles JH, Groth D, Mehlhorn I, James TL, et al. (1997) Identification of five allelic variants of the sheep prion protein gene and their association with natural scrapie. J Gen Virol 77: 2097–2101.

Author Contributions

Conceived and designed the experiments: DL. Performed the experiments: YW, JL, MX, WY, JH. Analyzed the data: YW, JL. Contributed reagents/materials/analysis tools: DL. Wrote the paper: YW, DL.
50. Bae SH, Legname G, Serban A, Prusiner SB, Wright PE, et al. (2009) Prion proteins with pathogenic and protective mutations show similar structure and dynamics. Biochemistry 48: 8120–8128.

51. Kay LE, Muhundiram DK, Wolf G, Shoelson SE, Forman-Kay JD (1998) Correlation between binding and dynamics at SH2 domain interfaces. Nat Struct Biol 5: 156–163.

52. Tzeng SR, Kalodimos CG (2009) Dynamic activation of an allosteric regulatory protein. Nature 462: 360–372.

53. Privalov PL, Makhatadze GI (1990) Heat capacity of proteins. II. Partial molar heat capacity of the unfolded polypeptide chain of proteins: protein unfolding effects. J Mol Biol 213: 305–391.

54. Alonso DO, Dill KA (1991) Solvent denaturation and stabilization of globular proteins. Biochemistry 30: 5974–5985.

55. Dill KA, Shortle D (1991) Denatured states of proteins. Annu Rev Biochem 60: 795–823.

56. Privalov PL, Makhatadze GI (1992) Contribution of hydration and non-covalent interactions to the heat capacity effect on protein unfolding. J Mol Biol 224: 715–723.

57. Zhang J (2010) Studies on the structural stability of rabbit prion probed by molecular dynamics simulations of its wild-type and mutants. J Theor Biol 264: 119–122.

58. Akke M, Bruschweiler R, Palmer AG, 3rd (1993) NMR Order Parameters and Free Energy: An Analytical Approach and Its Application to Cooperative Ca"²⁺ Binding by Calbindin D₉k. J Am Chem Soc 115: 9832–9833.

59. Yang D, Kay LE (1996) Contributions to conformational entropy arising from bond vector fluctuations measured from NMR-derived order parameters: application to protein folding. J Mol Biol 263: 369–382.

60. Yin SM, Zheng Y, Tien P (2003) On-column purification and refolding of recombinant bovine prion protein: using its octarepeat sequences as a natural affinity tag. Protein Expr Purif 32: 104–109.

61. Koradi R, Billeter M, Wuthrich K (1996) MOLMOL: a program for display and analysis of macromolecular structures. J Mol Graph 14: 51–55, 29–32.

62. Spyropoulos L (2006) A suite of Mathematica notebooks for the analysis of protein main chain 15N NMR relaxation data. J Biomol NMR 36: 215–224.

63. Cole R, Loria JP (2003) FAST-Modelfree: a program for rapid automated analysis of solution NMR spin-relaxation data. J Biomol NMR 26: 203–213.

64. Wishart DS, Sykes BD (1994) The 13C chemical-shift index: a simple method for the identification of protein secondary structure using 13C chemical-shift data. J Biomol NMR 4: 171–180.