Possible role of region 152–156 in the structural duality of a peptide fragment from sheep prion protein

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Abstract

The conformational conversion of the nonpathogenic “cellular” prion isoform into a pathogenic “scrapie” protease-resistant isoform is a fundamental event in the onset of transmissible spongiform encephalopathies (TSE). During this pathogenic conversion, helix H1 and its two flanking loops of the normal prion protein are thought to undergo a conformational transition into a β -like structure. A peptide spanning helix H1 and β -strand S2 (residues 142–166 in human numbering) was studied by circular dichroism and nuclear magnetic resonance spectroscopies. This peptide in aqueous solution, in contrast to many prion fragments studied earlier (1) is highly soluble and (2) does not aggregate until the millimolar concentration range, and (3) exhibits an intrinsic propensity to a β -hairpin-like conformation at neutral pH. We found that this peptide can also fold into a helix H1 conformation when dissolved in a TFE/PB mixture. The structures of the peptide calculated by MD showed solvent-dependent internal stabilizing forces of the structures and evidenced a higher mobility of the residues following the end of helix H1. These data suggest that the molecular rearrangement of this peptide in region 152–156, particularly in position 155, could be associated with the pathogenic conversion of the prion protein.

Keywords: NMR; structural duality; TFE; salt bridges; prion protein; peptide; helix H1; region 152–156

The nonpathogenic “cellular” isoform of the prion protein (PrP^C) is a strongly conserved cell surface glycoprotein expressed in all mammalian species studied so far (Bendheim et al. 1992). Its conformational conversion into a pathogenic “scrapie” isoform (PrP^{Sc}) is the fundamental event in the pathogenicity of transmissible spongiform encephalopathies (TSE) (Prusiner 1998). The major structural feature of prion

conversion manifests itself as an increase of the β -sheet content in PrP^{Sc} (Griffith 1967; Prusiner 1991; Pan et al. 1993). Transgenic studies argue that infectious PrP^{Sc} acts as a template (Prusiner et al. 1990; Telling et al. 1995) upon which the normal PrP^C is refolded into a pathogenic isoform through a process facilitated by an unknown as yet factor “X” (Kaneko et al. 1997).

The mammalian PrP^C contains 209 amino acid residues, from 23 to 231 in human numbering. The minimal prion fragment required for infectious propagation was mapped to residues 90–231 (Prusiner 1998). NMR studies of the recombinant mouse, hamster, bovine, and human prion proteins (Riek et al. 1997, 1998) showed that all these molecules have very similar 3D structures, including a flexible unstructured “tail” composed of residues 23–120 and a mostly α -helical globular core part 121–231. This globular

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Abbreviations: PrP, prion protein; PrP^C, “cellular” isoform; PrP^{Sc}, “scrapie” isoform; TFE, trifluoroethanol; PB buffer, 10 mM sodium phosphate buffer (pH 6.5); MD, molecular dynamics.

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PrP is composed of two short antiparallel β -strands (S1 and S2) and three α -helices (H1–H3) (Riek et al. 1996). This globular domain can further be divided into two subdomains (Jamin et al. 2002), one long hairpin subdomain (helix H1 and the β -sheet) and one purely α -helical subdomain (helices H2 and H3).

Within the globular domain of the molecule, the region containing helix H1 is known to be one of the most flexible of the prion proteins (Viles et al. 2001). The fragment containing helix H1 and strand S2 is the most probable site for conformational conversion of PrP^C (Riek et al. 1996; Prusiner 2001). The question of how helix H1 in particular can undergo such a major structural rearrangement from helical conformation to a structure involving a significant amount of β -sheet remains unsolved. The deletion of this region (helix H1 and strand S2) has been recently shown to inhibit formation of the PrP^{Sc} (Vorberg et al. 2001). A recent study indicates a bipartite function of helix H1 in the maturation and aggregation of PrP (Winklhofer et al. 2003). As helices H2 and H3 are stabilized by a disulfide bond and form the C-terminal scaffold, they probably have nearly the same conformation in PrP^{Sc} and PrP^C (Muramoto et al. 1996). However, structural studies of PrP^{Sc} have been limited because of its aggregated state (Prusiner et al. 1983; Caughey et al. 1991; Gasset et al. 1993; Safar et al. 1993). The exact role of helix H1 and strand S2 in the conformational conversion process remains to be clearly elucidated.

Many prion-derived peptides were analyzed (Gasset et al. 1992; Come et al. 1993; Tagliavini et al. 1993; De Gioia et al. 1994; Nguyen et al. 1995; Zhang et al. 1995; Heller et al. 1996; Inouye and Kirschner 1997; Pillot et al. 1997; Ragg et al. 1999) in an attempt to clarify the molecular basis that might be involved in promoting the PrP^C to PrP^{Sc} conformational transition. Most of them belong to the 90–145 region, and have intrinsic propensity to produce insoluble intermolecular aggregates of an extended β -like structure.

We investigated by CD and NMR spectroscopies the solution structure of a linear 26-mer peptide (hereafter referred to as peptide n3) (Kozin et al. 2000, 2001). Its sequence **GNDYE⁵DRYYR¹⁰ENMYR¹⁵YPNQV²⁰YYRPV²⁵C** contains 25 residues corresponding to the domain 145–169 of sheep prion protein (Goldmann et al. 1990) (142–166 in human prion protein numbering) and a C-terminal cysteine (the bold letters represent the segments corresponding to helix H1 [residues 144–154] and β -strand S2 [residues 161–164], respectively). In contrast to the prion-derived peptides studied earlier, peptide n3 (1) remains soluble in aqueous solution and (2) does not aggregate until the millimolar concentration range, and (3) exhibits an intrinsic propensity to a β -hairpin like conformation at neutral pH.

This peptide has also been recently studied by fluorescence measurements, and has been suggested to act as a potential inhibitor that could prevent the formation of PrP^{Sc} (Pato et al. 2004).

The experimental results obtained in the present work show that this peptide can also fold into the helix H1 conformation when dissolved in a TFE/PB mixture. This conversion from a β -like to helix structure was studied by CD and NMR. The structures of the peptide calculated by MD showed solvent-dependent internal stabilizing forces of the structures, and evidenced a higher mobility of the residues following the end of helix H1. This structural duality of the peptide is reminiscent of the overall conformational transition of PrP from helix to β -sheet. We propose that the potential nucleation site for the molecular rearrangement of the prion protein may be localized within this peptide.

Results

Secondary structure analysis

The peptide secondary structure was analyzed in different experimental conditions by far-UV CD spectroscopy. Early study (Kozin et al. 2000) in aqueous buffered solution showed that the peptide produced a broad negative Cotton effect at 208 nm, with a shoulder at 216 nm, and two smaller signals, namely, a positive one at 233 nm and a negative one at 241 nm, which are characteristic of nonrandom coil conformation (Venyaminov and Yang 1996). We showed that the increase of TFE concentration results in a transformation of the CD signal (Fig. 1). Indeed, in the range of 20–99.5% 2,2,2-trifluoroethanol (TFE), CD spectra of the peptide showed two negative peaks at 208 nm and 218 nm and a positive peak at 195 nm, typical of α -helix structure. An isodichroic point at 205 nm indicates equilibrium between two distinct conformations.

Figure 1. Far-UV CD spectra of the n3 peptide obtained in mixed PB–TFE solutions at 298 K. TFE concentrations expressed v/v: 0% (black), 10% (blue), 20% (cyan), 30% (magenta), 40% (green), 80% (yellow), and 96% (red). The inset demonstrates the changes in the mean residue weight ellipticity, Θ , at 222 nm.

NMR solution structure

The absence of time-dependent aggregation of the n3 peptide at millimolar concentrations at neutral pH allowed us to study conformational features of the peptide by NMR spectroscopy. All the NMR experiments were carried out at 500.13 MHz, using a 4-mM peptide concentration in an 87/13 (v/v) TFE/"PB" buffer, (10 mM sodium phosphate buffer [pH 6.5]). Spin systems were determined using two-dimensional TOCSY spectra with 35–70-msec mixing times. Assignments were confirmed by NOESY (Fig. 2) and ROESY experiments.

The sequential assignment of all backbone amide resonances was carried out by using the standard protocol developed by Wüthrich (1986). All experiments were performed at three different temperatures (278, 293, and 310 K) to solve assignment ambiguities resulting from signal overlaps. All $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ resonances were assigned, and most of $^{13}\text{C}'$ resonances were found.

Secondary structures were identified using the CSI protocol, involving $^1\text{H}^\alpha$, $^{13}\text{C}'$, $^{13}\text{C}^\alpha$, and $^{13}\text{C}^\beta$ chemical shifts (Tables 1,2) (Wishart and Sykes 1994). We applied the criteria for secondary structure and the differences between observed and random-coil $^1\text{H}^\alpha$ and $^{13}\text{C}^\beta$ chemical shifts from Asp 3 to Tyr 14 showed negative values, consistent with an α -helical arrangement. Also for these residues, the same calculation for $^{13}\text{C}'$ and $^{13}\text{C}^\alpha$ results in positive values, confirming their helical propensity (Fig. 3).

Moreover, the $^3\text{J}\text{-H}\alpha\text{HN}$ scalar coupling constants indicated a helical structure for this region of the peptide (Fig.

4). Indeed, these values are weak (less than 5 Hz), which means that these residues tend to populate α -helical ϕ angles.

The amide signal shift temperature coefficients ($\Delta [\delta \text{NH}]/\Delta T$, in ppb K^{-1}) were derived for all backbone amide protons of the peptide (Fig. 4). For the residues involved in intramolecular hydrogen-bonding network and/or hidden in a structural core protected from solvent, one can expect low absolute values of this coefficient compared to the range of values measured for random-coil peptides (Blanco et al. 1994). In our study, only three out of 23 measured residues show values >-8 ppb K^{-1} , which indicates a good structuration of the peptide in TFE and suggests the presence of secondary structures protecting the amide protons from external water molecules. However, these values are globally higher than those typically found in helical structures protected from solvent. These results are consistent with the fact that helix H1 has been shown to undergo hydrogen-deuterium exchange more easily than the central portions of helices H2 and H3 (Liu et al. 1999b), suggesting a looser, less compact structure that might unfold more readily.

All distance restraints used in the structure calculation were derived from NOEs observed at 278 K in TFE/ H_2O buffer during NOESY experiments at a 250-msec mixing time. The NOE pattern observed was typical of a helical structure (Fig. 4A). Extensive unambiguous medium-range NOEs $\alpha\text{N}(i, i+3)$ and NOEs $\alpha\beta(i, i+3)$ (Fig. 2) were observed for residues 3–14 (residues 144–155 in human numbering) and indicated helix conformation for this region.

Structure description

A 3D structure of the n3 peptide was obtained following the molecular dynamic protocol described in the Materials and Methods section using a final set of 252 NOE-derived distance constraints (153 sequential, 97 medium-range, and two long-range ($i-j \geq 5$) residues, Table 3) at 278 K. No intraresidual distance constraints or hydrogen bonds were included in the calculations. Thirteen dihedral angle constraints, deduced from $^3\text{J}\text{-H}\alpha\text{HN}$ coupling constants as described in the Materials and Methods section, were imposed and coupling constants were directly used as constraints.

After minimization, 20 structures based on low residual distance and dihedral angle violations and lower overall energies were selected to compute the solution structure of the n3 peptide. A ribbon model of the mean structure is shown in Figure 5A, whereas the 20 final structures superimposed for the minimum backbone deviations between residues 3 and 15 are displayed in Figure 5B.

Structural statistics are presented in Table 3. The structural models fit the NMR data well, with less than one violation of NOE constraints per structure, and a maximal violation of 0.55 Å. The Ramachandran plot can be consid-

Figure 2. Expansion of the fingerprint region of a 500-MHz two-dimensional ^1H , ^1H -NOESY NMR spectrum of the n3 peptide in 87/13 (v/v) TFE/PB buffer at 310 K. Assignments of cross-peaks are denoted with the sequence number. The sequential assignment is shown from residues 142–157 (2–16 in peptide numbering). The unambiguous medium-range NOEs $\alpha\text{N}(i, i+3)$, characteristic of a helical conformation, are pointed with arrows.

Table 1. Proton chemical shifts assignments [δ in ppm from Sodium 3-triméthylsilyl (2,2,3,3- 2H_4) propionate, 278 K (pH 6.5), in an 87/13 (v/v) TFE/PB solution], amide signal shift temperature coefficients ($\Delta(\delta \text{ NH})/\Delta T$, in ppb K^{-1}), and $^3J\text{-H}\alpha\text{HN}$ scalar couplings constants (in Hz) of the n3 peptide

Residue		NH	C α H	C β H	Other	$\Delta(\delta \text{ NH})/\Delta T$	$^3J\text{H}\alpha\text{HN}$
1 Gly	(G142)		3.92				
2 Asn	(N143)	8.33	4.86	2.94	N γ H 7.26, 6.45	−2.8	7.4
3 Asp	(D144)	8.38	4.65	2.87		−5.3	3.9
4 Tyr	(Y145)	7.94	4.32	3.13	Ar2, 6H 7.12; Ar3,5H 6.87	−2.8	3.4
5 Glu	(E146)	8.16	4.12	2.18	C γ H 2.56	−6.1	3.1
6 Asp	(D147)	8.11	4.62	3.00, 2.95		−6.9	3.4
7 Arg	(R148)	7.83	4.07	1.89	C γ H 1.68, 1.61; C δ H 3.16; N ϵ H 7.05; N η H 6.51	−3.7	3.1
8 Tyr	(Y149)	7.97	4.24	3.09, 2.98	Ar2,6H 6.99; Ar3,5H 6.82	−7.9	3.1
9 Tyr	(Y150)	8.15	4.31	3.20	Ar2,6H 7.18; Ar3,5H 6.88	−10.0	^a
10 Arg	(R151)	8.06	4.08	1.99, 1.89	C γ H 1.74; C δ H 3.26; N ϵ H 7.16; N η H 6.55	−8.2	2.8
11 Glu	(E152)	8.17	4.22	2.18	C γ H 2.62, 2.58	−8.1	3.7
12 Asn	(N153)	7.75	4.61	2.67	N γ H 6.98, 6.02	−1.6	4.9
13 Met	(M154)	7.82	4.25	1.96	C γ H 2.40; C ϵ H 2.03	−3.3	4.3
14 Tyr	(Y155)	7.57	4.53	3.01	Ar2,6H 7.13; Ar3,5H 6.84	−4.1	4.9
15 Arg	(R156)	7.48	4.30	1.70	C γ H 1.48; C δ H 3.15; N ϵ H 6.98; N η H 6.51	−2.3	7.4
16 Tyr	(Y157)	7.68	4.92	3.15, 2.96	Ar2,6H 7.17; Ar3,5H 6.85	−4.8	5.9
17 Pro	(P158)		4.89	2.31, 2.07	C γ H 2.05; C δ H 3.84, 3.62		
18 Asn	(N159)	7.91	4.69	2.88	N γ H 7.22, 6.42	−6.4	4.9
19 Gln	(Q160)	8.19	4.28	2.13	C γ H 2.42; N ϵ H 7.17, 6.42	−5.7	4.3
20 Val	(V161)	7.66	3.98	2.05	C γ H 0.89, 0.80	−5.0	6.5
21 Tyr	(Y162)	7.46	4.54	3.06, 2.89	Ar2,6H 7.06; Ar3,5H 6.83	−5.5	5.6
22 Tyr	(Y163)	7.52	4.56	3.03	Ar2,6H 7.10; Ar3,5H 6.83	−5.6	5.6
23 Arg	(R164)	7.39	4.68	1.86, 1.75	C γ H 1.67; C δ H 3.26, 3.20; N ϵ H 7.04; N η H 6.55	−4.4	6.8
24 Pro	(P165)		4.49	2.31, 2.07	C γ H 2.05; C δ H 3.64		
25 Val	(V166)	7.48	4.22	2.17	C γ H 1.01	−6.2	7.7
26 Cys		7.68	4.65	3.02, 2.10		−6.6	7.1

^a Not determined.

ered as satisfactory, with only 5% of residues outside of the allowed regions.

The total number of NOE constraints observed per residue is illustrated in Figure 6A. The reduced number of interresidue NOEs observed between P158 (residue 17) and Q160 (residue 19) suggests a break in structure between two well-structured regions. This is consistent with the values observed for the average local root-mean-square deviation (RMSD) per residue of the n3 peptide (backbone), shown in Figure 6B. This graphic highlights a higher mobility of residues 157 and 158 (16 and 17 in peptide numbering), according to the reduced NOEs number.

The RMSD to the mean structure for all backbone atoms is 1.5 ± 0.6 Å. This value drops to 0.3 ± 0.1 and 0.2 ± 0.1 if one only considers the residues of the 144–156 and 157–165 regions (3–15 and 18–24 in peptide numbering, respectively) (Fig. 7). This demonstrates that the structure of the n3 peptide is well defined for the 144–156 and the 157–165 regions, separated by a more flexible hinge, mainly corresponding to residues 157 and 158. The overall structure of the n3 peptide in TFE shows the absence of intermolecular contacts between segments H1 and S2. Superimposition of the structure of the n3 peptide in 87/13 (v/v) TFE/PB buffer

and of the corresponding fragment from the entire bovine protein (Fig. 8) shows how very similar the two structures of the residues implicated in helix H1 are.

Discussion

For the mean structure and the majority of the 20 best conformers, the Molmol software (Koradi et al. 1996) identifies an α -helix from residues 144 to 151, followed by a 3_{10} helix, from residues 152 to 155. The same secondary structures are obtained for these residues in the bovine prion protein (PDB code 1DX1; Lopez Garcia et al. 2000) and in the human prion protein at pH 7.0 (PDB code 1HJN; Calzolari and Zahn 2003). Interestingly, previous studies (Sharma et al. 1998; Ziegler et al. 2003) have shown that several synthetic prion peptides encompassing helix H1 and β -strand S2 were soluble in water solution only under acidic condition and precipitated at ($\pm$) neutral pH. Those peptides differ mostly from peptide n3 in the total electrostatic charge in the pH 6.5–7.5 range. The n3 peptide is highly soluble at aforementioned pH range, for which the total net charge of peptide n3 is 0.

Table 2. ^{13}C chemical shifts assignments of the n3 peptide (δ in ppm from DSS and TSPD₄) using ^1H - ^{13}C chemical shift correlation, 310 K (pH 6.5), in an 87/13 (v/v) TFE/PB solution

Residue		$^{13}\text{C}'$	$^{13}\text{C}^\alpha$	$^{13}\text{C}^\beta$	Other
1 Gly	(G142)	^a	44.2		
2 Asn	(N143)	173.2	53.8	39.4	
3 Asp	(D144)	174.6	54.8	39.2	
4 Tyr	(Y145)	175.6	57.5	39.0	C δ 133.6, C ϵ 118.9
5 Glu	(E146)	^a	59.2	29.0	C γ 33.6, C δ^a
6 Asp	(D147)	175.3	56.0	39.1	
7 Arg	(R148)	176.1	59.8	31.2	C γ 27.5, C δ 44.2, C ζ 133.7
8 Tyr	(Y149)	174.4	62.4	39.1	C δ 133.7, C ϵ 118.9
9 Tyr	(Y150)	179.9	61.4	39.1	C δ 133.8, C ϵ 118.9
10 Arg	(R151)	176.4	59.4	30.8	C γ 31.7, C δ 44.2, C ζ 133.8
11 Glu	(E152)	176.3	58.6	29.1	C γ 33.6, C δ^a
12 Asn	(N153)	^a	55.5	40.2	C γ 133.7
13 Met	(M154)	^a	57.9	33.4	C γ 133.7, C ϵ 17.0
14 Tyr	(Y155)	174.6	58.9	39.2	C δ 133.6, C ϵ 118.9
15 Arg	(R156)	^a	57.7	31.7	C γ 27.9, C δ 44.0, C ζ 133.7
16 Tyr	(Y157)	^a	56.9	39.1	C δ 133.8, C ϵ 118.9
17 Pro	(P158)	173.8	64.5	32.3	C γ 27.8, C δ 51.1
18 Asn	(N159)	173.7	54.6	39.1	C γ^a
19 Gln	(Q160)	173.5	57.9	29.6	C γ 34.7, C δ 133.8
20 Val	(V161)	174.3	64.7	32.8	C γ 21.1
21 Tyr	(Y162)	173.8	58.9	39.3	C δ 133.6, C ϵ 118.9
22 Tyr	(Y163)	173.1	59.0	39.2	C δ 133.7, C ϵ 118.9
23 Arg	(R164)	171.9	54.7	31.5	C γ 27.4, C δ 44.2, C ζ 133.7
24 Pro	(P165)	174.7	64.5	32.4	C γ 27.9, C δ 51.1
25 Val	(V166)	^a	62.8	34.0	C γ 21.1
26 Cys		^a	58.2	28.7	

^a Not determined.*Structural similarity of helix H1 segment in peptide n3 and in PrP^C*

Analysis of the structures obtained made it possible to identify the hydrogen bonds that appear to stabilize the peptide conformation. The H-bonding network was determined by using the CNS software (Brünger et al. 1998). Five backbone amide-carboxyl hydrogen bonds were found between the following amino acids: 143–147, 144–148, 146–150, 147–151, and 148–152 in the α -helix structure. Four were detected in the 3_{10} helix: 150–153, 151–154, 152–155, and 153–156. Therefore, the H-bonding network in the α -helical fragment of the n3 peptide is similar to that observed in helix H1 of native prion proteins. The C-terminal fragment of the peptide possesses an explicit but irregular conformation, which is characterized by two H-bonds between residues 154–159 and 159–162.

The particular role of charged residues in the stabilization of helix H1 in both the peptide fragment in TFE and the whole prion protein PrP^C is also to be elucidated. As helix H1 is the most hydrophilic helix in all the known protein structures, such hydrophilicity implies that intermolecular electrostatic interactions play a significant role in stabilizing the structure of helix H1 (Morrissey and Shakhnovich 1999). Interestingly, another common structural feature of

the α -helical region found in the peptide fragment studied in TFE and the known structures of prion protein PrP^C is the relative distance between charged groups of selected side chains for residue pairs: 147–151, 148–152, and 152–156 (Table 4). Such positions provide one with the possibility of producing a network of salt bridges in helix H1, as was previously assumed (Morrissey and Shakhnovich 1999).

Effect of solvent on peptide conformation

The peptide conformations in TFE and in phosphate buffer solution are very different. The main difference concerns the structure of segment H1, which, in water solution, adopts an extended conformation stabilized by specific intramolecular contacts with amino acid residues of region S2. These interactions were shown to be very specific and stable (Kozin et al. 2001). However, they are disrupted in the presence of TFE. The way in which the medium surrounding a polypeptide chain affects its structure is still poorly understood (Chitra and Smith 2001). However, TFE has a lower dielectric constant than water, suggesting that electrostatic interactions will be strengthened in a TFE/water mixture. In this study, it appears that, contrary to phosphate buffer, the TFE medium simulates the protein envi-

Figure 3. Deviation of chemical shift values ($\Delta\delta$, in ppm), determined for the peptide in TFE, from those characteristic of the random coil, derived (A) from $^1\text{H}^\alpha$ chemical shifts, and (B) from $^{13}\text{C}^\alpha$ (black), $^{13}\text{C}^\beta$ (gray), and $^{13}\text{C}'$ (white) chemical shifts. Sequence-dependent corrections were added. Any group of three or more consequent residues is considered to form a helical structure, if for each residue within the group corresponding, $\Delta\delta$ $^1\text{H}^\alpha$ and $\Delta\delta$ $^{13}\text{C}^\beta$ are negative, whereas $^{13}\text{C}^\alpha$ and $^{13}\text{C}'$ are positive. The significant values indicative of a helix conformation are shown within the shadowed box.

ronment in which the segment corresponding to helix H1 adopts its native conformation. Hence, the conformational behavior of the n3 peptide is reminiscent of the $\alpha \rightarrow \beta$ prion structural conversion.

Structural duality of peptide n3

Earlier (Liu et al. 1999a), it was found that the synthetic hexadecapeptide mPrP(143–158) encompassing prion segment H1 showed significant intrinsic helical propensity in both H_2O and a 1:1 mixture of H_2O and TFE. Our results are in keeping with the fact that, in the absence of contacts with other prion segments like L3 or S2, the H1 segment always adopts its native-like helical conformation.

Wüthrich (Riek et al. 1996; Korth et al. 1997) and Prusiner (2001) have proposed prion conversion models in which helix H1 underwent transconformation into extended β -sheet structure upon intra- or/and intermolecular interactions with a preexisting β -sheet (strands S1 and S2). The conformation behavior of peptide n3 reported here and in our previous study (Kozin et al. 2001) suggests that peptide n3 could be a useful model system to work for a better

understanding of the $\alpha \rightarrow \beta$ conformational transition occurring in the prion protein.

Implication of the role of the 152–156 region in peptide structural rearrangement

A particular feature of the n3 peptide is the salt bridge E152–R156 found in water. The interaction between E152 and R156, which could thus increase both the length and the stability of helix H1, does not exist in PrP and in peptide n3 in TFE. Further inspection of the structure of the n3 peptide in TFE shows that the aromatic ring of Y155 resides at the end of helix H1, between the side chains of E152 and R156 (Fig. 9). These data suggest that the tyrosine at position 155 may act as a barrier to the E152–R156 salt bridge (Table 4). However, this ionic interaction has only been observed in the structure of the n3 peptide in PB buffer solution (Kozin et al. 2001).

The absence of the E152–R156 salt bridge is also observed in all the known structures of PrP (Riek et al. 1996; Liu et al. 1999b; Lopez Garcia et al. 2000; Zahn et al. 2000). The nature of the residue at position 155, which is a tyrosine for sheep and mice, an asparagine for the Syrian hamster, and a histidine for humans and bovine, could play a par-

Figure 4. (A) The observed sequential and medium-range NOE connectivities are indicated by lines connecting the two residues that are related by the NOE. Thick and medium bars indicate strong and medium NOE intensities, respectively. Thin bars indicate the weak and very weak NOE intensities. (B) The $^3\text{J-H}\alpha\text{HN}$ couplings are indicated by squares. Filled squares identify residues with $^3\text{J-H}\alpha\text{HN} < 4$ Hz, and 2/3 filled squares correspond to $^3\text{J-H}\alpha\text{HN}$ between 4 Hz and 5 Hz. This indicates a local α -type conformation from residues 144–155 (3–14 in peptide numbering). The 1/3 filled squares identify residues with $^3\text{J-H}\alpha\text{HN}$ between 5 Hz and 6 Hz, and the open squares correspond to $^3\text{J-H}\alpha\text{HN} > 6$ Hz. (C) The $\Delta(\delta\text{NH})/\Delta T$ are represented by circles. The filled circles identify residues with $\Delta(\delta\text{NH})/\Delta T > -5$ ppb/K. The half-filled circles represent residues with $\Delta(\delta\text{NH})/\Delta T$ between -5 and -8 ppb/K. The open circles correspond to $\Delta(\delta\text{NH})/\Delta T < -8$ ppb/K.

Table 3. NMR restraints and structural statistics for the 20 top-ranked peptide n3 conformers (obtained by simulated annealing) in an 87/13 (v/v) TFE/PB solution

NOE restraints		
Total restraints	252	
Sequential	153	
Medium range ($i - j < 5$)	97	
Long range ($i - j \geq 5$)	2	
NOE constraints per residue	9.7	
NOE violations		
Maximum individual violation (Å)	0.551	
Average number of violations (≥ 0.5 Å) per structure	0.5	
Other restraints		
Number of ^3J H α HN coupling constants	22	
Number of H α CSI constraints	25	
Number of C α and C β CSI constraints	25	
Ramachandran analysis		
Residues in most favored regions (%)	63.2	
Residues in additional allowed regions (%)	16.1	
Residues in generously allowed regions (%)	15.7	
Residues in disallowed regions (%)	5.0	
Global rmsd to a mean structure		
All backbone atoms (Å)	1.5 $\pm$ 0.6	
All nonhydrogen atoms (Å)	2.2 $\pm$ 0.8	
Local rmsd to a mean structure (res. 144–156 and 159–165, respectively)		
All backbone atoms (Å)	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1
All nonhydrogen atoms (Å)	1.3 $\pm$ 0.3	0.9 $\pm$ 0.3

ticular role relative to the possible formation of the E152–R156 salt bridge in PrP^{Sc}.

Interestingly, Priola (Priola et al. 2001) has found that mutation at position 155 strongly influences the rate of conversion from PrP^C to PrP^{Sc}. In the hamster sequence, introduction of a tyrosine instead of an asparagine at position 155 (154 in hamster numbering) significantly reduced the formation of protease-resistant PrP. This is consistent with

Figure 5. Ribbon models of the n3 peptide structure in 87/13 (v/v) TFE/PB buffer. (A) Ribbon model of the mean molecule calculated from the 20 best structures. (B) Superimposition of the 20 best structures calculated. The helical part of the backbone is shown in red. The corresponding side chains are shown in blue. **Figure 6.** (A) Number of NOE restraints per residue (intrasidual NOEs were neglected). (B) Backbone heavy atom local rmsd values per residue for the family of 20 structures relative to the average structure. (C) The position of the helix is indicated at the bottom as a filled bar.

the fact that Y155 may block the possibility of a stabilization of helix H1 by the E152–R156 salt bridge (Fig. 9).

Moreover, in human prion protein, the protonation of H155 and H187 presumably contributes to these structural changes (Calzolari and Zahn 2003). In view of our results,

Figure 7. Views of the backbone (N, C α , C') atoms of the 20 best structures of the n3 peptide and superimpositions of structures for best fit on backbone atoms. Fit (A) for all residues; (B) for residues 144–156 (3–15 in peptide numbering); (C) for residues 159–165 (18–24 in peptide numbering).

Figure 8. Superimposition of the structure of the n3 peptide in 87/13 (v/v) TFE/PB buffer (in green) and of the corresponding fragment from the entire bovine protein (in gray and blue). The extended C-terminal part of the bovine protein is strand S2 (stabilized by strand S1 in the globular domain of the PrP^C). The two structures are fitted from residue 142 to residue 155.

histidine 155 could act as a possible “pH-dependent switch” to prion conversion.

Thus, the nature of the residue located at position 155 may play a key role in stabilizing helix H1. Interestingly, this residue may be involved in the TSE species barrier (Billeter et al. 1997; Priola et al. 2001).

Several mutations of residues implicated in putative salt bridges thought to stabilize helix H1 have been reported (Speare et al. 2003; Ziegler et al. 2003). Only a small local destabilization of helix H1 in the D147A and E152A mutants of human prion was observed, which implies the existence of a helix-stabilizing interaction in the wild-type peptide (Ziegler et al. 2003).

In conclusion, this study highlights the structural duality of the n3 peptide, which had been previously assumed (Kozin et al. 2001). Peptide n3 adopts in TFE/PB mixture a well-defined helical conformation, which is stabilized by a specific network of H-bonds. The measured distances between the charged groups of residues such as D147–R151, R148–E152, and E152–R156 are also very different from those observed in PB buffer.

Figure 9. Schematic model of the ionic interaction (gray arrows) involved in the structure of peptide n3: On the *left*, peptide in TFE showing Y155 located at the end of helix H1, between the side chains of E152 and R156 and probably acting as a barrier to the E152–R156 salt bridge. On the *right*, ionic interactions observed in PB buffer solution. The gray arrows indicate distances <3.5 Å as shown in Table 4. Thus, the nature of the residue located in position 155 could play a role in the stability of helix H1.

Another potential application of the n3 peptide has been recently suggested. The peptide n3 could serve as a template to develop an inhibitor to the formation of PrP^{Sc} (Pato et al. 2004).

According to these results, other mutants in the 152–156 region, particularly at position 155 could now be considered.

Materials and methods

Peptide synthesis and purification

The n3 peptide was synthesized and purified as previously described (Kozin et al. 2001). Mass spectrometry and amino acid analysis were performed to check the peptide purity and verify its sequence. The purity exceeded 99%.

Circular dichroism (CD) spectroscopy

CD spectra of the 150 μM n3 peptide for PB buffer and various concentrations of TFE samples (0%, 10%, 20%, 30%, 40%, 80%, and 96% expressed in v/v) were measured at 298 K using a JASCO J-710 instrument. Samples were studied in quartz cells with path lengths of 0.5 mm or 1 mm, following the protocol previously described (Kozin et al. 2001).

Nuclear magnetic resonance (NMR) spectroscopy

NMR samples were dissolved in TFE-*d*2OH/“PB” buffer (10 mM sodium phosphate buffer [pH 6.5]) 87:13 (v/v). A crystal of

Table 4. Comparison of distances (Å) between charged groups of selected side chains inside peptide segment corresponding to 142–166 human prion protein sequence

Residues	Peptide n3		PrP ^{Ca}
	in TFE (mostly helical conformation)	in PB buffer (mostly β-hairpin-like conformation)	
R148–E152 (Arg7–Glu11)	6.5	13.4	5.7
E152–R156 (Glu11–Arg15)	9.5	2.7	9.6
D147–R151 (Asp6–Arg10)	6.5	3.3	6.8

^a Example given for the bovine PrP^C (Lopez Garcia et al. 2000).

TSPD4, 3-(trimethylsilyl)[2,2,3,3-d₄] propionic acid, sodium salt, was used as internal reference for the proton shifts. The experiments were run at 500.13 MHz for ¹H on a Bruker AMX 500 spectrometer equipped with a Silicon Graphics workstation. The WATERGATE method (Piotto et al. 1992) was used in all experiments to eliminate the water signal rather than the presaturation method. 1D, 2D-TOCSY, 2D-ROESY, and 2D-NOESY spectra were recorded at several temperatures within the 278–310 K range. Mixing times of 35–70 msec were used for 2D-TOCSY. For 2D-ROESY experiments, a spin-lock of 200–400 msec was used. 2D phase-sensitive NOESY experiments were carried out using the States-TPPI method with a mixing time in the 50–800 msec range. The ³J-H α HN scalar coupling constants were measured in 1D spectrum and extracted from 2D-TOCSY. The ¹³C NMR chemical shifts were carefully calibrated using DSS (4,4-dimethyl 4-silapentane sodium sulfonate) and TSPD4. The assignments of ¹³C were made at 310 K using two ¹H-¹³C Chemical Shift Correlation spectra: PFG-HSQC (Pulse Field Gradient-Heteronuclear Single Quantum Correlation) phase-sensitive using sensitive enhancement (Hurd and John 1991), and PFG-HMBC (Pulse Field Gradient-Heteronuclear Multiple Bond Correlation) (Bax and Summers 1986).

Structural calculations and data deposition

Model structures were calculated by simulated annealing (SA) using torsion angle dynamics as implemented in the program CNS (Brünger et al. 1998). Calculations were performed on a Silicon Graphics Indigo² workstation. Distance constraints were derived from cross-peaks in NOESY spectra recorded at 500 MHz and 278 K (solvent: 87% TFE, 13% PB) with a mixing time of 250 msec. The NOE cross-peaks classified as strong, medium, weak, and very weak were converted into 252 interresidual distance restraints of 1.8–2.5 Å, 1.8–3.5 Å, 1.8–4.5 Å, and 1.8–5.5 Å, respectively. Appropriate pseudotom corrections were applied to non-stereospecifically assigned protons (Wüthrich 1986). Several rounds of structure calculations and assignments were performed to resolve ambiguities. ³J-H α HN coupling constants were used directly as constraints. Backbone dihedral restraints for ϕ angle were used as $-60 \pm 30^\circ$ for the 13 residues presenting a ³J-H α HN value less than 5 Hz. Chemical shift index of H α , C α , and C β , were calculated and modified for all residues but the N terminus Gly, applying some sequence-dependent corrections (Schwarzinger et al. 2001) and used directly as constraints.

Finally, the 20 best-minimized models with the lowest overall energies obtained with the standard CNS simulated annealing protocol were retained for analysis. Structures were displayed with the Molmol program (Koradi et al. 1996) and evaluated using Procheck-NMR (Laskowski et al. 1996). The atomic coordinates have been deposited in the Protein Data Bank (available at <http://www.rcsb.org>) (PDB code 1M25). Proton chemical shifts table and the ³J-H α HN scalar coupling constants of the n3 peptide have been deposited with the BioMagResBank (<http://www.bmrb.wisc.edu>) (code BMRB-5405).

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