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Effect of salts on the structural behavior of hPrP α2-helix-derived analogues: the counterion perspective

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Abstract: Both theoretical studies and direct experimental evidence have emphasized the importance of electrostatic interactions in the general phenomenon of spontaneous amyloid fibril formation. A number of observations have recently spurred interest in the contribution of these interactions to the conformational behavior of the prion protein. In this paper, we show how salt addition and pH change can modify the conformation of two peptide analogues derived from the human prion protein helix 2 according to a Hofmeister-series-type dependence. Employment of various sodium salts allowed us to highlight the fact that chaotropic anions favor unstructured conformation, whereas kosmotropic anions promote the formation of compact structures like α-helix and β-sheet, which may ultimately facilitate fibril formation. This finding should warn people engaged in ion-based research on prion and derived peptides about cation-bound effects, which have been almost exclusively investigated to date, being easily confounded with modifications that are actually caused by anion activity, thus leading researchers into misunderstand ion-specific effects. To avoid the common complication of ion confounding, it is highly desirable that experiments be designed so that the species causing the modification can be unequivocally perceived. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: amyloid; prion structure; transmissible spongiform encephalopathies; prion helix 2 derived peptides; Hofmeister series; anions; cations; counterions

INTRODUCTION

The unfolding of the cellular prion protein (PrP^C) and its refolding to the amyloid fibril prone scrapie isoform (PrP^{Sc}) are related to prion diseases [1]. Actually, the presence of proteinaceous amyloid deposits in organs and tissues, which may cause cell death and tissue damage, is common to all amyloid disorders. Thus, amyloid formation is viewed as a generic property of the polypeptide chain, with no relation to sequence or structural similarity. In fact, under appropriate conditions any protein can form amyloid fibrils, in which the monomeric unit is characterized by a common cross-β-sheet backbone [2]. It is therefore apparent that understanding the general phenomenon of spontaneous amyloid assembly, both *in vivo* and *in vitro*, is of fundamental importance. Knowledge of the molecular basis of neurodegenerative diseases provides the foundation for rational structure-based drug design, aiming at the early diagnosis and protective treatment of amyloid disorders. Along this route, the behavior of the full-length prion protein and its fragments has been thoroughly investigated at different pHs and in the presence of denaturants [3–10]. A number of observations have recently spurred interest in the contribution of electrostatic interactions to the conformational behavior of the prion protein. Both theoretical studies [11] and direct experimental evidence [12] have emphasized the importance of electrostatic interactions in the general phenomenon of spontaneous amyloid fibril formation. It has even been shown that ionic solutes play a role in the conversion of PrP^C into PrP^{Sc} and its subsequent oligomerization [13]. Recently, it has been found that an increase in salt concentration destabilizes the structure of the full-length prion protein, but is ineffective in perturbing the *N*-terminus truncated form [14]. The authors suggested that this different behavior could depend on the interaction between the *N*-terminal region and the protein globular core, hypothesizing the involvement of helix 1. In reality, this helix is stabilized by intrinsic electrostatic interactions and behaves as an autonomous structural unit [11]. Because of its high stability, it could even contribute to a barrier that prevents the spontaneous conversion of PrP^C to PrP^{Sc} isoform [15]. On the other hand, we have previously reported that the fragment corresponding to helix 2 of the human prion protein, hPrP[173–195], displays conformational ambiguity [16]. This led us to agree with others that full-length helix 2 could be involved in the nucleation process of prion misfolding

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and oligomerization [16,17]. On this ground, we were prompted to investigate whether and how the nature of the salt employed to adjust the ionic strength may affect the conformational behavior of this fragment. Contrary to recent studies on cation binding to PrP protein [18–21], we focus on the usually unexplored anion effect.

MATERIALS AND METHODS

Peptide Synthesis and Characterization

The acetylated-*N*-terminus and amidated-*C*-terminus peptides with sequences AcVNITIKQHTVTTTTKGNH$_2$ and AcVNITIKQATVTTTTKGNH$_2$, henceforth identified as hPrP[180–195] and hPrP[H187A], respectively, were synthesized in batch by Fmoc standard chemistry protocol on Rink-amide 4-methylbenzhydrylamine (MBHA) resins. After peptide assembling, acetylation was carried out by 1 M acetic anhydride in dimethylformamide containing 5% diisopropylethylamine. Cleavage from the solid support was performed by treatment with a trifluoroacetic acid (TFA)/triisopropylsilane/water (90 : 5 : 5, v/v/v) mixture for 90 min at room temperature. The peptides were then precipitated in cold diethyl ether, dissolved in water/acetonitrile (1 : 1, v/v) mixture, lyophilized, and purified by RP-HPLC using a C18 Jupiter (250 × 22 mm) column applying a linear gradient of 0.1% TFA/acetonitrile in 0.1% TFA/water. Peptide purity and integrity were confirmed by RP-HPLC analysis and by MALDI-TOF mass measurements (Voyager-DE Biospectrometry Workstation, PerSeptive Biosystems).

Chemicals

All solvents were reagent grade. HPLC chemicals were purchased from Lab-Scan (Dublin, Ireland), and the other organic reagents were from Sigma–Aldrich (Milan, Italy). Analytical grade sodium salts were obtained from Carlo Erba (Milan, Italy). N$^{\alpha}$-Fmoc-protected amino acids and activating agents were purchased from Inbios (Pozzuoli, Italy). Resin for peptide synthesis was from Novabiochem (Läufelfingen, Switzerland). Columns for peptide purification and characterization were from Phenomenex (Torrance, CA, USA).

CD Spectroscopy

All working solutions were prepared in Milli-Q water, adjusting the pH by using small amounts of 0.1 M HCl or NaOH. Spectropolarimetric titrations as a function of salt concentration were carried out at 20 °C on a Jasco J-810 spectropolarimeter equipped with a thermostated water bath, adding small aliquots from a 200 mM salt solution to 3 μM peptide samples in 1-cm path length quartz cells. After each addition, spectra were acquired from 260 to 190 nm, using 20 nm min^{-1} scan speed, 1.0 nm bandwidth, 0.2 nm resolution, 50 mdeg sensitivity, and 4 s response. Final spectra were obtained after averaging three scans, subtracting the contributions from salts, and converting the signal to mean residue ellipticity in units of deg cm^2 dmol^{-1} res^{-1}. Concentration-dependent experiments were performed at 20 °C by keeping the product of protein concentration and optical path length constant, which ensured that the number of molecules in the optical path was constant. In thermal experiments, hPrP[H187A] dissolved in 20 mM Na_2SO_4 at pH 4.5 was heated from 20 to 80 °C at the rate of 1 °C min^{-1} and recooled at the same rate. In time-dependent experiments, different amounts of hPrP[H187A] were dissolved in 50 mM Na_2SO_4 at pH 7.0.

RESULTS

As shown in Figure 1(A), in the absence of any added salt, the shape of the circular dichroism (CD) spectra of both peptides was suggestive of an unordered structure. Neither the addition of NaCl or $NaClO_4$ nor the addition of small amounts of HCl or NaOH to adjust the pH caused any substantial modification. Spectra in sodium phosphate solution at pH 4.5, in which $H_2PO_4^-$ is the most populated anion, were also reminiscent of disordered conformation. By contrast, on addition of either sodium phosphate or sulfate at pH 7.0, where bivalent HPO_4^{2-} or SO_4^{2-} anions are dominant, the spectrum of hPrP[180–195] was characterized by a pronounced negative maximum around 220 nm and a positive maximum around 200 nm, which are characteristic of β-sheet conformation (dashed lines in Figure 1(B) and (C), respectively), whereas hPrP[H187A] exhibited spectral features typical of the α-helix conformation, with a positive maximum around 195 nm and two negative maxima around 208 nm and 222 nm (bold lines in Figure 1(B) and (C), respectively). Finally, spectra of hPrP[H187A] and hPrP[180–195] were modified to those typical of the β-sheet and disordered structure, respectively, by acidifying the Na_2SO_4 solution to pH 4.5 (Figure 1(D)). Table 1 summarizes the above described CD effects.

The thermodynamic processing of these salt-induced structural changes is outside the scope of the present paper. Nevertheless, it is worth noting that they show features typical of most structural transitions and are therefore suited to be treated by some thermodynamic model that hinges on the structuring effect of the anion activity. For example, the isodichroic point that can be noticed around 207 nm in the spectra of hPrP[H187A] at neutral pH (see inset of Figure 1(C)) suggests a two-state equilibrium between the unstructured peptide and the structured peptide, whose onset is accompanied by a sharp ellipticity change around 35–40 mM of salt (Figure 2). Notably, hPrP[H187A] in Na_2SO_4 at acidic pH assumes a β-sheet-like conformation, which is irreversibly destroyed by heating, with an apparent midpoint temperature (T_m) of about 47 °C.

Concentration-dependent experiments performed in the 6–300 μM range show that the peptide behavior on salt addition is more complex than that described

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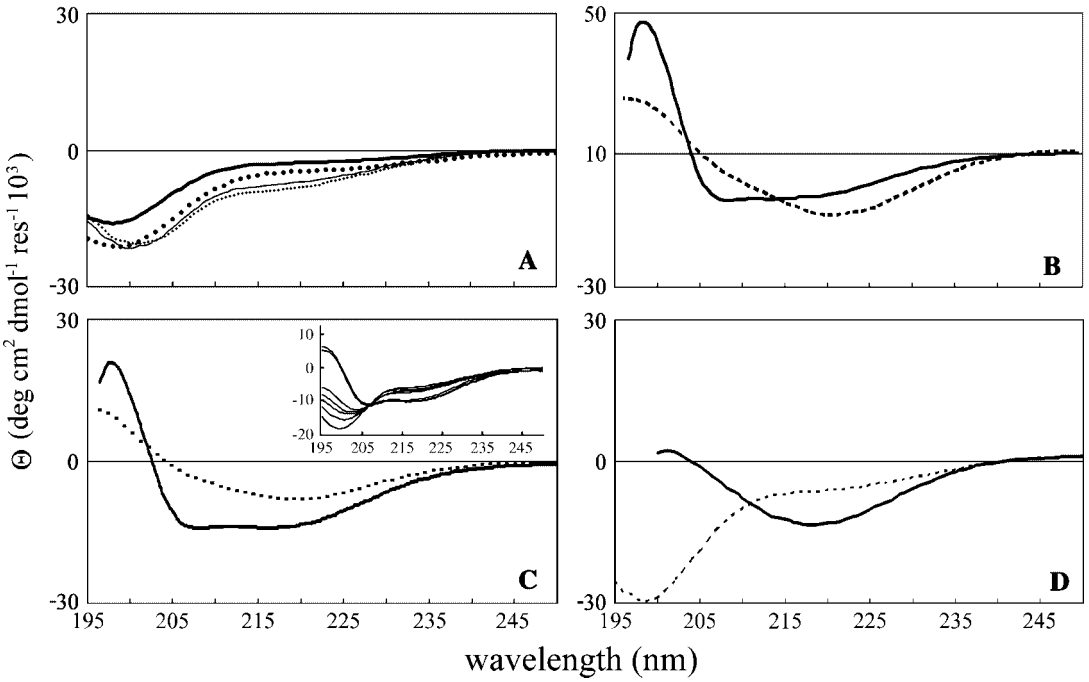

Figure 1 Effect of salts on circular dichroism (CD) spectra of hPrP[180–195] and hPRP[H187A]. (A) hPRP[H187A] dissolved in H_2O (bold line), 100 mM NaCl (thin line), 100 mM $NaClO_4$ (dotted line), and 30 mM NaH_2PO_4 (dashed line). CD spectra of hPrP[180–195] under the same conditions (not shown) were superimposable on those of hPRP[H187A]. (B) hPrP[180–195] (dashed line) and hPRP[H187A] (bold line) dissolved in 30 mM NaH_2PO_4/Na_2HPO_4 (pH 7.0). (C) hPrP[180–195] (dashed line) and hPRP[H187A] (bold line) dissolved in 50 mM Na_2SO_4 (pH 7.0); the inset shows spectra of hPRP[H187A] as obtained by titration with Na_2SO_4 up to 50 mM (pH 7.0). (D) hPrP[180–195] (dashed line) and hPRP[H187A] (bold line) dissolved in 50 mM Na_2SO_4 (pH 4.5).

Table 1 Effect of anions and pH on peptide conformation

Medium	C (mM)	pH	hPrP [180–195]	hPrP [H187A]
Water	—	4.5–7.0	Disordered	
Cl^-	0–100	4.5–7.0	Disordered	
ClO_4^-	0–100	4.5–7.0	Disordered	
$H_2PO_4^-$	0–50	4.5	Disordered	
$HPO_4^{2-}/H_2PO_4^-$	0–50	7.0	β-strand	α-helix
SO_4^{2-}	0–50	7.0	β-strand	α-helix
SO_4^{2-}	0–50	4.5	Disordered	β-strand

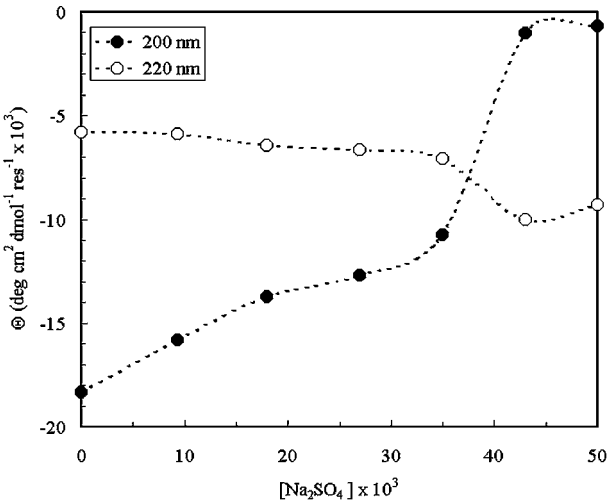

Figure 2 Effect of Na_2SO_4 on the ellipticity values of hPrP[H187A] at pH 7.0. The peptide concentration was 3 μM.

above. As can be appreciated from Figure 3, the observed ellipticity shows a hyperbolic trend in 50 mM Na_2SO_4, whereas it is expected to depend linearly on the peptide concentration, according to the Beer–Lambert's law. Such a deviation from linearity can be interpreted in terms of structural rearrangements that lead to self-association. Furthermore, pseudo first order rate constants show an anomalous increase with the peptide concentration. This suggests a kinetic behavior different from the first order expected for a simple conformational rearrangement involving monomers (Figure 4). We infer therefore that both peptides self-associate. At least in the case of hPrP[H187A] it is apparent that this follows secondary structure rearrangements because it occurs in the presence of 50 mM Na_2SO_4, which is higher than the salt concentration at which structural modifications were observed (Figure 2). In general, it cannot be excluded that structural reorganization and self-association are to some extent concomitant.

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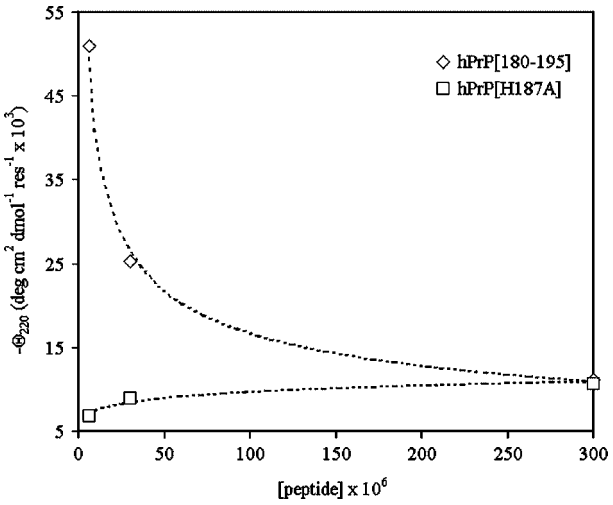

Figure 3 Peptide self-association at pH 7.0. Concentration-dependent experiments were performed at 20 °C in 50 mM Na_2SO_4 maintaining the number of molecules in the optical path constant (equimolecular condition). This was achieved by keeping the product of the protein concentration and optical path length constant.

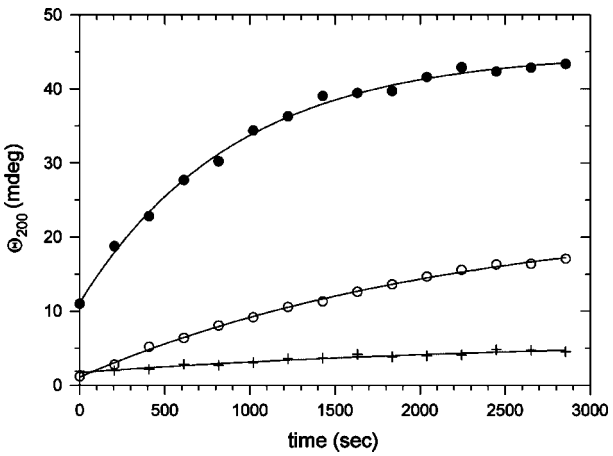

Figure 4 Time-dependence of hPrP[H187A] reorganization at pH 7.0. Experiments were performed at 20 °C on 2.5 μM (+), 5.0 μM (○), and 10 μM (●) peptide dissolved in 50 mM Na_2SO_4, monitoring the dichroic signal at 200 nm. Rate constants were calculated by best fitting to a pseudo first order kinetic equation, which gave 3.8×10^{-4}, 4.5×10^{-4}, and 11.0×10^{-4} s for 2.5, 5.0, and 10 μM peptide, respectively.

DISCUSSION

Environmental conditions, like pH, salts, and presence of nucleic acids or glycosaminoglycans, seem to affect the structural stability of prion proteins to a much larger extent than other proteins [9,14,22–26]. It was suggested that this unusual behavior could be ascribed to the ability of PrP^C *N*-terminal region to interact with anions, which leads to destabilization of the prion core structure [14]. Also, in an extensive analysis on the interaction of anti-prion compounds and amyloid-binding dyes with a carboxy-terminal domain of prion protein, it has been found that sulfonates, like Congo red and phthalocyanine tetrasulfonate, bind with high affinity [27].

Here, we have shown that the conformational properties of two PrP^C helix 2-derived analogues, hPrP[180–195] and hPrP[H187A], are affected by anion identity. Such large differences in the CD spectra of both peptides with anion identity can be rationalized via the ion charge density dependence that is typical of Hofmeister effects [28,29] (Figure 5). Anions like Cl^-, ClO_4^-, and $H_2PO_4^-$ are weakly hydrated because of their low charge density, and their interaction with water molecules is weaker than that of water with itself. This causes them to behave as water structure breakers (chaotropes), which make the bulk solution a better solvent. As a consequence, both hPrP[180–195] and hPrP[H187A] maximize their solvent accessible surface area, favoring the formation of the unstructured conformation. On the other hand, multiply charged ions, like SO_4^{2-} and HPO_4^{2-}, exhibit stronger interactions with water molecules than water with itself because of their high charge density. These ions are water structure makers (kosmotropes) and make the bulk solution a poorer solvent. Thus, they encourage hPrP[180–195] and hPrP[H187A] to

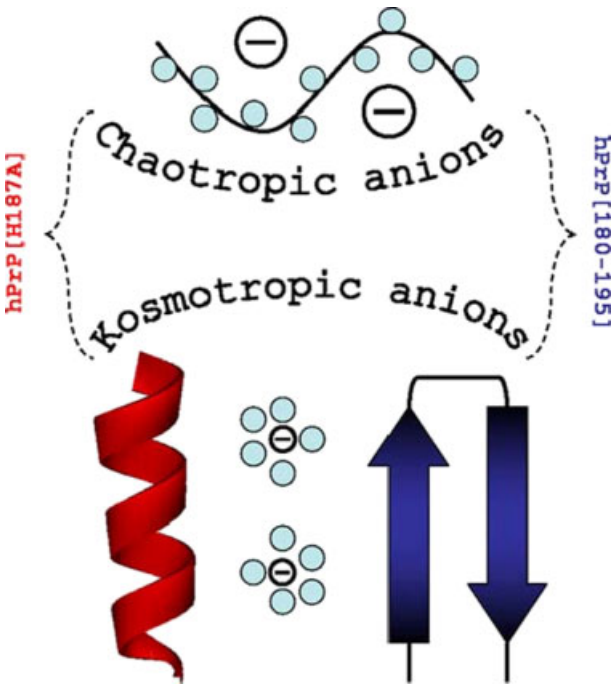

Figure 5 Weakly hydrated chaotropic anions (top) maximize the solvent accessible surface area and favor the unordered structure in both hPrP[H187A] and hPrP[180–195]. Strongly hydrated kosmotropic anions (bottom) make bulk water a poorer solvent and cause hPrP[H187A] and hPrP[180–195] to fold to α-helix and β-structure, respectively, to minimize their solvent accessible surface area.

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minimize their solvent accessible surface area and assume β-sheet-like and α-helix-like conformations, respectively. It is likely that the compactness of these structures predisposes both peptides to self-association (Figure 3), which may be a preliminary step toward fibril formation. This is also suggested by preliminary heat irreversible denaturation experiments on hPrP[H187A], which assumes a β-sheet-like conformation when dissolved in Na_2SO_4 at acidic pH.

The different conformational behavior of the wild type peptide as compared to the mutant peptide in the presence of kosmotropic anions at neutral pH is likely caused by the His side chain, which displays an α-inducing ability lower than the Ala side chain. Nevertheless, in the prion protein, the segment containing the His^{187} residue is still able to retain an α-helical conformation owing to tertiary interactions [16]. Furthermore, in acidic solution, the His protonation and increased proton exchange [25] could play a role in causing hPrP[180–195] and hPrP[H187A] to assume disordered structure and β-conformation, respectively. Also, our results confirm the chameleonlike character of the helix 2 domain [16], suggesting that preferential binding with naturally occurring anions, rather than nonspecific interactions, such as ionic strength-dependent interactions, plays an important role in prion protein misfolding and amyloid fibril growth.

Understanding ion-specific effects is a central theme of biology. Unfortunately, the complication of 'ion confounding' is extremely common in all disciplines concerned with ion-based research because ions are generally manipulated through the use of salts. It occurs because changing the concentration of a single cation or anion using a single salt results in a simultaneous change of the associated co-ion, which causes the main effect associated with that ion to be confounded with the effects caused by changing the concentration of the co-ion [30]. It is therefore worth stressing that, even in studies on prion and derived peptides, anion-bound effects may overlap the largely explored cation-bound effects. Unfortunately, experiments are not always designed so that the species that cause the modification can be unequivocally perceived. In conclusion, we highlight that the sensitivity of our peptides, as well as the entire prion proteins [18–21] or other amyloidogenic systems [31], to environmental modifications suggests that the complication of anion involvement cannot be neglected anymore, either in investigating metal effects on peptide conformation [18–21,31] or in checking the inhibition of amyloid formation by unusual agents [32,33].

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REFERENCES

1. Carrel RW, Lomas DA. Conformational disease. *Lancet* 1997; **350**: 134–138.
2. Temussi PA, Masino L, Pastore A. From Alzheimer to Huntington: why is a structural understanding so difficult? *EMBO J.* 2003; **22**: 355–361.
3. Swietnicki W, Petersen RB, Gambetti P, Surewicz WK. pH-Dependent stability and conformation of the recombinant human prion protein PrP(90–231). *J. Biol. Chem.* 1997; **272**: 27517–27520.
4. Swietnicki W, Morillas M, Chen SG, Gambetti P, Surewicz WK. Aggregation and fibrillization of the recombinant human prion protein huPrP90-231. *Biochemistry* 2000; **39**: 424–431.
5. Liemann S, Glockshuber R. Influence of amino acid substitutions related to inherited human prion diseases on the thermodynamic stability of the cellular prion protein. *Biochemistry* 1999; **38**: 3258–3267.
6. Hornemann S, Glockshuber R. A scrapie-like unfolding intermediate of the prion protein domain PrP(121–231) induced by acidic pH. *Proc. Natl. Acad. Sci. U.S.A.* 1998; **95**: 6010–6014.
7. Wildegger G, Liemann S, Glockshuber R. Extremely rapid folding of the C-terminal domain of the prion protein without kinetic intermediates. *Nat. Struct. Biol.* 1999; **6**: 550–553.
8. Rezaei H, Marc D, Choiset Y, Takahashi M, Hoa GHB, Haertle T, Grosclaude J, Debey P. High yield purification and physicochemical properties of full-length recombinant allelic variants of sheep prion protein linked to scrapie susceptibility. *Eur. J. Biochem.* 2000; **267**: 2833–2839.
9. Zanusso G, Farinazzo A, Fiorini M, Gelati M, Castagna A, Righetti PG, Rizzuto N, Monaco S. pH-Dependent prion protein conformation in classical Creutzfeldt-Jakob disease. *J. Biol. Chem.* 2001; **276**: 40 377–40 380.
10. Baskakov IV, Legname G, Prusiner SB, Cohen FE. Folding of prion protein to its native α-helical conformation is under kinetic control. *J. Biol. Chem.* 2001; **276**: 19 687–19 690.
11. Morrissey MP, Shakhnovich EI. Evidence for the role of PrP^C helix 1 in the hydrophilic seeding of prion aggregates. *Proc. Natl. Acad. Sci. U.S.A.* 1999; **96**: 11 293–11 298.
12. Apetri AC, Surewicz WK. Atypical effect of salts on the thermodynamic stability of human prion protein. *J. Biol. Chem.* 2003; **278**: 22 187–22 192.
13. Morillas M, Vanik DL, Surewicz WK. On the mechanism of alpha-helix to beta-sheet transition in the recombinant prion protein. *Biochemistry* 2001; **40**: 6982–6987.
14. Nandi PK, Leclerc E, Marc D. Unusual property of prion protein unfolding in neutral salt solution. *Biochemistry* 2002; **41**: 11 017–11 024.
15. Ziegler J, Sticht H, Marx UC, Müller W, Rösch P, Schwarzinger S. CD and NMR studies of prion protein (PrP) helix 1: novel implications for its role in the PrP^C to PrP^{Sc} conversion process. *J. Biol. Chem.* 2003; **278**: 50 175–50 181.
16. Tizzano B, Palladino P, De Capua A, Marasco D, Rossi F, Benedetti E, Pedone C, Ragone R, Ruvo M. The human prion protein alpha2 helix: a thermodynamic study of its conformational preferences. *Proteins* 2005; **59**: 72–79.
17. Haire LF, Whyte SM, Vasisht N, Gill AC, Verma C, Dodson EJ, Dodson GG, Bayley PM. The crystal structure of the globular domain of sheep prion protein. *J. Mol. Biol.* 2004; **336**: 1175–1183.
18. Brown DR, Guantieri V, Grasso G, Impellizzeri G, Pappalardo G, Rizzarelli E. Copper(II) complexes of peptide fragments of the

DOI: 10.1002/psc

prion protein. Conformation changes induced by copper(II) and the binding motif in C-terminal protein region. *J. Inorg. Biochem.* 2004; **98**: 133–143.
19. Brown DR, Qin K, Herms JW, Madlung A, Manson J, Strome R, Fraser PE, Kruck T, von Bohlen A, Schulz-Schaeffer W, Giese A, Westaway D, Kretzschmar H. The cellular prion protein binds copper in vivo. *Nature* 1997; **390**: 684–687.
20. Millhauser GL. Copper binding in the prion protein. *Acc. Chem. Res.* 2004; **37**: 79–85.
21. Cereghetti GM, Schweiger A, Glockshuber R, van Doorslaer S. Stability and Cu(II) binding of prion protein variants related to inherited human prion diseases. *Biophys. J.* 2003; **84**: 1985–1997.
22. Zuegg J, Gready JE. Molecular dynamics simulations of human prion protein: importance of correct treatment of electrostatic interactions. *Biochemistry* 1999; **38**: 13 862–13 876.
23. Wong C, Xiong L-W, Horiuchi M, Raymond L, Wehrly K, Chesebro B, Caughey B. Sulfated glycans and elevated temperature stimulate PrP^{Sc}-dependent cell-free formation of protease-resistant prion protein. *EMBO J.* 2001; **20**: 377–386.
24. Cordeiro Y, Machado F, Juliano Neto L, Juliano MA, Brentani RR, Foguel D, Silva JL. DNA converts cellular prion protein into the beta-sheet conformation and inhibits prion peptide aggregation. *J. Biol. Chem.* 2001; **276**: 49 400–49 409.
25. Calzolai L, Zahn R. Influence of pH on NMR structure and stability of the human prion protein globular domain. *J. Biol. Chem.* 2003; **278**: 35 592–35 596.
26. De Simone A, Dodson GG, Verma CS, Zagari A, Fraternali F. Prion and water: tight and dynamical hydration sites have a key role in structural stability. *Proc. Natl. Acad. Sci. U.S.A.* 2005; **102**: 7535–7540.
27. Kawatake S, Nishimura Y, Sakaguchi S, Iwaki T, Doh-ura K. Surface plasmon resonance analysis for the screening of anti-prion compounds. *Biol. Pharm. Bull.* 2006; **29**: 927–932.
28. Collins KD. Charge density-dependent strength of hydration and biological structure. *Biophys. J.* 1997; **72**: 65–76.
29. Collins KD. Ions from the hofmeister series and osmolytes: effects on proteins in solution and in the crystallization process. *Methods* 2004; **34**: 300–311.
30. Niedz RP, Evens TJ. A solution to the problem of ion confounding in experimental biology. *Nat. Methods* 2006; **3**: 417.
31. Chen YR, Huang HB, Chyan CL, Shiao MS, Lin TH, Chen YC. The effect of Aβ conformation on the metal affinity and aggregation mechanism studied by circular dichroism spectroscopy. *J. Biochem. (Tokyo)* 2006; **139**: 733–740.
32. Roberts MF. Organic compatible solutes of halotolerant and halophilic microorganisms. *Saline Syst.* 2005; **1**: 1–30.
33. Arora A, Ha C, Park CB. Inhibition of insulin amyloid formation by small stress molecules. *FEBS Lett.* 2004; **564**: 121–125.

DOI: 10.1002/psc