ARTICLE

OBC
www.rsc.org/obc

Conformational properties of peptide fragments homologous to the 106–114 and 106–126 residues of the human prion protein: a CD and NMR spectroscopic study†‡

Giuseppe Di Natale,[a] Giuseppe Impellizzeri[a] and Giuseppe Pappalardo*[b]

[a] *Dipartimento di Scienze Chimiche, Università di Catania, V.le A. Doria 6, 95125, Catania*

[b] *Istituto di Biostrutture e Bioimmagini-Sezione di Catania, CNR, V.le A. Doria 6, 95125, Catania. E-mail: pappalardo.cnr@unict.it; Fax: +39 (0) 95 337678; Tel: +39 (0) 95 7385016*

Received 26th May 2004, Accepted 10th November 2004
First published as an Advance Article on the web 21st December 2004

Two peptide fragments, corresponding to the amino acid residues 106–126 (PrP[Ac-106–126-NH_2]) and 106–114 (PrP[Ac-106–114-NH_2]) of the human prion protein have been synthesised in the acetylated and amide form at their N- and C-termini, respectively. The conformational preferences of PrP[Ac-106–126-NH_2] and PrP[Ac-106–114-NH_2] were investigated using CD and NMR spectroscopy. CD results showed that PrP[Ac-106–126-NH_2] mainly adopts an α-helical conformation in TFE–water mixture and in SDS micelles, while a predominantly random structure is observed in aqueous solution. The shorter PrP[Ac-106–114-NH_2] fragment showed similar propensities when investigated under the same experimental conditions as those employed for PrP[Ac-106–126-NH_2]. From CD experiments at different SDS concentrations, an α-helix/β-sheet conformational transition was only observed in the blocked PrP[Ac-106–126-NH_2] sequence. The NMR analysis confirmed the helical nature of PrP[Ac-106–126-NH_2] in the presence of SDS micelles. The shorter PrP[Ac-106–114-NH_2] manifested a similar behaviour. The results as a whole suggest that both hydrophobic effects and electrostatic interactions play a significant role in the formation and stabilisation of ordered secondary structures in PrP[Ac-106–126-NH_2].

Introduction

Prion-related disorders which include sheep scrapie, bovine spongiform encephalopathy (BSE) in cattle, Creutzfeldt–Jakob disease (CJD), Gerstmann–Straussler–Scheinker syndrome (GSS) and fatal familial insomnia (FFI) in humans, are associated with the conversion of a normal cellular isoform of prion protein (PrP^C) into an abnormal pathological scrapie isoform (PrP^{Sc}). The conversion of the protein is purely conformational and involves a reduction in the α-helices and an increase in the β-sheet content. Unlike PrP^C, PrP^{Sc} is partly resistant to protease digestion and able to polymerise into amyloid fibrils that accumulate in the brain parenchima.[1]

NMR studies carried out on several recombinant proteins from human,[2] cow,[3] hamster[4] and mouse,[5] show that PrP^C is composed of two structurally different domains: an extended N-terminal segment (which approximately extends residues 23–125) with features of a flexible and disordered peptide chain and a well defined globular region (residues 126–231) with three α-helices (residues 144–151, 172–193, 200–227) and two short β-strands (residues 129–134 and 159–165).[2–5] On the other hand, the protease-resistant core of PrP^{Sc} is less characterised and appears to be mostly β-sheet with less α-helical structure.[6]

The highly conserved region encompassing residues 106–126 of PrP^C is believed to play a key role in such conformational conversion. Furthermore, it has been shown that this peptide exhibits some of the pathogenic properties of PrP^{Sc}, including neurotoxicity, protease-resistant properties, induction of hypertrophy and proliferation of astrocytes.[7–9]

In this respect, *in vitro* studies carried out on the synthetic peptide homologous with this sequence have indicated remarkable conformational polymorphism, acquiring different secondary structures in various conditions including pH, ionic strength, hydrophobic or membrane-like environments.[10] The solution conformation of this peptide sequence, both in water and 50% TFE–water, has been previously described, by means of nuclear magnetic resonance (NMR) spectroscopy, by Ragg *et al.*[11] The authors found that the peptide possesses an intrinsic propensity to adopt an α-helical conformation in the hydrophobic region starting from Met112, in the presence of TFE as well as in deionised water.

Furthermore, previous spectroscopic work showed that the PrP(106–126)'s conformational plasticity, as well as its aggregation and amyloidogenic propensity, might be regulated by the hydrophobic palindrome sequence AGAAAAGA, which was also found to be necessary for the neurotoxic effect of the peptide.[12,13]

Thus, PrP(106–126) has been widely used by different groups as a useful model to mimic the neurotoxicity as well as the biophysical properties of PrP^{Sc}, even though this peptide is not actually present in the brain during the course of prion diseases.

The primary structure of PrP(106–126) is characterised by an N-terminal hydrophilic region (KTNMKH–) followed by a long hydrophobic tail (–MAGAAAAGAVVGGLG), which enables the peptide to interact with the cellular membrane. In this regard, previous studies have shown that PrP(106–126) forms single ion channels in artificial membrane made up of a lipid bilayer.[14–17] The results suggested that PrP(106–126)'s ion channel-forming ability might be the mechanism of action by which this amyloidogenic peptide fragment exerts its cytotoxicity.[18] In addition, PrP(106–126)'s primary sequence is an integral part of the domain that spans the endoplasmic reticulum membrane in ^{Ctm}PrP, a transmembrane form of the prion protein, that in some circumstances has been associated with neurodegeneration without detectable PrP^{Sc}.[19]

† Electronic supplementary information (ESI) available: CD spectrum of PrP[Ac-106–126-NH_2]. See http://www.rsc.org/suppdata/ob/b4/b407928k/

‡ One-letter symbols for the amino acids: A, Ala; G, Gly; H, His; K, Lys; L, Leu; M, Met; N, Asn; T, Thr; V, Val; CD, circular dichroism; COSY, correlation spectroscopy; TOCSY, total correlation spectroscopy; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotating-frame Overhauser effect spectroscopy;. TFE, 2,2,2-trifluoroethyl alcohol; SDS, sodium dodecyl sulfate; NOS, nitric oxide synthase.

DOI: 10.1039/b407928k

Recently, it has been reported that when human microglial cells were challenged with the fibrillogenic peptide PrP(106–126), induction of NOS-II was observed together with an increase of nitrite levels in the supernatants of cultures. On the contrary, the scramble sequence of PrP(106–126) and the non-fibrillogenic C-terminally amidated form of PrP(106–126) were unable to determine any significant increase in nitrite levels in these cells.[20]

It should be said that the majority of the studies conducted on PrP(106–126) have been carried out by using samples of the peptide fragment with unblocked N- and C-termini. Conversely, the acetylation of the N-terminus and the amidation of the C-terminus should provide a peptide model that more properly mimics the sequence inserted within the primary structure of the parent protein.

In the present paper we investigate the solution conformation of two peptides corresponding to residues 106–126 and 106–114 of the human prion protein. In both the peptide fragments the C-terminus was synthesised in the amide form, and the N-terminus amino group was acetylated. The conformational preferences of these peptides were investigated in aqueous solution, in mixed solvents obtained by addition of variable amounts of TFE, or in the presence of different concentration of SDS by means of circular dichroism. In addition, NMR experiments were carried out in the presence of SDS micelles to investigate the solution conformation of these two peptides at a membrane mimicking environment. The comparison of the results obtained for the hydrophilic PrP[Ac-106–114-NH_2] (Ac-KTNMKHMAG-NH_2) peptide fragment and the whole PrP[Ac-106–126-NH_2] sequence (Ac-KTNMKHMAGAAAAGAVVGGLG-NH_2) of this study may provide insight into the possible mechanism of interaction at membrane-like environments in addition to detailed information on the secondary structure of these new model peptides.

Experimental

Materials

All *N*-fluorenylmethoxycarbonyl (Fmoc)-protected amino-acids, 2-(1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), *N*-hydroxybenzotriazole (HOBT) and Novasyn TGR resin were purchased from Novabiochem (Switzerland). *N*,*N*-Dimethylformamide (DMF, peptide synthesis grade) and 20% piperidine–DMF solution, were from Perseptive Biosearch. *N*,*N*-Diisopropyl-ethylamine (DIEA), triisopropylsilane (TIS), trifluoroacetic acid (TFA), ethanedithiol (EDT), TFE, SDS, sodium dodecyl$_{-d25}$ sulfate (SDS$_{-d25}$, >98% isotopic enrichment), were from Sigma Aldrich. All other chemicals were of the highest available grade and were used without further purification.

Peptide synthesis and purification

The peptides PrP[Ac-106–126-NH_2] and PrP[Ac-106–114-NH_2] were synthesised on a Milligen Model 9050 peptide synthesizer using N^{α}-fluorenylmethoxy-carbonyl (Fmoc) amino acids. The following L-amino acid derivatives were used: Fmoc-Lys(Boc)–OH, Fmoc-Thr(tBu)–OH, Fmoc-Asn(Trt)–OH, Fmoc-Met–OH, Fmoc-His(Trt)–OH, Fmoc-Ala–OH, Fmoc-Val–OH, Fmoc-Leu–OH and Fmoc-Gly–OH. All residues were introduced according to the TBTU/HOBT/DIEA method. The synthesis was carried out under a fourfold excess of amino acid at every cycle and each amino acid re-circulated through the resin for 30 min. In the synthesis of PrP[106–126] double coupling cycles were introduced for Ala120, Val121, Val122, and Leu 125, amino acid residues.

Each peptide was cleaved from the resin by treatment with a TFA–H_2O–TIS–EDT mixture (94 : 2.5 : 1 : 2.5 by volume) over 1.5–2.0 h.

The solution containing the free peptide was filtered off from the resin, concentrated *in vacuo* at a temperature not exceeding 30 °C and then precipitated with cold freshly distilled diethyl ether.

The precipitate was filtered off and desiccated under vacuum. The crude products were lyophilised before purification.

The PrP[Ac-106–126-NH_2] was purified by preparative RP-HPLC on a Vydac C_{18} (2.2 × 25 cm; 10 μm particle size and 300 Å pores) chromatographic column with a linear gradient of 100 : 0 water (containing 0.1% TFA)–acetonitrile (containing 0.1% TFA) to 65 : 35 water (containing 0.1% TFA)–acetonitrile (containing 0.1% TFA) over 35 min at a flow rate of 10 mL min^{-1}.

The PrP[Ac-106–114-NH_2] was purified by ion exchange chromatography on a CM-sephadex C-25 (NH_4^+ form) column. The column was eluted initially with water and then with a linear gradient of aqueous NH_4HCO_3 (0–0.4 M; 1600 mL). Fractions were assayed by TLC and those containing the desired product (eluent: PrOH–H_2O–EtOAc–NH_4OH 5 : 3 : 5 : 1 v/v/v/v and BuOH–AcOH–H_2O 60 : 15 : 25 v/v/v) were combined, concentrated to dryness under vacuum at 40 °C, repeatedly dissolved in water and dried to decompose any remaining ammonium hydrogencarbonate. The purity of both peptides was checked by analytical RP-HPLC using a Vydac C_{18} chromatographic column (150 × 4.6 mm; 5 μm particle size and 300 Å pores). Two gradients of acetonitrile (containing 0.1% TFA) and water (containing 0.1% TFA) were used: for PrP[Ac-106–126-NH_2] a linear gradient of 85 : 15 water–acetonitrile to 70 : 30 water–acetonitrile over 25 min and at a flow rate of 1 ml min^{-1} was used. In the case of PrP[Ac-106–114-NH_2] the water–acetonitrile gradient started from 95 : 5 to 70 : 30 water–acetonitrile over 30 min and at a flow rate of 1 ml min^{-1} Peptide elution was monitored at 222 nm. Purity was greater than 98% for both peptides.

The products were characterised by ESI-MS PrP[Ac-106–126-NH_2]: m/z 1953.5 $(M + H)^+$, calculated for $C_{82}H_{141}N_{27}O_{24}S_2$ 1952.0; PrP[Ac-106–114-NH_2]: m/z 1058.3 $(M + H)^+$, calculated for $C_{43}H_{75}N_{15}O_{12}S_2$ 1057.5.

Spectroscopic measurements

Circular dichroism (CD). The CD spectra were obtained at 300 K under a constant flow of nitrogen on a Jasco model J-810 spectropolarimeter, calibrated with an aqueous solution of (1*R*)-(−)-10-camphorsulfonic acid, ammonium salt.[21] Experimental measurements were carried out under a variety of experimental conditions including different pH, different percentages of aqueous TFE or different SDS concentrations. The CD spectra were recorded in the UV region (190–260 nm) using 1 mm path length cuvettes with peptide concentrations of 2.0×10^{-4} M. The spectra represent the average of 8–20 scans. CD intensities are expressed as mean residue ellipticity [θ] (deg cm^2 dmol^{-1}).

NMR spectroscopy. All NMR spectra were acquired at 300 K on a Varian INOVA Unity-plus spectrometer operating at 499.884 MHz. Lyophilised samples of *ca.* 2 mM concentration were dissolved in 90 : 10 H_2O (with 0.17 M SDS$_{d25}$) : D_2O, and 90 : 10 H_2O (with 0.17 M of SDS$_{d25}$–SDS in a 3 : 1 ratio) : D_2O. Trimethylsilylpropionic acid (TSP) was used as an internal standard. The pH of the solution was adjusted to pH 5.0 by adding the appropriate acid or base solution. The electrode-measured pH values are uncorrected for the isotope effect.

1D spectra were generally acquired with 32768 data points over a spectral width of 6000 Hz. 2D experiments were typically acquired with 2048 data points in the t_2 dimension and 512 t_1 increments. Water saturation was achieved by low power irradiation during the relaxation delay. TOCSY spectra were acquired with a spin locking field of 7 kHz at a mixing time of 80 ms. ROESY spectra were run using a 2 kHz spin locking field at a mixing time of 250 ms. Mixing times of 300 and 500 ms were used in the NOESY experiments.

Results and discussion

Circular dichroism analysis

The solution conformation of PrP[Ac-106–126-NH_2] and PrP[Ac-106–114-NH_2] at different solvent environments was investigated by CD spectroscopy. The CD spectra of both peptides, recorded in aqueous solutions, exhibited a strong negative band below 200 nm and weaker broad negative ellipticity around 222 nm typical of peptides in a random coil conformation.[22] In both peptide samples, varying the pH of the solution from 4.0 to 11.0 did not significantly modify the shape of the CD curves toward the β-sheet spectral pattern that may be a prelude to the aggregation into amyloid fibrils (Fig. 1).[22] Moreover, incubating the PrP[Ac-106–126-NH_2] sample solutions over a period of 92 h in water at pH 7.0, or in 100 mM phosphate buffer at pH 5.0 or 7.0, did not result in spectral modifications that could indicate conformational change toward the β-sheet structure (data not shown). These results indicated that blocking both N- and C-termini completely inhibited the aggregation propensity in PrP[Ac-106–126-NH_2] and are in agreement with data previously reported for the C-terminus amidated peptide analogue.[23] In the presence of increasing concentration of TFE, the shape of the CD spectrum of PrP[Ac-106–126-NH_2] becomes progressively more similar to that of the helical conformation showing double minima at 206 and 222 nm and a positive band at 192 nm (Fig. 2).[24] Above 40% and up to 70% TFE a helix fraction of 0.17 was independently estimated by the CONTIN/LL program provided within the CDPro software package[25] and by the $[\theta]_{222}$ value.[26] However, the lack of an isodichroic point might reflect the presence of other intermediate conformers that make the equilibrium not easily interpretable in terms of simple random coil–helix transition. In this respect, it should be mentioned that the CONTIN/LL calculation indicates a significant fraction of β-sheet structure that reaches its maximum (around 0.30) at 20–30% of TFE, then it declines to 0.25 in the 40–70% TFE range. The effect of pH on helix stabilisation was investigated in 50% aqueous TFE. The resulting CD spectra indicated that the helical content increases as the pH increases. In particular, the $[\theta]_{222}$ values plotted as a function of the pH indicated that the neutralisation of the positively charged ε-amino groups of the Lys residues, that occurred above pH 10, remarkably stabilized the helix dipole thus enhancing the α-helical content (Fig. 3).[27] A similar spectroscopic behaviour was observed for the PrP[Ac-106–114-NH_2] peptide fragment containing the polar and hydrophilic amino acid residues of the PrP[Ac-106–126-NH_2] sequence. In fact, although to a lesser extent with respect to PrP[Ac-106–126-NH_2], the CD experiments carried out in the increasing percentages of TFE in water, showed that the inherent α-helical propensity is maintained even in such a short peptide sequence (Fig. 4). In this case, the presence of an isodichroic point

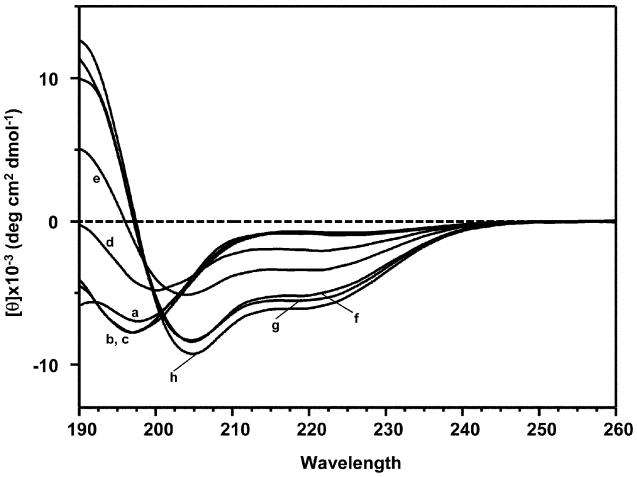

Fig. 2 CD spectra of PrP[Ac-106–126-NH2] ($C = 2.0 \times 10^{-4}$ M) recorded at different concentrations of TFE at pH 7.0: (a) H_2O; (b) 5% TFE; (c) 10% TFE; (d) 20% TFE; (e) 30% TFE; (f) 40% TFE; (g) 50% TFE; (h) 70% TFE.

around 203 nm suggests that the PrP[Ac-106–114-NH_2] peptide is mainly adopting two conformational states and is consistent with the presence of mixed helix and coil conformations.[28] Based on CONTIN/LL calculation and $[\theta]_{222}$ value a helical content around 10% has been estimated for this system at 70% TFE. In the same way as PrP[Ac-106–126-NH_2], the CD experiments carried out at 50% TFE revealed the stabilising effect of the increasing pH toward the peptide's helical transition (Fig. 5). Again, major effects were observed above pH 10 as a result of the neutralisation of the Lys ε-amino groups (see Fig. 5). The whole of the above CD data demonstrate the good propensity of PrP[Ac-106–126-NH_2] to fold into the α-helix conformation at a more hydrophobic environment. Moreover, the capability of the short peptide fragment PrP[Ac-106–114-NH_2] to undergo a coil to α-helix transition suggests that the conformational changes observed for PrP[Ac-106–126-NH_2] might not be exclusively confined within the hydrophobic region.

To obtain further information on the conformational properties of PrP[Ac-106–126-NH_2] in a membrane-mimicking environment, we extended our CD study by examining PrP[Ac-106–126-NH_2] samples in aqueous solutions containing sodium dodecyl sulfate. SDS has been extensively used for the structural investigations of membrane peptides as it provides a hydrophobic environment that mimics either biological membranes or the interior of proteins.[29]

In 0.17 M SDS–water mixture, PrP[Ac-106–126-NH_2] clearly adopts an α-helical conformation (Fig. 6). However, unlike the behaviour observed in 50% TFE (Fig. 3), varying the pH of the solution resulted in negligible modification in the intensity

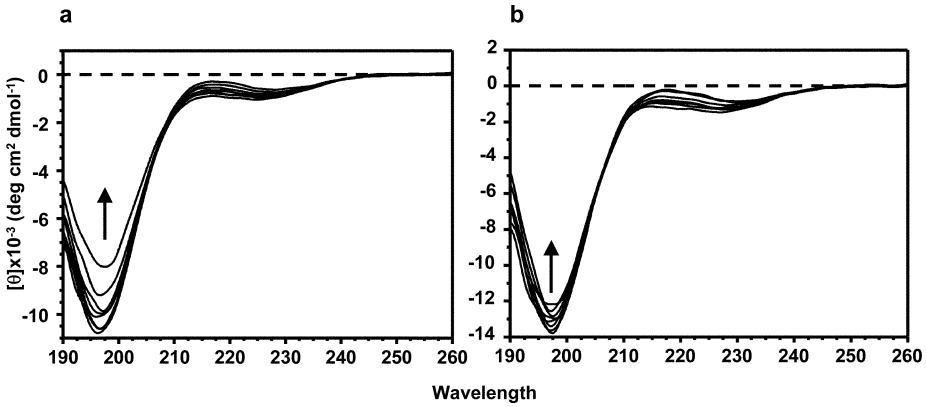

Fig. 1 CD spectra of (a) PrP[Ac-106–126-NH_2] ($C = 2.0 \times 10^{-4}$ M) and (b) PrP[Ac-106–114-NH_2] ($C = 2.0 \times 10^{-4}$ M); in H_2O at different pH values. From bottom to top (arrow): pH = 4.0; pH = 5.0; pH = 6.0; pH = 7.0; pH = 8.0; pH = 9.0; pH = 10.0; pH = 11.0.

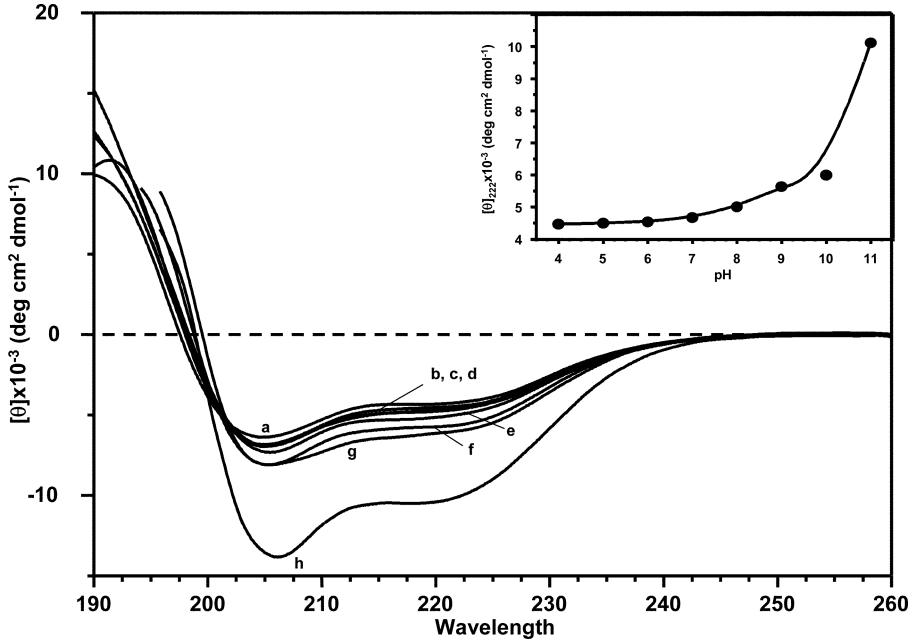

Fig. 3 CD spectra of PrP[Ac-106–126-NH_2] ($C = 2.0 \times 10^{-4}$ M) in 50% H_2O–TFE at different pH values: (a) pH = 4.0; (b) pH = 5.0; (c) pH = 6.0; (d) pH = 7.0; (e) pH = 8.0; (f) pH = 9.0; (g) pH = 10.0; (h) pH = 11.0. Inset: plot of $[\theta]_{222}$ *vs.* pH.

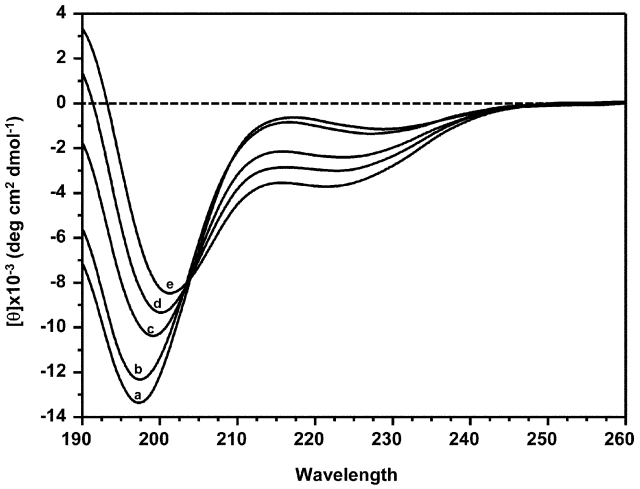

Fig. 4 CD spectra of PrP[Ac-106–114-NH_2] ($C = 2.0 \times 10^{-4}$ M) recorded at different concentrations of TFE at pH 7.0: (a) H_2O; (b) 10% TFE; (c) 30% TFE; (d) 50% TFE; (e) 70% TFE.

of the spectra, suggesting that in the presence of SDS micelles peptide's helicity already reaches its maximum at pH 4; this in turn implies that in such experimental conditions, the folding of the peptide is mostly driven by hydrophobic effects. In sharp contrast, when the same experiment was carried out for the PrP[Ac-106–114-NH_2] fragment, the observed partial helical conformation was progressively stabilised by increasing the pH of the solution. In this case, however, the expected enhancement of the helical population above pH 10 was not observed (Fig. 7).

It has been reported that at concentrations below its critical micellar concentration (c.m.c., 1–10 mM depending on the ionic strength), SDS promotes β-sheet structure in peptides with β-sheet structure propensity.[30] The CD spectra reported in Fig. 8 were recorded at pH 7.0 and show that in the SDS concentration range of 50.0 to 500.0 μM (which is below the c.m.c.) PrP[Ac-106–126-NH_2] remained in a random coil conformation. Between 1.0 and 5.0 mM of SDS, that are concentration values close to the c.m.c., the peptide underwent a conformational transition toward the β-sheet structure as suggested by the appearance of a positive band at 195 nm and a negative ellipticity at 218 nm in the related CD spectra. However, at these SDS concentrations, PrP[Ac-106–126-NH_2] showed a reduced solubility and the sample solution became slightly turbid. To verify whether the shape of these spectra could be affected by the presence of scattering artefacts the dichroic activity, as well as the absorption spectra, were measured in the 350–600 nm wavelength range. Neither appreciable ellipticity nor absorption bands were detected at these wavelengths. Far UV CD experiments (190–260 nm range) with the cuvette positioned at different distances from the spectropolarimeter's photomultiplier were also performed. It turned out that the CD spectrum, recorded with the cuvette in contact with the photomultiplier's window, just exhibited a less pronounced positive ellipticity in the range of 190–210 nm whereas for the remaining part it was identical to that one recorded with the cuvette placed in its appropriated slot (see supplementary materials†). Both spectra retained the typical profile of the β-sheet conformation. These results indicate that even if some light-scattering can occur, due to the turbidity of the sample solution, this does not affect significantly the shape of the corresponding spectra. This in turn validates the consistency of the curves (c) and (d) reported in Fig. 8. Further increasing the SDS concentration to 0.01 M (just above the c.m.c.) resulted in the complete dissolution of the peptide, and the recorded CD spectra were typical of the α-helix conformation. No change in the shape of the CD curves was observed up to 0.17 M of SDS. The β-sheet structure formation and the consequent aggregation that occurred at low SDS concentration, may result from the attractive electrostatic interaction of the negatively charged SDS's sulfate group with the cationic side chains of Lys, and to a certain extent His, amino acid residues. This phenomenon may lead to an enhancement of the intermolecular interactions between the hydrophobic region of the peptide.[31] In this regard, and unlike the behaviour observed for PrP[Ac-106–126-NH_2], the CD spectra of the shorter peptide PrP[Ac-106–114-NH_2] did not show any β-sheet structure or aggregation tendencies at any concentration of the detergent and at neutral pH (data not shown). Such a different behaviour brings into evidence the pivotal role of the PrP[Ac-106–126.NH_2]'s hydrophobic core sequence in the observed conformational polymorphism.

NMR spectroscopy

More precise data on the conformational preferences of both PrP[Ac-106–126-NH_2] and PrP[Ac-106–114-NH_2] in micellar SDS environment were obtained by NMR spectroscopy.

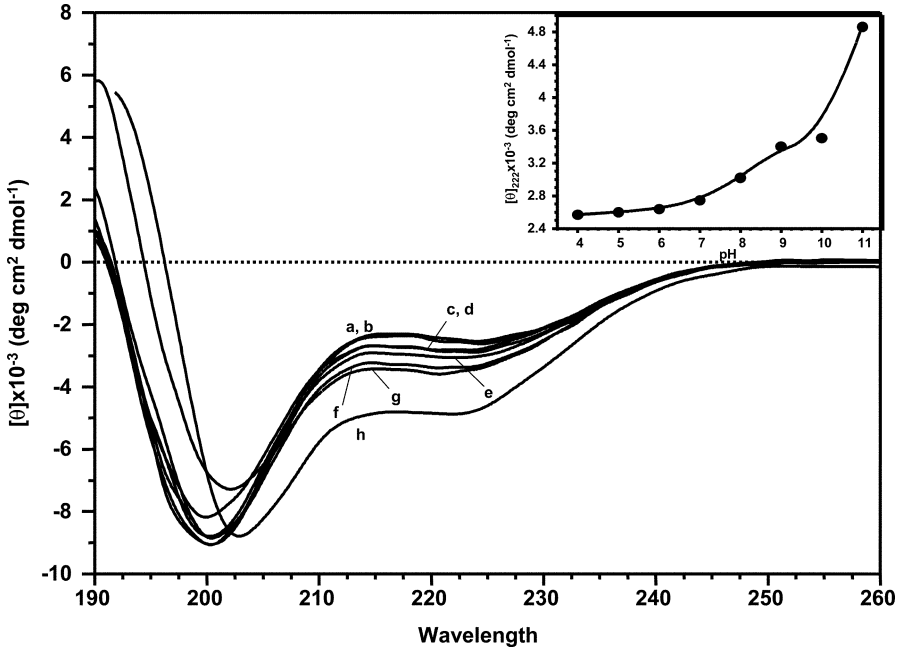

Fig. 5 CD spectra of PrP[Ac-106–114-NH_2] ($C = 2.0 \times 10^{-4}$ M) in 50% H_2O–TFE at different pH values: (a) pH = 4.0; (b) pH = 5.0; (c) pH = 6.0; (d) pH = 7.0; (e) pH = 8.0; (f) pH = 9.0; (g) pH = 10.0; (h) pH = 11.0. Inset: plot of $[\theta]_{222}$ *vs.* pH.

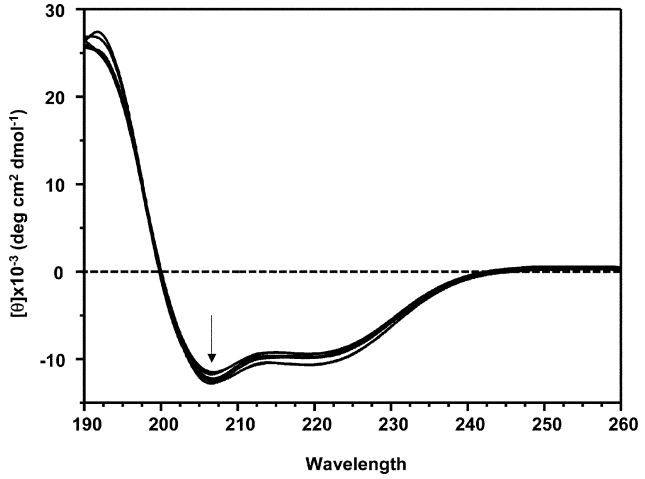

Fig. 6 CD spectra of PrP[Ac-106–126-NH_2] ($C = 2.0 \times 10^{-4}$ M) in 0.17 M aqueous SDS at different pH values: From top to bottom (arrow): pH = 4.0; pH = 5.0; pH = 6.0; pH = 7.0; pH = 8.0; pH = 9.0; pH = 10.0; pH = 11.0.

All the NMR experiments were performed at pH 5.0. Sequence-specific assignment of the proton resonances were achieved by spin-system identification from COSY and TOCSY spectra, followed by sequential assignments through NOE connectivities.[32] The complete assignment of the proton chemical shifts of PrP[Ac-106–126-NH_2] and PrP[Ac-106–114-NH_2] in 0.17 M SDS is reported in Table 1.

The presence of helical secondary structure was verified by examining the ROESY and NOESY spectra for distinctive sequential and medium-range NOEs as well as by measuring the variation of the H_α chemical shift values relative to their random coil shifts ($\Delta\delta_{H\alpha}$).[32,33]

The helix conformation is readily identified by a series of strong consecutive $d_{NN(i,i+1)}$ NOEs concomitant with $d_{\alpha N(i,i+3)}$. The short- and medium-range NOE interactions observed for PrP[Ac-106–126-NH_2] in H_2O/SDS 0.17 M are shown in Fig. 9. These include strong $d_{NN(i,i+1)}$ and $d_{\alpha N(i,i+1)}$ sequential NOEs together with evident $d_{\alpha N(i,i+3)}$ and $d_{\alpha N(i,i+4)}$ medium range connectivities. These data suggest that the PrP[Ac-106–126-NH_2] can fold into a predominantly α-helical conformation. Moreover, the observation of some $d_{\alpha N(i,i+2)}$ and $d_{NN(i,i+2)}$ connectivities in the region encompassing the residues Ala115 and Gly123, may reflect the presence of mixed 3_{10} helix and α-helix conformation in this part of the peptide sequence. This last observation is in agreement with the reported ability of Ala-rich peptide sequences to adopt a 3_{10} helical conformation.[34]

The same NMR experiments were conducted for the shorter peptide PrP[Ac-106–114-NH_2] and the partial helical character of this peptide could be only inferred from the presence of $d_{NN(i,i+1)}$ connectivities (Fig. 9).

The $\Delta\delta_{H\alpha}$ secondary shifts can provide a straightforward way for assessing local helix content. Relative to a random coil conformation, an increase of helicity results in an upfield shifts of $^1H_\alpha$ resonances, which in turn results in a negative variation of the chemical shifts.[33]

The negative secondary shift values reported in Fig. 10 revealed the presence of considerable α-helical conformation between Met109 and Ala118 segment. The Gly119 and Ala120 residues seem to interrupt the propagation of the helical conformation notwithstanding the contrary effect of the next two Val residues.

Furthermore, the comparison of the $\Delta\delta_{H\alpha}$ for both peptides (Fig. 10) suggests that the hydrophobic tail in PrP[Ac-106–126-NH_2] has a remarkable effect in the propagation of the helical conformation also in the hydrophilic region. Indeed, the Hα protons relative to the longer PrP[Ac-106–126-NH_2] peptide are shifted upfield to greater degree than the same protons in the PrP[Ac-106–114-NH_2] sequence. Estimation of helicity based on $C_\alpha H$ upfield shift in the longer peptide PrP[Ac-106–126-NH_2] using the method suggested by Rizo *et al.*,[34] indicated a helical content around 45%. This value is not much different from the helicity value of 38% obtained by analysing the CD spectra shown in Fig. 6 with the CONTIN/LL program.

To observe direct contacts between SDS and PrP[Ac-106–126-NH_2] protons, NOESY spectra were carried out in the presence of a 0.17 M mixture of SDS_{d25}–SDS (in a 3 : 1 ratio).[28,35]

SDS peaks were assigned on the basis of previously reported NMR data and by comparison with authentic spectra of separate SDS samples.[36]

A number of intermolecular cross-peaks were observed (Fig. 11); these included the SDS C_{12}-H with His-βCH_2 and Met-γCH_2; SDS C_{4-11}-H with Asn-βCH_2, Lys-εCH_2 and Met-γCH_2; SDS C_2-H with Met-γCH_2. Additional peptide–SDS interactions, namely with the methyl protons of the Ala, Val

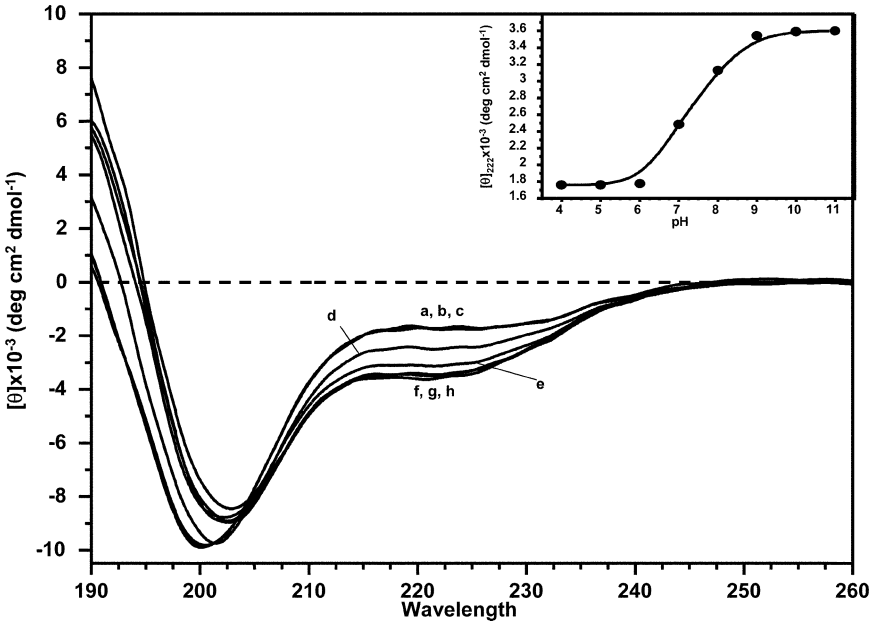

Fig. 7 CD spectra of PrP[Ac-106–114-NH_2] ($C = 2.0 \times 10^{-4}$ M) in 0.17 M aqueous SDS at different pH values: (a) pH = 4.0; (b) pH = 5.0; (c) pH = 6.0; (d) pH = 7.0; (e) pH = 8.0; (f) pH = 9.0; (g) pH = 10.0; (h) pH = 11.0. Inset: plot of $[\theta]_{222}$ *vs.* pH.

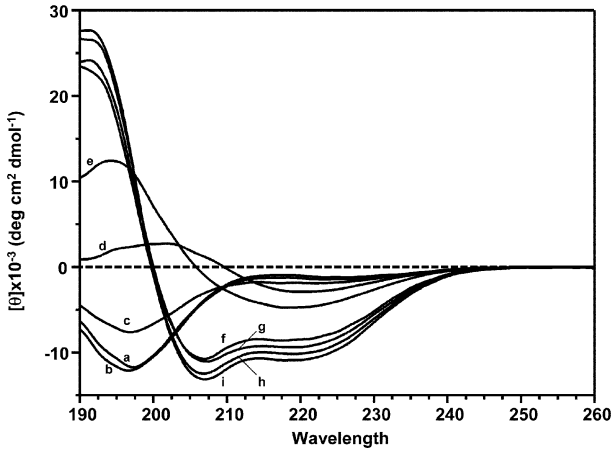

Fig. 8 CD spectra of PrP[Ac-106–126-NH_2] ($C = 2.0 \times 10^{-4}$ M) recorded at different concentrations of SDS at pH 7.0: (a) 5.0×10^{-5} M; (b) 1.0×10^{-4} M; (c) 5.0×10^{-4} M; (d) 1.0×10^{-3} M; (e) 5.0×10^{-3} M; (f) 1.0×10^{-2} M; (g) 5.0×10^{-2} M; (h) 0.1 M; (i) 0.17 M.

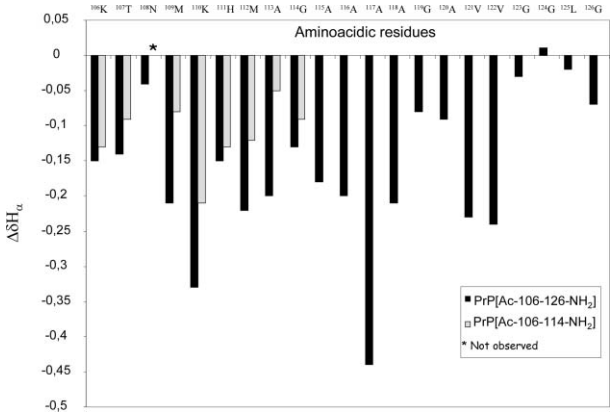

Fig. 10 A comparison of H_α chemical shift deviation from random coil values ($\Delta\delta_{H\alpha}$). The chemical shift values were measured in the experimental conditions reported in Table 1. $\Delta\delta_{H\alpha} = \delta_{obs} - \delta_{randomcoil}$. The random coil values were taken from Wishart *et al.*[33g]

Fig. 9 Sequential and medium range NOE connectivities in the peptides (a) PrP[Ac-106–126-NH_2] and (b) PrP[Ac-106–114-NH_2] in 0.17 M aqueous SDS at 300 K and pH 5.0. The thickness of the lines reflects the relative intensities of the NOEs within the individual plot (a) and (b). Solid lines indicate unambiguous NOEs. Dashed lines indicate possible NOEs where chemical shift degeneracy interferes with identification.

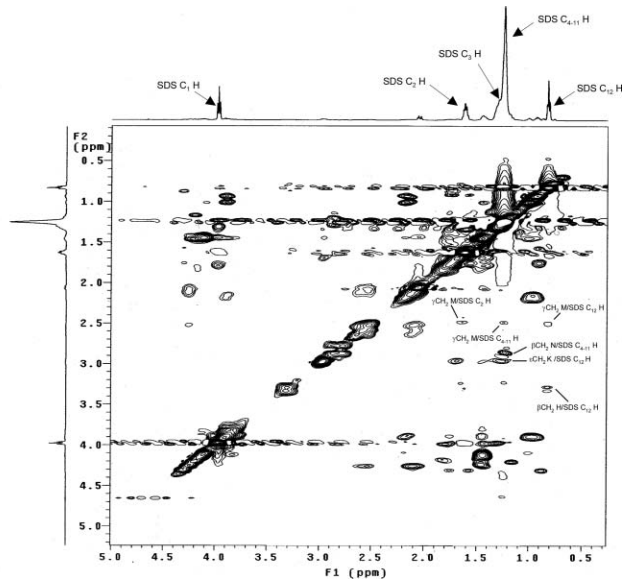

Fig. 11 Portion of the 300 ms NOESY spectrum of PrP[Ac-106–126-NH_2] in the presence of a 3 : 1 SDS_{d25}/SDS mixture at pH 5.0 and 300 K. Selected NOE cross-peaks between SDS and peptide protons are labelled.

Table 1 Proton chemical shifts of PrP[Ac-106–126-NH_2] and PrP[Ac-106–114-NH_2][a]

	PrP[Ac-106–126-NH_2]				PrP[Ac-106–114-NH_2]			
Residue	NH	α	β	Others	NH	α	β	Others
Ac	—	—	—	2.05	—	—	—	2.05
^{106}Lys	8.19	4.17	1.83	γCH_2 1.46; δCH_2 1.64; εCH_2 2.99	8.19	4.18	1.85	γCH_2 1.47; δCH_2 1.69; εCH_2 2.98
^{107}Thr	8.06	4.21	4.16	γCH_3 1.19	8.01	4.26	4.23	γCH_3 1.17
^{108}Asn	8.20	4.70	2.83	$CONH_2$ 7.53; 6.90	8.17	ND	2.79	$CONH_2$ 7.48; 6.88
^{109}Met	8.16	4.27	2.07	γCH_2 2.60; εCH_3 2.06	8.02	4.40	2.06	γCH_2 2.53;εCH_3 2.12
^{110}Lys	8.14	3.99	1.83	γCH_2 1.50; δCH_2 1.64; εCH_2 2.99	8.03	4.11	1.79	γCH_2 1.36; δCH_2 1.69; εCH_2 2.98
^{111}His	8.06	4.58	3.32	4H 7.38; 2H 8.65	8.13	4.60	3.24	4H 7.32; 2H 8.64
^{112}Met	8.12	4.26	2.07	γCH_2 2.60; εCH_3 2.06	8.02	4.36	2.06	γCH_2 2.53; εCH_3 2.12
^{113}Ala	8.26	4.12	1.45	—	8.08	4.27	1.40	—
^{114}Gly	8.25	3.83	—	—	8.13	3.87	—	—
^{115}Ala	7.97	4.14	1.45	—	—	—	—	—
^{116}Ala	8.11	4.12	1.45	—	—	—	—	—
^{117}Ala	8.05	3.88	1.45	—	—	—	—	—
^{118}Ala	8.03	4.11	1.45	—	—	—	—	—
^{119}Gly	8.12	3.88	—	—	—	—	—	—
^{120}Ala	7.86	4.23	1.45	—	—	—	—	—
^{121}Val	7.81	3.89	2.17	γCH_3 0.98	—	—	—	—
^{122}Val	7.92	3.88	2.17	γCH_3 0.98	—	—	—	—
^{123}Gly	8.18	3.93	—	—	—	—	—	—
^{124}Gly	7.96	3.97	—	—	—	—	—	—
^{125}Leu	7.90	4.32	1.74	γCH_2 1.58; δCH_3 0.90		—	—	—
^{126}Gly	8.17	3.89	—	—	—	—	—	—
NH_2	—	—	—	7.30; 7.00	—	—	—	7.32; 7.02

[a] Chemical shifts are expressed in δ and referenced to internal TSP [sodium(trimethylsilyl)]propionate]. Values were measured in 0.17 M aqueous SDS_{-d25} 90% and 10% D_2O at pH 5.0 and 300 K. ND = not detected.

and Leu amino acid residues, may be present but could not be observed due to the overlapping with the huge signals of SDS. In the aromatic region a weak cross-peak was assigned to the interaction of the SDS C_1-H proton (δ 3.98) with the imidazole 4H proton (δ 7.38) (data not shown).

The observed intermolecular NOE contacts support the hypothesis that SDS molecules can interact with the amino acid residues located in the hydrophilic N-terminal region of the PrP[Ac-106–126-NH_2] sequence. The attractive electrostatic interactions between the anionic groups of SDS and the cationic groups of lysine or histidine may drive the organisation of the micelles around the peptide chain. Such an interaction is particularly interesting because it can contribute to the α-helical structuring in the presence of SDS micelles, whereas it may promote the conformational transition toward the β-sheet structure when the SDS concentration is just below the critical micellar concentration.[31] Our findings are consistent with results reported for the unblocked peptide fragment MoPrP106–126 in which the central portion of the peptide spanning the palindrome sequence has been determined to be involved in the core region of the β-sheet structure observed in the fibrils.[37] In addition, the absence of conformational effects in the micellar environment recently reported for a prion peptide fragment encompassing the residues 92–113 (therefore lacking the hydrophobic region), further support the critical role of the PrP[Ac-106–126-NH_2]'s hydrophobic region in the α-helix/β-sheet conformational conversion.[38]

It should be said that the unblocked PrP(106–126) analogue has been already shown to be able to adopt β-sheet conformation,[10] however, to the best of our knowledge, the CD results we obtained in the presence of different concentration of SDS show for the first time the capability of the N-acetylated and C-amidated form of PrP(106–126) to undergo a α-helical/β-sheet conformational transition.

The results obtained in the present study are interesting in the view of suggestions about the possible role of membranes and/or membrane components in the pathological conversion of PrP^{C} [39] and may allow us to speculate that side chain charge shielding of ionic amino acid residues, in conjunction with the hydrophobic environment of the biological membranes or contacts with the inner part of the PrP^{C} structured region, might represent the early events that trigger the pathological conversion of the physiological PrP^{C} into the pathological PrP^{Sc}.

Acknowledgements

We thank Ministero dell'Istruzione Università e Ricerca (MIUR) Grant No. 196 D.M. 1105/2002 and PRIN Project 2003031424. We thank Prof. Enrico Rizzarelli for helpful comments on the manuscript. Mrs. Tiziana Campagna is also acknowledged for skilful technical assistance.

References

1 S. B. Prusiner, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **98**, 13363–13383.
2 R. Zahn, A. Liu, T. Luhrs, R. Riek, C. von Schroetter, F. Lòpez Garcia, M. Billeter, L. Calzolai, G. Wider and K. Wuthrich, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 145–150.
3 F. Lopez Garcia, R. Zahn, R. Riek and K. Wüthrich, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 8334–8339.
4 D. G. Donne, J. H. Viles, D. Groth, I. Mehlhorn, T. L. James, F. E. Cohen, S. B. Prusiner, P. E. Wright and H. J. Dyson, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 13452–13457.
5 R. Riek, S. Hornemann, G. Wider, R. Glockshuber and K. Wüthrich, *FEBS Lett.*, 1997, **413**, 282–288.
6 (*a*) S. B. Prusiner, *New Engl. J. Med.*, 2001, **20**, 1516–1526; (*b*) K. M. Pan, M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlhorn, Z. Huang, R. J. Fletterick and F. E. Cohen, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 10962–10966.
7 (*a*) G. Forloni, N. Angeretti, R. Chiesa, E. Monzani, M. Salmona, O. Bugiani and F. Tagliavini, *Nature*, 1993, **362**, 543–546; (*b*) C. N. O'Donovan, D. Tobin and T. G. Cotter, *J. Biol. Chem.*, 2001, **276**, 43516–43523; (*c*) M. Ettaiche, R. Pichot, J. P. Vincent and J. Chabry, *J. Biol. Chem.*, 2000, **275**, 36487–36490.
8 C. Selvaggini, L. De Gioia, L. Cantu, E. Ghibaudi, L. Diomede, F. Passerini, G. Forloni, O. Bugiani, F. Tagliavini and M. Salmona, *Biochem. Biophys. Res. Commun.*, 1993, **194**, 1380–1386.
9 D. R. Brown, *J. Neurochem.*, 1999, **73**, 1105–1113 and references cited therein.
10 L. De Gioia, C. Selvaggini, E. Ghibaudi, L. Diomede, O. Bugiani, G. Forloni, F. Tagliavini and M. Salmona, *J. Biol. Chem.*, 1994, **269**, 7859–7862.
11 E. Ragg, F. Tagliavini, P. Malesani, L. Ponticelli, O. Bugiani, G. Forloni and M. Salmona, *Eur. J. Biochem.*, 1999, **266**, 1192–1201.
12 D. R. Brown, *Mol. Cell. Neurosci.*, 2000, **15**, 66–78.

13 M. F. Jobling, L. R. Stewart, A. R. White, C. McLean, A. Friedhuber, F. Maher, K. Beyreuther, C. L. Masters, C. J. Barrow, S. J. Collins and R. Cappai, *J. Neurochem.*, 1999, **73**, 1557–1565.
14 L. Kaurie and A. Culversen, *J. Neurosci. Res.*, 2000, **62**, 120–133.
15 M. C. Lin, T. Mirzabekov and B. L. Kagan, *J. Biol. Chem.*, 1997, **272**, 44–47.
16 D. Grasso, D. Milardi, C. La Rosa and E. Rizzarelli, *New J. Chem.*, 2001, **25**, 1543–1548.
17 J. I. Kourie and A. A. Shorthouse, *Am. J. Physiol. Cell. Physiol.*, 2000, **278**, C1063–C1087.
18 J. I. Kourie, *Chem.-Biol. Interact.*, 2001, **138**, 1–26.
19 (*a*) R. S. Hedge, J. A. Mastrianni, M. R. Scatt, K. A. Defra, P. Tremblay, M. Torchia, S. J. De Armond, S. B. Prusiner and V. R. Lingappa, *Science*, 1998, **279**, 827–834; (*b*) R. S. Hedge, P. Tremblas, D. Groth, S. J. De Armond, S. B. Prusiner and V. R. Lingappa, *Nature*, 1999, **402**, 822–826.
20 C. Forloni, V. Silei, M. Menegazzi, M. Salmona, O. Bugiani, F. Tagliavini, M. Suzuki and G. M. Lauro, *J. Biol. Chem.*, 2001, **276**, 25692–25696.
21 G. C. Chen and J. T. Yang, *Anal. Lett.*, 1977, **10**, 1195–1207.
22 N. R. Kallenbach, P. C. Lyu and H. Zhou, in *Circular Dicroism and the Conformation Analysis of Biomolecules*, ed. G. D. Fasman, Plenum, New York, 1996, p. 201.
23 (*a*) M. F. Jobling, X. Huang, L. R. Stewart, K. J. Barnham, C. Curtain, I. Volitakis, M. Perugini, A. R. White, R. A. Cherny, C. L. Masters, C. J. Barrow, S. J. Collins, A. I. Bush and R. Cappai, *Biochemistry*, 2001, **40**, 8073–8084; (*b*) M. Salmona, P. Malesani, L. De Gioia, S. Gorla, M. Bruschi, A. Molinari, F. Della Vedova, B. Pedrotti, M. A. Marrani, T. Awan, O. Bugiani, G. Forloni and F. Tagliavini, *Biochem. J.*, 1999, **342**, 207–214.
24 G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, 1965, **87**, 218–228.
25 N. Sreerama and R. W. Woody, *Anal. Biochem.*, 2000, **287**, 252–260.
26 (*a*) Y. H. Chen, J. T. Yang and K. H. Chau, *Biochemistry*, 1974, **13**, 3350–3359; (*b*) G. Merutka, D. Morokis, R. Brüschweiler and P. E. Wrigth, *Biochemistry*, 1993, **32**, 13089–13097; (*c*) J. M. Scholtz, J. Qian, E. J. York, J. M. Stewart and R. L. Baldwin, *Biopolymers*, 1991, **31**, 1463–1470; (*d*) P. C. Lyu, M. I. Liff, L. A. Marky and N. R. Kallenbach, *Science*, 1990, **250**, 669–673.
27 J. M. Scholtz and R. L. Baldwin, in *Peptides, Synthesis, Structures and Application*, ed. B. Gutte, 1995, Academic Press, p. 171.
28 (*a*) A. Jasanoff and A. R. Fersht, *Biochemistry*, 1994, **33**, 2129–2135; (*b*) M. E. Holtzer and A. Holtzer, *Biopolymers*, 1992, **32**, 1589–1591.
29 R. MonSerret, M. J. Mc Leish, A. Bockmann, C. Geourjan and F. Penin, *Biochemistry*, 2000, **39**, 8362–8373.
30 R. M. Brito and W. L. Vaz, *Anal. Biochem.*, 1986, **152**, 250–255.
31 T. A. Pertinez, M. Bouchard, R. A. G. Smith, C. M. Dobson and L. J. Smith, *FEBS Lett.*, 2002, **529**, 193–197.
32 K. Wüthrich, *NMR of proteins and nucleic acid*, 1986, Wiley, New York.
33 (*a*) A. Pardi, G. Wagner and K. Wüthrich, *Eur. J. Biochem.*, 1983, **137**, 445–454; (*b*) A. Pastore and V. Saudek, *J. Magn. Reson.*, 1990, **90**, 165–176; (*c*) D. S. Wishart, B. D. Sykes and F. M. Richards, *FEBS Lett.*, 1991, **293**, 72–80; (*d*) D. S. Wishart, B. D. Sykes and F. M. Richards, *J. Mol. Biol.*, 1991, **222**, 311–323; (*e*) D. S. Wishart, B. D. Sykes and F. M. Richards, *Biochemistry*, 1992, **31**, 1647–1651; (*f*) J. Rizo, F. J. Blanco, B. Kobe, M. D. Bruch and L. M. Gierasch, *Biochemistry*, 1993, **32**, 4881–4894; (*g*) D. S. Wishart, C. G. Bigam, A. Holm, R. S. Hodges and B. D. Sykes, *J. Biomol. NMR*, 1995, **5**, 67–81.
34 J. Rizo, F. J. Blanco, B. Kobe, M. D. Bruch and L. M. Gierash, *Biochemistry*, 1993, **32**, 4881–4894.
35 G. L. Millhauser, C. J. Stenland, P. Hanson, K. A. Bolin and F. J. M. van de Ven, *J. Mol. Biol.*, 1997, **267**, 963–974.
36 G. Wong, W. D. Treleaven and R. J. Cushley, *Biochim. Biophys. Acta*, 1996, **1301**, 174–184.
37 K. Kuwata, T. Matumoto, H. Cheng, K. Nagayama, T. L. James and H. Roder, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 14790–14795.
38 C. Renner, S. Fiori, F. Fiorino, D. Landgraf, D. Delica, M. Mentler, K. Grantner, F. G. Parak, H. Kretzschmar and L. Moroder, *Biopolymers*, 2004, **73**, 421–433.
39 (*a*) M. Vey, S. Pilkuhn, H. Wille, R. Nixon, S. J. DeArmond, E. J. Smart, R. G. Anderson, A. Taraboulos and S. B. Prusiner, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 14945–14949; (*b*) A. Taraboulos, M. Scott, A. Semenov, D. Avrahami, L. Laszlo, S. B. Prusiner and D. Avrahami, *J. Cell Biol.*, 1995, **129**, 121–132.