FEBS Letters 405 (1997) 378–384 FEBS 18365

Conformational properties of the prion octa-repeat and hydrophobic sequences

Corinne J. Smith[a], Alex F. Drake[b], Beaulah A. Banfield[b], Graham B. Bloomberg[a], Mark S. Palmer[c], Anthony R. Clarke[a,*], John Collinge[c]

[a] *Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK*
[b] *EPSRC National Chiroptical Spectroscopy Service (Birkbeck College), 20 Gordon Street, London WC1H 0AJ, UK*
[c] *Neurogenetics Unit, Department of Biochemistry, St Mary's Medical School, Imperial College, Norfolk Place, London W2 1PG, UK*

Received 19 February 1997

Abstract We have used circular dichroism to study synthetic peptides from two important regions of the prion protein: the N-terminal octa-repeat domain and a highly conserved hydrophobic section. Our results show that the octa-repeat sequence in free solution can adopt a non-random, extended conformation with properties similar to the poly-L-proline type II left-handed helix. We also show that the conformation can be changed by temperature, organic solvents (e.g. acetonitrile) and on binding to phospholipid vesicles. We compared CD data from two peptides corresponding to the hydrophobic region between residues 106 and 136 which contained either methionine or valine at position 129. This variation represents a common polymorphism in humans which has been shown to influence predisposition towards iatrogenic and sporadic CJD. There was no detectable difference between the CD spectra of these peptides irrespective of the solvent conditions we used.

Key words: Circular dichroism; PrP^{Sc}; Peptide; Fluorescence; Scrapie

1. Introduction

The prion diseases are transmissible neurodegenerative conditions which include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and kuru in humans; and scrapie and bovine spongiform encephalopathy (BSE) in animals. Research interest in this area has been intense because of the unique properties of the transmissible agent or prion.

The nature of the prion has been the subject of strong debate for many years since, despite its infectious properties, no significant nucleic acid content has been found [1–6]. Instead, a wide body of data now supports the idea that the transmissible agent consists of an abnormal isoform of a host-encoded sialoglycoprotein, the prion protein (PrP), designated PrP^{Sc} (for review see reference [7]). In all the prion diseases PrP^{Sc} accumulates in affected brains and forms partially protease-resistant aggregates and sometimes amyloid deposits. The normal form of the protein, PrP^{C}, is found predominantly on the surface of neurones, attached by a glycosylphosphatidylinositol (GPI) anchor [8–11]. It has therefore been suggested that it may have a role in cell adhesion or signalling. There is little insight into its function although electrophysiological studies in PrP-null mice have shown that PrP is necessary for normal synaptic function [12].

PrP^{Sc} is derived from PrP^{C} by a post-translational mechanism [13–16]. Systematic study of known covalent post-translational modifications has shown no consistent differences between PrP^{C} and PrP^{Sc} [17]. By exclusion, therefore, it has been assumed that the difference lies in a conformational change. Evidence for differences in secondary structure content between the two isoforms has been provided by circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopic methods. These techniques show PrP^{C} to be rich in α-helix while PrP^{Sc} is seen to have a high β-sheet content [18].

The current model of prion propagation is that PrP^{Sc} interacts directly with PrP^{C}, promoting its conversion to additional PrP^{Sc} [19,20]. Such a pathogenic mechanism was originally proposed as a result of transgenetic and human molecular genetic studies which demonstrate the importance of PrP sequence identity for the initiation and progression of prion diseases [19,21–25]. The production of protease-resistant PrP from PrP^{C} has now been shown in vitro although it has not yet been possible to demonstrate infectivity of the protease-resistant form [26].

Recently a solution structure of a core region of mouse PrP corresponding to residues 121–231 has been determined [27]. However this does not include the N-terminal octa-repeat region of the protein and lacks part of the hydrophobic region. Here we report studies on these two unusual segments of polypeptide. Extensive human molecular genetic data indicates that these regions are of key importance in the pathogenesis of prion diseases. The first region consists of 4 glycine-rich, tandemly repeating, octapeptide units following a non-apeptide of related sequence. This sequence represents a unique structural motif, having no significant homology to other sequences in the protein database. Expansions of this region, by the insertion of additional integral numbers of tandem octa-repeat elements, are seen in families with inherited prion diseases. Kindreds with an additional 2, 4, 5, 6, 7, 8, and 9 octa-repeats have been described [28–34]. Conversely, deletional mutations, deleting a single octa-repeat element, are non-pathogenic and are uncommon polymorphisms in the normal population [35,36]. Interestingly, comparison with chicken PrP, which shows an overall similarity of only around 40%, [37,38] shows the chicken sequence to contain a series of tandem hexapeptide repeats at the same position in the sequence as the octapeptide repeats in mammalian species implying an evolutionary requirement for somewhat similar repeating units across mammalian and avian species at this position.

*Corresponding author. Fax: (44) (1179) 288274.

Abbreviations: TEA, triethanolamine hydrochloride; CD, circular dichroism; SDS, sodium dodecyl sulphate

PII S0014-5793(97)00220-2

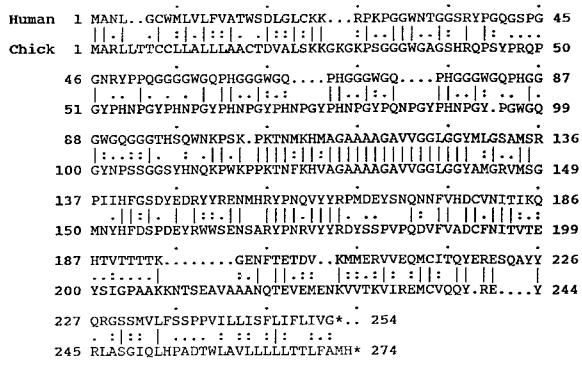

Fig. 1. Comparison of human and chicken sequences using a Wisconsin GCG alignment program. The percentage similarity was 59% and the percentage identity was 41%. Of particular note is the region of almost exact identity within the hydrophobic domain (residues 126–141 of the chicken sequence). Although there is no direct homology to the human octa-repeat region the chicken sequence contains an array of sexapeptide repeats at the same position.

The second region we study is a hydrophobic region between residues 113 and 128 (in Fig. 1) which is totally conserved across chicken and mammalian sequences presently known. Before experiments indicated that PrP^C was releasable from the cell surface it was proposed that this formed a transmembrane helix [39–41]. The region has been predicted to be helical in the native protein while the recent NMR structure of the core section (residues 121–231) of mouse PrP shows that part of it forms a β strand. Short peptides produced corresponding to residues 106–126 have a number of interesting properties in that they show amyloid-like features and are toxic to cultured neuronal cells [42,43]. Some of the following studies are based on the knowledge that individuals homozygous for the common polymorphism of methionine or valine at codon 129, which falls within the region proposed to be a hydrophobic helix, are more likely to succumb to either iatrogenic or the sporadic forms of prion disease [21]. This suggests that a highly specific interaction must take place between the infectious particle and the host reserve of PrP^C and that the interaction is influenced by the presence of methionine or valine at codon 129.

Interestingly, a sub-type of inherited prion disease has been described with a point mutation in codon 145 resulting in a Tyr→STOP change in the protein [44]. Affected individuals therefore express a truncated PrP which consists almost entirely of the octa-repeat region and the hydrophobic sequence. This genetic evidence further highlights their functional importance.

2. Materials and methods

2.1. Materials

Triethanolamine hydrochloride was from Boehringer Mannheim, sodium chloride, acetic acid and sodium acetate, Analar grade and HPLC grade acetonitrile were from BDH Chemicals. Grade I egg lecithin and phosphatidylserine from bovine spinal cord were from Lipid Products, South Nutfield, UK.

2.2. Preparation of peptides

The four peptides were synthesised by the Bristol University Peptide synthesis unit. The 16-residue octa-repeat peptide was acetylated at the N-terminus. The peptides were made on a Milligen 9050 automatic peptide synthesiser using Fmoc chemistry with HBTU (*o*-benzotriazol-*N*,*N*,*N*′,*N*′-tetra-methyl-uronium-hexafluoro-phosphate) activation on a Milligen PEG-PS resin using customised protocols. Side-chain protection was as follows: trityl for Gln and His, *t*-butyl for tyrosine and protonation for arginine. After completion of the synthesis the resin was washed with methanol and dichloromethane and dried under high vacuum overnight. Peptides were cleaved from the resin using TFA:ethanediol:anisole:H_2O in the ratio 94:2:2:2 for 2 h. The peptides were recovered by ether precipitation. The short (16-residue) octa-repeat peptide was purified by HPLC to over 90% purity. Plasma desorption mass spectrometry was conducted on an Applied Biosystems Bio-Ion spectrometer and showed a major peak of molecular weight 1649. The long (46 residue) octa-repeat peptide was of sufficient purity (above 90%) to be used directly. Electrospray mass spectrometry performed on a VG Quattro spectrometer showed this peptide to be one major component of molecular weight 4481 ± 0.64. The 31-residue 'hydrophobic' peptides were of sufficient purity to be used directly (about 80%). All peptides were freeze-dried before further use and all peptides were soluble in water (e.g. with concentrations of over 15 mg/ml obtainable for both hydrophobic peptide variants).

The amino acid sequences of the synthetic peptides are as follows (reading from N- to C- terminus).

(1) Short octa-repeat peptide
*R*YPPQGGGGWGQP*H*GG
(2) Long octa-repeat peptide
*R*YPPQG(GGGWGQP*H*)$_4$GGGWGQGG
(3) Met variant of hydrophobic peptide
*K*TNM*KH*MAGAAAAGAVVGGLGGYMLGSAMS*R*
(4) Val variant of hydrophobic peptide
*K*TNM*KH*MAGAAAAGAVVGGLGGYVLGSAMS*R*

Highlighted in bold are the residues representing the polymorphism at codon 129 in humans and highlighted with italics are those residues which contribute to the charge of the peptides. Overall, all the peptides carry a positive charge at neutral pH.

2.3. Circular dichroism measurements

2.3.1. Single octa-repeat peptide. Circular dichroism measurements were conducted using a Jasco J600 spectropolarimeter. Samples of the single-repeat peptide were at 0.5 mg/ml in 50 mM TEA buffer containing at least 50 mM acetate at pH 7.4 unless stated otherwise. The pH was adjusted using acetic acid or sodium hydroxide. A cell of path length 0.5 mm was used except where stated otherwise. Scans were conducted between 190 and 260 nm at a scan speed of 10nm/min with a sensitivity of 10 mdeg. The temperature was measured to an accuracy of ±2° with a probe directly inserted in the sample.

2.3.2. Long octa-repeat peptide and hydrophobic peptides. Circular dichroism studies were performed using a Jobin-Yvon CD6 spectropolarimeter. Typically data were collected in a 1 mm path length cell containing 0.2 mg/ml peptide dissolved in water or in a 0.5 mm path length cell with a protein concentration of 0.4 mg/ml. Scans were conducted at a resolution of 0.2 nm with an integration time per step of 1 s unless stated otherwise.

2.3.3. Fluorescence studies. Fluorescence work was performed on a Spex FluoroMax spectrofluorimeter. Fluorescence emission scans were conducted using an excitation wavelength of 278 nm. The peptide concentration used was 0.025 mg/ml. The measurements were conducted either in water or 50 mM triethanolamine hydrochloride/50 mM acetate buffer pH 6.0.

2.3.4. Preparation of phospholipid vesicles. Phosphatidylcholine (PC) and phosphatidylserine (PS) were mixed in the ratio 1:4 PS to PC. 20 mg of lipid (concentration deduced from product details) were taken and the solvent removed firstly by flushing with N_2 gas and then by desiccation under vacuum (for 10 min). The lipid was resuspended in 1 ml of water and the mixture sonicated for 5×10 s using a microtip at 75% of the maximum power for that tip or until the milky solution became clearer and opalescent. The vesicle suspension was spun at 13 500 rpm in a microfuge for 2 min to remove larger particles and stored at 4°C.

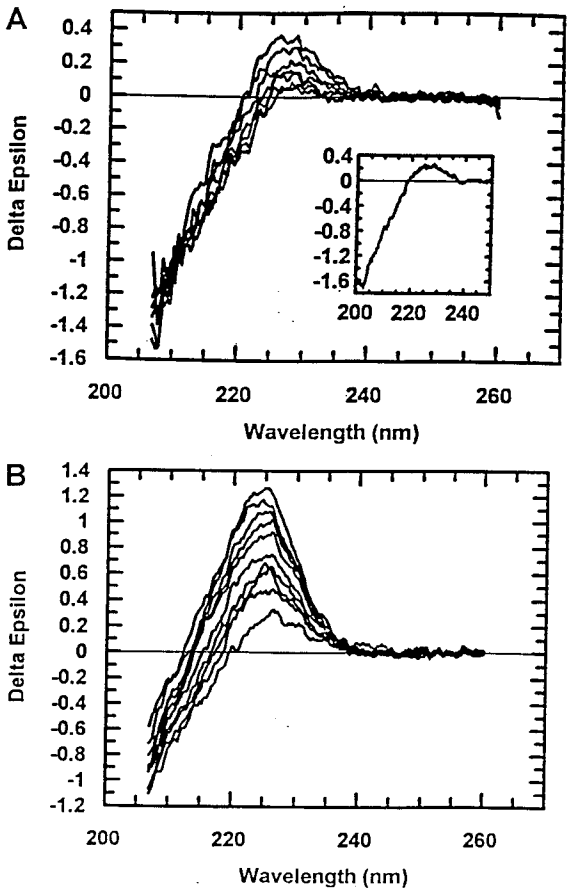

Fig. 2. A: Circular dichroism spectra of the short octa-repeat peptide showing the effect of temperature on the spectrum. The peptide concentration was 0.5 mg/ml and the cell was of path length 0.5 mm. All samples were dissolved in 50 mM TEA and 50 mM acetate pH 7.4. The temperatures were (highest peak) 24.6°, 33.9°, 44.0°, 53.9°, 61.7°, 70.4°, and 79.1°C (lowest peak). Inset: Spectrum of short octa-repeat peptide in water alone. The peptide concentration was 0.4 mg/ml and the cell path length was 0.5 mm. The axis labels are identical to those in the main diagram. B: Cryogenic spectra of single repeat peptide in ethanediol:water mixture (2:1). The peptide concentration was 0.5 mg/ml and the cell was of path length 0.5 mm. The temperature for each spectrum is as follows (highest peak): −91.3°, −78.5°, −68.1°, −57.4°, −48.7°, −32.3°, −22.8°, 19.4°, −13.1° and −3.1°C (lowest peak). The units of delta epsilon are cm^{-1} mol^{-1}.

3. Results

3.1. Circular dichroism studies on the single and full-length octa-repeat peptides

3.1.1. Aqueous conditions. Fig. 2A shows CD spectra given by the 16-residue octa-repeat peptide in buffer at pH 7.4 with inset showing the spectrum in water. Interestingly the short peptide (of 16 residues) gives a distinctive CD spectrum which is highly unusual for a short peptide at room temperature. The shape of the curve is unlike any CD spectra characteristic of α-helix, β-sheet or random coil secondary structure. There is a broad maximum between 225 and 230 nm which decreases towards 210 nm in water, and there is a minimum at around 202 nm. Fig. 3 shows the CD spectrum of the full-length (46-residue) octa-repeat peptide in water at room temperature. It too has a broad maximum peak between 225 and 230 nm with a trough between 202 and 207 nm. Comparison of spectra of the long octa-repeat peptide taken at 0.4 mg/ml in 0.5 mm path length cell and 0.02 mg/ml in a 10 mm path length cell in water showed no concentration dependence of this CD signal within experimental error, suggesting no alteration in conformation as a result of association of the peptide (data not shown).

Raising the temperature resulted in a decrease in the peak at 228 nm for both peptides which gave a spectrum more similar to a random coil conformation (Figs. 2A and 3). In both peptides the peak shifted 2 or 3 nm towards longer wavelengths. The peak at 228 nm was restored on cooling the short peptide to room temperature.

Cooling the shorter peptide to cryogenic temperatures (Fig. 2B) in an ethanediol/water mixture (2:1) caused the peak at 228 to become more intense and to shift to a lower wavelength (225 nm). The effect of cryogenic temperatures on this conformation was fully reversed when the sample returned to room temperature. The large increase in signal at low temperature shows that only a small proportion of the conformation detected is present at room temperature and even at the lowest temperature (−91°C) the increase had not reached its end-point.

The data given in Figs. 2 and 3 suggest that both peptides adopt some kind of non-random conformation which can be melted out at higher temperatures and (in the case of the shorter peptide) stabilised by cooling. We next added the protein denaturants, urea and guanidine hydrochloride, to the shorter peptide to determine whether this conformation could be denatured. Interestingly (see Fig. 4A) these agents induced an increase rather than a decrease in the signal at 228 nm implying that such a conformation was stabilised by the action of these chaotropic agents. This in turn suggests an unusually extended conformation. Varying the pH between 4 and 8 did not significantly affect the spectrum of the shorter peptide. Spectra of the long octa-repeat in 20 mM TEA/20 mM acetate buffer at pH 4 and pH 8 showed that the maximum at around 228 nm was reduced by a small amount at pH 8 compared to pH 4 while the minimum at around 202 nm was slightly raised at pH 8 compared to that at pH 4 (data

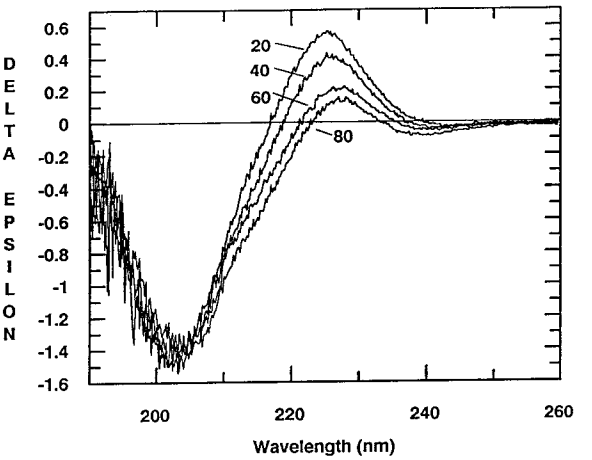

Fig. 3. Effect of temperature on the long octa-repeat peptide. The spectra were obtained at 20, 40, 60 and 80°C. The peptide concentration was 0.4 mg/ml and the cell was of path length 0.5 mm. The spectra collected were the result of 20 accumulated scans at a resolution of 0.2 nm with an integration time of 1 s. The units of delta epsilon are cm^{-1} mol^{-1}.

not shown). The four histidine residues present in this peptide could explain this observation which suggests that their protonation has a weak stabilising effect on the conformation of the long octa-repeat peptide.

3.1.2. Effect of non-polar solvents. In view of the importance of a conformational change in prion pathogenesis we decided to investigate the possibility that a second conformation is available to the octa-repeat region. We investigated the effect of more hydrophobic solvents on the conformation of the single repeat peptide. Fig. 4B shows the effect of the solvents, *n*-propanol, trifluoroethanol and acetonitrile on the CD spectrum of the 16-residue octa-repeat. These solvents caused a reduction in the intensity of the positive CD signal at around 228 nm and an increase in the negative CD signal at approximately 202 nm. It is worthy of note that 100% trifluoroethanol does not induce α-helix in this peptide. In acetonitrile the spectrum contains two weak maxima at around 209 and 232 nm, the minimum between occurring at 217 nm. The spectrum does not resemble spectra given by any one type of secondary structure [45–48] nor is it close to spectra generally seen for proteins. Of the standard spectra, those for various types of β-turn [47] and the type I poly-L-proline [45] spectra

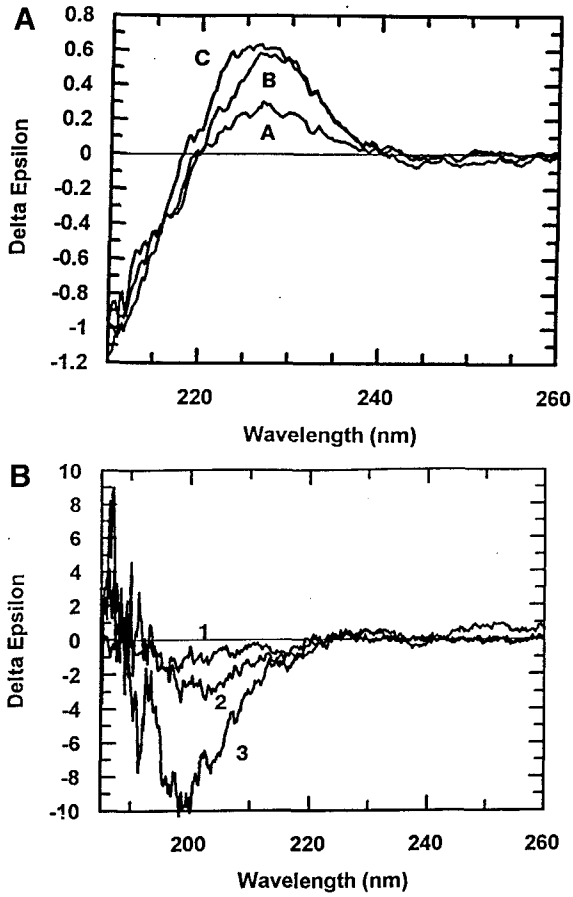

Fig. 4. A: The effect of chemical denaturants on the CD spectrum of the single repeat peptide. **A** is recorded in 50 mM TEA/50 mM acetate buffer pH 7.4 alone, **B** in 8 M guanidine hydrochloride dissolved in the same buffer and **C** in 10 M urea dissolved in the same buffer. B: Circular dichroism spectra of single-repeat peptide in organic solvents. **1** is 98% acetonitrile (in water), **2** 100% *n*-propanol and **3** 100% trifluoroethanol. The units of delta epsilon are cm^{-1} mol^{-1}.

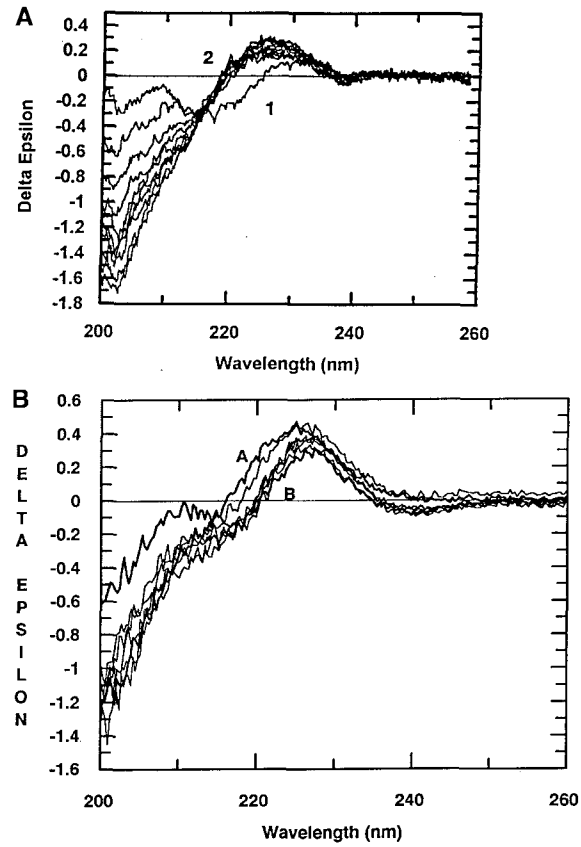

Fig. 5. A: Acetonitrile/water titration of single octa-repeat peptide. The acetonitrile concentrations (from 1 to 2) were 95, 90, 80, 70, 60, 50, 40, 30, 20, 9 and 0% (v/v acetonitrile/water). The peptide concentration was 0.4 mg/ml and the cell was of path length 0.5 mm. B: Circular dichroism of long octa-repeat peptide. The graph shows an acetonitrile titration with concentrations of 0, 20, 40, 60, 70, 80 and 90% acetonitrile in water (v/v). **A** indicates the 0% acetonitrile spectrum (shown in bold print) and **B** the 90% acetonitrile spectrum (shown in bold print). The cell was of path length 1 mm and the peptide concentration was 0.2 mg/ml. Spectra were recorded at 25°C and displayed as a spline fit for clarity. The units of delta epsilon are cm^{-1} mol^{-1}.

have a strong maximum at around 202–206 nm and at 215 nm respectively. These features could explain the development of a maximum at 209 nm in the octa-repeat peptide. The acetonitrile titration (Fig. 5A) of the peptide exhibited an isodichroic point at around 215 nm which indicates an equilibrium between two conformational states. The effect of acetonitrile therefore appears to be to stabilise an alternative conformation to that present in aqueous solution.

Fig. 5B also shows the CD spectrum of the full-length (46-residue) octa-repeat peptide in water/acetonitrile mixtures. The spectrum given by the longer peptide in water is similar to that given by the shorter octa-repeat peptide. The effect of acetonitrile in the full-length octa-repeat peptide resembles the titration using the single octa-repeat peptide in that a peak at around 210 nm can be seen to develop as the concentration of acetonitrile increases.

3.2. Interaction of full-length octa-repeat peptide with vesicles

Since PrP^C is a membrane-anchored protein we investigated the interaction of the longer peptide with membranes. To this end we used phospholipid vesicles as a simple analogue of a

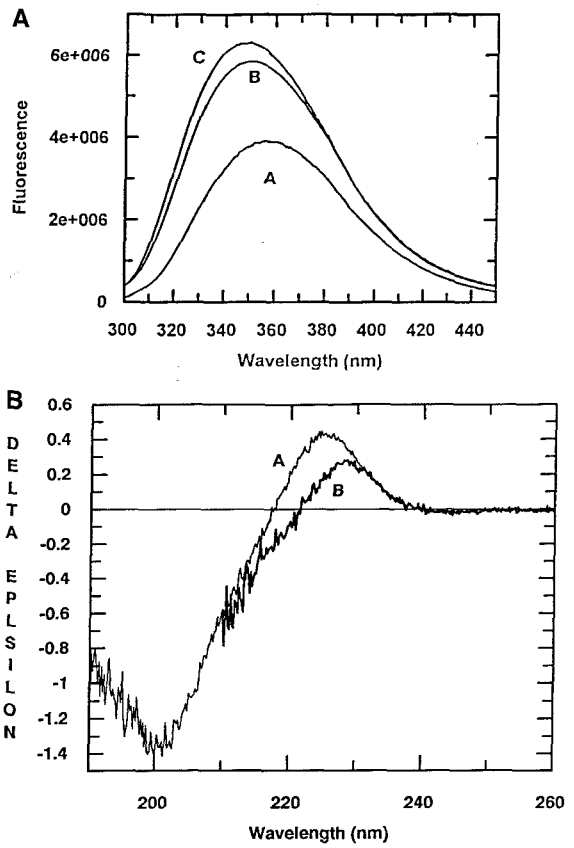

Fig. 6. A: Fluorescence (arbitrary units) emission spectra of long octa-repeat peptide in the presence of phospholipid vesicles. The excitation wavelength was 278 nm and the peptide concentration was 0.025 mg/ml. **A** is the emission spectrum of peptide alone, **B** is the corrected spectrum in the presence of 10 μl (approx 0.1 mg/ml) of the PS/PC vesicle suspension whilst **C** is the emission spectrum with 20 μl of vesicle suspension at which the effect saturates. B: Circular dichroism spectra of long octa-repeat peptide in the absence (**A**) and presence (**B**) of vesicles containing phosphatidylcholine and phosphatidylserine. **A** is the spectrum given by 0.2 mg/ml peptide with the water baseline subtracted. **B** is given by the same concentration of peptide in the presence of 30 μl PS/PC vesicles (approx 2 mg/ml) with the vesicle background subtracted. The spectra collected were the result of 5 accumulated scans at a resolution of 0.2 nm with an integration time of 1 s. The units of delta epsilon are cm^{-1} mol^{-1}.

cell membrane. We added phospholipid vesicles containing 20% phosphatidylserine and 80% phosphatidylcholine to the 46-residue octa-repeat and obtained a saturable enhancement of fluorescence emission intensity, the peak of which was blue-shifted from 357 nm to 345 nm (see Fig. 6A). The blue shift observed on binding is indicative of a change in the environment of the tryptophan residues within the peptide from highly aqueous surroundings to a more hydrophobic environment. The effect was most marked in water at below neutral pH. In buffered solutions the higher ionic strength weakened the binding; in 20 mM TEA no binding was observed at pH 7.4. In buffer at pH 6 (which contained 50 mM TEA and 50 mM sodium acetate) binding was observed, with an associated smaller fluorescence blue shift and smaller enhancement of fluorescence. Binding could not be demonstrated with vesicles containing only phosphatidylcholine. Hence the 46-residue octa-repeat binds to phosphatidylserine in vesicles at below neutral pH and low ionic strength. Since phosphatidylcholine is neutral and phosphatidylserine is negatively charged it seems likely that this binding is aided by a charge interaction dependent on protonation of the four histidine residues present in this peptide.

Fig. 6B shows the effect of phospholipid vesicles containing phosphatidylcholine and phosphatidylserine on the conformation of the long octa-repeat peptide under conditions in which vesicles have been shown to bind. Both spectra are corrected for the background effects of vesicles and water. Addition of vesicles causes the spectrum to alter significantly, implying that a conformational change is induced. These data do not permit full interpretation of the structure but it is interesting to note that this spectrum has similar characteristics to that obtained with the hydrophobic solvent, acetonitrile.

3.3. *The hydrophobic polypeptide corresponding to residues 106–136*

CD spectra of both variants of the hydrophobic peptide in water were characteristic of a structureless peptide (see Fig. 7) with a minimum at around 197 nm and no evidence of minima between 210 and 250 nm which might imply the presence of α-helix or β-sheet. In 1% SDS the CD spectra suggested a proportion of α-helical content (see Fig. 7) displaying two minima at around 207 and 220 nm and a maximum at around 192 nm.

In order to determine the effect of the presence of methionine or valine at codon 129 on the conformation of the 'hydrophobic' peptides made for this study we obtained CD spectra for each peptide in water and in 1% SDS (which induces helix formation and provides a membrane-like environment). Spectra were identical within the limits of experimental error allowing us to conclude that the difference in amino acid sequence did not affect the conformation adopted by the peptides as detected by circular dichroism.

Since it is thought that this region of the protein may be involved in dimerisation we mixed the two variants in a ratio

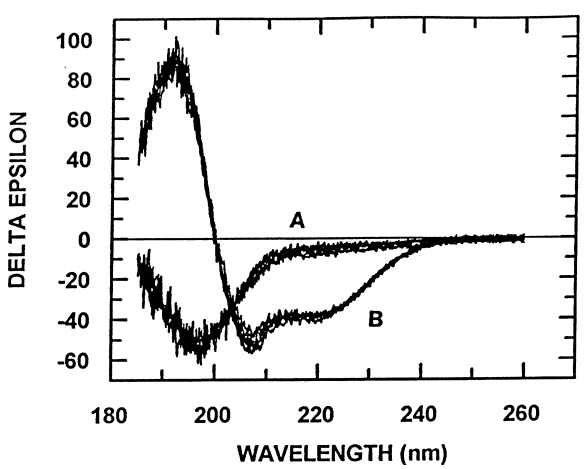

Fig. 7. Hydrophobic peptide 106–136 spectra overlaid to show their similarity. A: Spectra obtained in water for 0.4 mg/ml of the methionine variant of the hydrophobic peptide 106–136 alone, the valine variant of the peptide alone and a 1:1 mixture of the methionine and valine variants (containing 0.2 mg/ml of methionine variant and 0.2 mg/ml of valine variant). B: The same spectra as A but in this case 1% SDS in water was used as the solvent. The cell was of path length 0.5 mm in all cases. Spectra were collected at a resolution of 0.2 nm with an integration time of 2 s. The units of delta epsilon are cm^{-1} mol^{-1}.

of 1:1 and compared the resulting spectra both in water and 1% SDS. Again the spectra were identical within experimental error to those obtained for unmixed peptide.

We also investigated the concentration dependence of the shape of the spectra of these peptides in water by taking measurements at a concentration of 0.4 mg/ml in a 0.5 mm path length cell and 0.02 mg/ml in a 10 mm path length cell. There was no significant effect of this 20-fold difference in concentration on the shape of the spectrum of either the methionine variant of the valine variant in water implying that association or dissociation of the peptide chains either did not occur or did not affect their conformation (data not shown). Spectra of each of these peptides obtained in buffer (20 mM TEA, 20 mM acetate) at pH 4 and pH 8 at 0.4 mg/ml in a 0.5 mm cell also showed no significant variation within experimental error.

4. Discussion

4.1. Octa-repeat peptides

Although a solution structure for a core section of mouse PrP [27] has now been determined this does not include the N-terminal octa-repeat region of the protein. While we do not present a determination of the structure of this region these data provide some clues to its nature. It is interesting to note that the spectra we have obtained strongly resemble spectra obtained for a poly-L-proline type II left-handed extended helix [49] which has a minimum at 207 nm and a weak broad maximum at 227 nm (our results for the long octa-repeat peptide show a minimum between 202 and 207 nm and a weak broad maximum between 225 and 230 nm). Our spectra are also very similar to those obtained by Tathamet al. [50] who suggest that a peptide corresponding to a repeating region within the protein C hordein and C hordein itself can adopt the poly-proline type II helix at low temperature. The sequence of the C hordein repeat does contain proline but also has glutamine and phenylalanine. Our sequence is not rich in proline but there is a high content of glycine and a similar collagen-like structure has been reported for poly-glycine peptides [51]. Unfortunately, NMR studies carried out by us to confirm the presence of this conformation were complicated by the presence of overlapping glycine resonances. However, the CD spectra of both long and short octa-repeat peptides are affected by changes in temperature which implies the presence of a proportion of non-random structure.

What is striking about this structure is its apparent stability. A detectable proportion of the characteristic poly-proline type II spectrum is seen at room temperature even with a peptide of 16 residues. Where similar spectra have been observed [50] these were obtained only at temperatures lower than 0°C. Reassuringly the spectra obtained for the longer peptide which corresponds to essentially all the octa-repeat region have similar characteristics to those given by the short peptide corresponding to only a single repeat. The chaotropic agents urea and guanidine hydrochloride, which normally destroy ordered structure by reducing the hydrophobic effect, appear to stabilise rather than disrupt the structure of the short peptide. Also an environment which is thought to promote the formation of backbone hydrogen bonds, namely 100% TFE, causes its spectrum to change to one reminiscent of random coil. The fluorescence emission maximum of the tryptophan residues in the long peptide is at 357 nm which implies a highly solvated environment consistent with an extended conformation. Whether or not the octa-repeat adopts a poly-proline type II left-handed helix these results show the peptide to be able to adopt a non-random, extended conformation which is resistant to the formation of helix in trifluoroethanol.

In the less polar environment provided by acetonitrile both octa-repeat peptides alter conformation. This suggests that a hydrophobic environment may induce a conformational change in the octa-repeat region. A further property of the longer octa-repeat peptide is its ability to bind to phospholipid vesicles in conditions of low salt and below neutral pH with a change in tryptophan fluorescence consistent with its protection from the aqueous solvent. The CD spectrum also changes for the vesicle-bound peptide implying an accompanying conformational change with binding.

The ability of the mouse PrP 121–231 domain [27] to fold independently implies that the octa repeat is not required for stability. Furthermore, since this peptide does not fold in a classical way and cannot be induced to fold by trifluoroethanol it is possible that a requirement of this domain is specifically not to fold but to remain extended, perhaps in order to promote non-specific binding to a membrane or to other proteins. We know that in chicken the analogous repeat region is required for endocytosis [52] of PrP^{C}, a fact which would suggest that the repeat region may be involved in binding to other proteins.

The octa-repeat region has no direct homologue in other proteins, however, it is interesting to note that the synaptic vesicle-associated proteins synapsin I and VAMP-I contain repeating sequences rich in glycine, glutamine or proline which are thought to have a role in binding to vesicles and the cytoskeleton [53–55]. Given the finding [12] that PrP is necessary for normal synaptic function and its known localisation at the surface of neuronal cells it is possible that these repeating sequences, although not directly homologous, share a similar function with the octa-repeat region of PrP.

4.2. The hydrophobic polypeptide corresponding to residues 106–136

In order to investigate possible structural effects of the polymorphism at codon 129 on disease susceptibility we compared the CD spectra of two peptides to the hydrophobic region identical but for a single change corresponding to either the methionine or the valine variant at codon 129. We chose to study a sequence longer than other peptides studied so far [56], so as to include the whole 23 amino acid hydrophobic sequence flanked by the more hydrophilic surrounding residues. In this way we hoped to minimise the effects of studying the sequence in isolation from the whole protein. Much of this region (106–120) is not included in the mouse prion domain 121–231 NMR structure.

The structural behaviour of this hydrophobic sequence resembles that of transmembrane helical peptides which become helical in SDS and phospholipid vesicles. There are also similarities to the behaviour of the shorter peptide (PrP 106–126) studied previously [56]. In SDS PrP106–126 adopts an α-helical conformation while in water a random coil conformation dominates the spectrum (around 60%). There is a difference, however, in that PrP 106–126 in water does possess a proportion of secondary structure(around 13% α-helix and around 20% β-sheet) while our peptide (corresponding to residues

106–136) gives no strong indication of adopting ordered secondary structure in water.

It might be expected that the possibility of an interaction between PrP^{C} and PrP^{Sc} in the region of codon 129 would be reflected in differences in the structural behaviour of the two peptides containing this change. This turns out not to be the case. Comparing the spectra of the methionine and valine peptide variants shows no effect within experimental error of the substitution on the conformation of the peptides either in SDS or in water. Mixing these peptides or carrying out measurements at different concentrations provided no evidence for self-association or dissociation which might influence conformation under these conditions.

Acknowledgements: This work was funded by the Wellcome Trust. A.R.C. is a Lister Fellow. We are grateful for access to the EPSRC National Chiroptical Spectroscopy Service.

References

[1] T. Alper, D.A. Haig, M.C. Clarke, Biochem. Biophys. Res. Commun. 22 (1966) 278–284.
[2] T. Alper, W.A. Cramp, D.A. Haig, M.C. Clarke, Nature 214 (1967) 764–766.
[3] G.D. Hunter, J. Infect. Dis. 125 (1972) 427–440.
[4] S.B. Prusiner, Science 216 (1982) 136–144.
[5] N. Meyer, V. Rosenbaum, B. Schmidt, et al. J. Gen. Virol. 72 (1991) 37–49.
[6] K. Kellings, N. Meyer, C. Mirenda, S.B. Prusiner, D. Riesner, J. Gen. Virol. 73 (1992) 1025–1029.
[7] S.B. Prusiner, Science 252 (1991) 1515–1522.
[8] D.R. Borchelt, M. Scott, A. Taraboulos, N. Stahl, S.B. Prusiner, J. Cell Biol. 110 (1990) 743–752.
[9] N. Stahl, D.R. Borchelt, S.B. Prusiner, Biochemistry 29 (1990) 5405–5412.
[10] N. Stahl, D.R. Borchelt, K. Hsiao, S.B. Prusiner, Cell 51 (1987) 229–240.
[11] D. Westaway, S.B. Prusiner, Nucleic Acids Res. 14 (1986) 2035–2044.
[12] J. Collinge, M.A. Whittington, K.C.L. Sidle, et al. Nature 370 (1994) 295–297.
[13] D.R. Borchelt, M. Scott, A. Tarabolous, N. Stahl, S.B. Prusiner, J. Cell Biol. 110 (1990) 743–752.
[14] D.R. Borchelt, A. Tarabolous, S.B. Prusiner, J. Biol. Chem. 267 (1992) 16188–16199.
[15] B. Caughey, R.E. Race, D. Ernst, M.J. Buchmeier, B. Chesebro, J. Virol. 63 (1989) 175–181.
[16] B. Caughey, G.J. Raymond, J. Biol. Chem. 226 (1991) 18217–18223.
[17] Stahl, N., Baldwin, M.A., Teplow, D. et al. (1992) in: Prion Diseases of Humans and Animals (Prusiner, S.B., Collinge, J., Powell, J. and Anderton, B., Eds.), pp. 361–379, Ellis Horwood, London.
[18] K. Pan, M.A. Baldwin, J. Nguyen, et al. Proc. Natl. Acad. Sci. USA 90 (1993) 10962–10966.
[19] S.B. Prusiner, M. Scott, D. Foster, et al. Cell 63 (1990) 673–686.
[20] C. Weissmann, Nature 349 (1991) 569–571.
[21] M.S. Palmer, A.J. Dryden, J.T. Hughes, J. Collinge, Nature 352 (1991) 340–342.
[22] J. Collinge, M.S. Palmer, Curr. Opin. Genet. Dev. 2 (1992) 448–453.
[23] K.K. Hsiao, M. Scott, D. Foster, D.F. Groth, S.J. DeArmond, S.B. Prusiner, Science 250 (1990) 1587–1590.
[24] K. Hsiao, H.F. Baker, T.J. Crow, et al. Nature 338 (1989) 342–345.
[25] Hsiao, K.K., Groth, D., Scott, M. et al. (1992) in: Prion Diseases of Humans and Animals (Prusiner, S.B., Collinge, J., Powell, J. and Anderton, B., Eds.), pp. 120–128, Ellis Horwood, London.
[26] D.A. Kocisko, J.H. Come, S.A. Priola, B. Chesebro, G.J. Raymond, P.T. Lansbury, B. Caughey, Nature 370 (1994) 471–474.
[27] R. Riek, S. Hornemann, G. Wider, M. Billeter, R. Glockshuber, K. Wüthrich, Nature 382 (1996) 180–182.
[28] F. Owen, M. Poulter, T. Shah, et al. Mol. Brain Res. 7 (1990) 273–276.
[29] F. Owen, M. Poulter, R. Lofthouse, et al. Lancet 1 (1989) 51–52.
[30] J. Collinge, J. Brown, J. Hardy, et al. Brain 115 (1992) 687–710.
[31] M. Poulter, H.F. Baker, C.D. Frith, et al. Brain 115 (1992) 675–685.
[32] F. Owen, M. Poulter, J. Collinge, et al. Mol. Brain Res. 13 (1992) 155–157.
[33] L.W. Duchen, M. Poulter, A.E. Harding, Brain 116 (1993) 555–567.
[34] L.G. Goldfarb, P. Brown, B.W. Little, et al. Neurology 43 (1993) 2392–2394.
[35] J.L. Laplanche, J. Chatelain, J.M. Launay, C. Gazengel, M. Vidlaud, Nucleic Acids Res. 18 (1990) 6745.
[36] M.S. Palmer, S.P. Mahal, T.A. Campbell, et al. Hum. Mol. Gen. 2 (1993) 541–544.
[37] D.A. Harris, D.L. Falls, F.A. Johnson, G.D. Fischbach, Proc. Natl. Acad. Sci. USA 88 (1991) 7664–7668.
[38] J.M. Gabriel, B. Oesch, H. Kretzschmar, M. Scott, S.B. Prusiner, Proc. Natl. Acad. Sci. USA 89 (1992) 9097–9101.
[39] B. Caughey, K. Neary, R. Buller, D. Ernst, L.L. Perry, B. Chesebro, R.E. Race, J. Virol. 64 (1990) 1093–1101.
[40] N. Stahl, D.R. Borchelt, S.B. Prusiner, Biochemistry 29 (1990) 5405–5412.
[41] J.F. Bazan, R.J. Fletterick, M.P. McKinley, S.B. Prusiner, Protein Eng. 1 (1987) 125–135.
[42] C. Selvaggini, L. De Gioia, L. Cantù, E. Ghibaudi, L. Diomede, F. Passerini, G. Forloni, O. Bugiani, F. Tagliavini, M. Salmona, Biochem. Biophys. Res. Commun. 194 (1993) 1380–1386.
[43] G. Forloni, N. Angeretti, R. Chiesa, E. Monzani, M. Salmona, O. Bugiani, F. Tagliavini, Nature 362 (1993) 543–546.
[44] T. Kitamoto, R. Iizuka, J. Tateishi, Biochem. Biophys. Res. Commun. 192 (1993) 525–531.
[45] Timasheff, S.N., Susi, H., Townend, R., Stevens, L., Gorbunoff, M.J. and Kumosinski, T.F. (1967) in: Conformation of Biopolymers, Vol. I (G.N. Ramachandran, Ed.), pp. 173–196, Academic Press, London.
[46] A.F. Drake, G. Siligardi, W.A. Gibbons, Biophys. Chem. 31 (1988) 143–146.
[47] S. Brahms, J. Brahms, J. Mol. Biol. 138 (1980) 149–178.
[48] W.C. Johnson Jr., Proteins Struct. Funct. Genet. 7 (1990) 205–214.
[49] E.W. Ronish, S. Krimm, Biopolymers 13 (1974) 1635–1651.
[50] A.S. Tatham, A.F. Drake, P.R. Shewry, Biochem. J. 259 (1989) 471–476.
[51] F.H.C. Crick, A. Rich, Nature 176 (1955) 780–781.
[52] S.L. Shyng, K.L. Moulder, A. Lesko, D.A. Harris, J. Biol. Chem. 270 (1995) 14793–14800.
[53] M.P. Williamson, Biochem. J. 297 (1994) 249–260.
[54] C.A. McCaffery, L.J. DeGennaro, EMBO. J. 5 (1986) 3167–3173.
[55] W.S. Trimble, D.M. Cowan, R.H. Scheller, Proc. Natl. Acad. Sci. USA 85 (1988) 4538–4542.
[56] L. De Gioia, C. Selvaggini, E. Ghibaudi, L. Diomede, O. Bugiani, G. Forloni, F. Tagliavini, M. Salmona, J. Biol. Chem. 269 (1994) 7859–7862.