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Sonication induced sheet formation at the air–water interface†

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A hydrophobic pentadecapeptide, AGAAAA-GAVVGGLGG (1), part of the prion sequence PrP (106-127), on fresh aqueous dissolution takes a mixture of random and sheet conformations which forms a stable monolayer with a high β-sheet content when compressed at the air–water interface. This also develops into a kinetically stabilized β-sheet structure on sonication.

The secondary structure conversion of the prion protein in the normal form (PrP^c) into the abnormal form (PrP^{sc}) is reported to be a cause of several human and animal diseases, such as Creutzfeldt–Jacob disease, Gerstmann–Straussler–Scheinker syndrome, fatal familial insomnia, sheep scrapie, and bovine spongiform encephalopathy.[1] PrP^c has high α-helix and low β-sheet content[2] in contrast to PrP^{sc}, which is rich in β-sheets.[3,4] The reason for this conformational transition is still completely unknown despite numerous investigations.[5] In this work, we have shown that peptide 1 on ultrasonic treatment and compression at the air–water interface forms a stable β-sheet assembly. Insoluble or sparingly soluble polypeptides and proteins which form monolayers at the air–liquid interface are very applicable as models for biological systems to study structural transitions and orientation effects due to 'molecular crowding'.[6–9]

The sequence of the peptide used in this study is AGAAAA-GAVVGGLGG (1), which corresponds to H1 of human prion protein (residues 113-127). Peptide 1 was prepared by a solid phase method using Boc chemistry. The purity and composition were determined by amino acid analysis and were identified by MALDI-TOF MS analysis. Peptide 1 forms a stable film at the air–water interface. Fig. 1 shows a pressure–area isotherm for peptide 1. It is seen that the isotherm of peptide 1 is of the liquid expanded type (85 Å^2) indicating the fairly rigid nature of the film. The value of the molecular area of the monolayer can be directly evaluated from the intersection of the *x*-axis with the tangent line of the isotherm at the onset of the condensed phase of the film, and is found to be 30 Å^2 per molecule. The collapse pressure is in the region of 28 mN m^{-1}. The film formed by peptide 1 could be transferred to a quartz plate by *y*-type (vertical) deposition with a transfer ratio around 0.95. The circular dichroism (CD) spectrum of the transferred film (20 layers) is shown in the inset of Fig. 1. It is found that the peptide 1 solution spread at the air–water interface results in monolayers containing predominant sheet conformation, as shown by the negative CD at 220 nm and the crossover at 213 nm. This indicates a profound effect of oriented molecular crowding on β-sheet assembly.

The CD spectrum of peptide 1 in PBS at pH 7.4, (Fig. 2; curve a) exhibits a mixture of random, helix, sheet and turn conformations. The sheet content is ~ 30 %. Peptide 1 solution *in vitro* must be incubated for several days at ~ 37 °C and under basic conditions (pH 10.6) in order to build a significant β-sheet content. The CD spectrum of incubated protofibrils is given in the ESI.† On sonication it is known to produce energy rich vapour–liquid interfaces in the liquid with shear force (cavitation effect). We thought it would be worth investigating the effect of sonication on the conformation of peptide 1. To our surprise we found that sonication leads to kinetically stabilized β-sheet formation.

The CD spectrum of peptide 1 in PBS at pH 7.4 is shown in Fig. 2 (curve a). The peptide solution was sonicated and the CD spectrum was recorded at 30 s intervals (Fig. 2, curves a to d). It is inferred from this study that upon sonication the sheet conformation predominates. This may be due to a highly energetic cavitational effect, which leads to interfacial effects on the aggregates. The plot of % sheet structure *versus* sonication time is shown in the inset of Fig. 2. It is tacit that the sheet structure of peptide 1 increases with sonication time. This

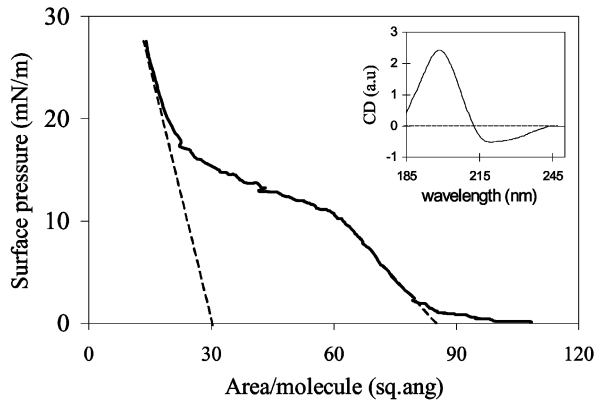

Fig. 1 Surface area–pressure isotherm for peptide 1. Inset: Circular dichroism (CD) spectrum of a 20 layer film of peptide 1.

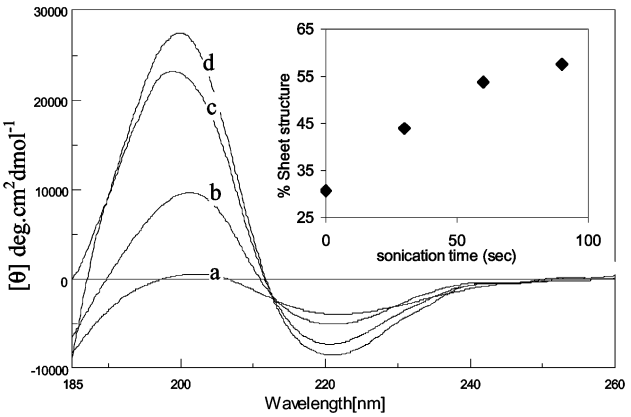

Fig. 2 CD spectra of peptide 1 in PBS at pH 7.4 and at different sonication time intervals: (a) 0 s, (b) 30 s, (c) 60 s, (d) 90 s. Inset: Plot of % sheet structure *versus* sonication time. % of secondary structure motifs of peptide 1 estimated by the SELCON program.[11]

† Electronic supplementary information (ESI) available: CD spectra and table of secondary structure motifs of peptide 1. See http://www.rsc.org/suppdata/cc/b2/b206886a/

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implies that the self-assembly process at the air–water interface seems to be a kinetically stable phenomenon. El-Agnaf *et al*.[10] have reported that sonication of Aβ(25-35) promotes the sheet structure which is irreversible, which may be attributed to oxidation of the methionine residue.

The central message of this work is that a stable β-sheet enriched state of the amyloid is formed at the air–water interface and the interface produced by cavitation effects, in contrast to the initial bulk solution containing large amounts of random coil and small amounts of β-sheet structures. The reason for the structural transition may be due to the intrinsic hydrophobicity of the air–water interfaces, which form a hydrophobic–hydrophilic system with air as the hydrophobic content. To the best of our knowledge this is the first attempt to observe the effect of interfacial phenomenon on structural transitions in prion peptides.

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Notes and references

1 S. B. Prusiner, *Science*, 1997, **278**, 245.
2 P. J. Selkoe, *Trends Neurosci.*, 1993, **16**, 403.
3 K.-M. Pan, M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlron, Z. Huang, J. Fletterick and F. E. Cohen, *Proc. Nat. Acad. Sci. USA*, 1993, **90**, 10962.
4 J. Safar, P. P. Roller, D. C. Gajdusek and C. J. Gibbs, *J. Biol. Chem.*, 1993, **268**, 20276.
5 F. Edenhofer, S. Weiss, E.-L. Winacker and M. Famulok, *Angew. Chem.*, 1997, **109**, 1748.
6 S. A. Riou, S. L. Hsu and H. D. Stidham, *Biophys. J.*, 1998, **75**, 2451.
7 J. Benjamins and F. Van Voorst Vader, *Colloids. Surf.*, 1992, **65**, 161.
8 D. E. Grahams and M. C. Phillips, *J. Colloid Interface Sci.*, 1979, **70**, 403.
9 R. Jayakumar and M. Murugesan, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1055.
10 O. M. A. El-Agnaf, G. B. Irvine and J. S. Gulthrie, *J. Neurochem.*, 1997, **68**, 437.
11 N. Sreerama and R. W. Woody, *Anal. Biochem.*, 1993, **209**, 32.