Supporting Information
for

Conformational Stability of Syrian Hamster Prion Protein PrP(90-231)

Megan Grabenauer,[1] Thomas Wyttenbach[1], Narinder Sanghera,[2] Susan E. Slade,[2] Teresa J.T. Pinheiro,[2] James H. Scrivens,[2] and Michael T. Bowers[1,*]

[1] Department of Chemistry and Biochemistry, University of California, Santa Barbara, California, 93106, USA; [2]Department of Biological Sciences, University of Warwick, Gibbet Hill Rd, Coventry, CV4 7AL, UK;

** To whom correspondence should be addressed*

Biological relevance of this study with respect to prion amyloid formation:

Prion diseases are the only transmissible diseases that have proteins as the disease element being transmitted. These must be extremely stable species to survive the rigors of transmission and even sterilization processes. They must also be small, soluble, and somehow avoid the many defenses of the brain both to get there and survive there. To our knowledge this study reveals the only prion species shown to be stable enough to survive in these conditions. It needs to be considered as potentially biologically important.

Here, we are not focusing on the aggregation of the prion protein, as PrP^C is the non-amyloid forming version of the protein. However, we contend still there is valuable information to be obtained from studies of this form. Perhaps when the "misfolding" occurs, this extremely stable intermediate structure is involved or maybe the equilibrium shifts to favor this structure. There is also still debate as to what the toxic species are in amyloid diseases. An abundance of recent data suggests that the amyloid fibrils formed are not toxic, and may serve as the body's defense mechanism by sequestering more toxic monomers and small oligomers.[1-3]

ATD Peak Assignments:

A portion of each monomer peak in the mass spectrum may be composed of multiply charged oligomer (a monomer with 8 charges and a dimer with 16 charges have the same *z/n* value) and higher order oligomers with the same *z/n* value as monomers always appear at shorter arrival times than the monomer.[4] Therefore it is possible that the peaks we have assigned as compact structures are actually oligomers. However, if this were true, then the relative intensity of the early arrival time peak would increase with increasing oligomer presence in the mass spectrum, which is not the case. ATDs from acidic solutions (Figure S2) with significant oligomer presence look qualitatively the same as ATDs from neutral solutions, no increase in relative intensity of the early arrival time features is observed. Therefore, the early arrival time features have been assigned as compact monomer structures. Any contribution from oligomers must be buried under the much more intense monomer signal.

Under biologically relevant solution conditions of 10 mM ammonium acetate pH 7.5, we expect to preserve solution structure. There is no indication in the cross section measurements that some unusual gas phase rearrangement is taking place. Charge state +9 is the protonation state expected in solution and our very gentle sampling conditions are consistent with retention of these structures in our IMS experiment. In contrast, earlier IMS work on peptides[5] and small proteins[6-8] focused on solution conditions yielding maximum signal (water/methanol/acetic acid) completely neglecting the denaturing effect of solvents. This is clearly reflected in charge state distributions - bimodal in our system +7 to +14, 16 kDa vs. a single distribution from +3 to +20 for cytochrome c 12 kDa, for example.[6]

The intermediate and elongated structures Jarrold and others[6-8] observed in the model systems by IMS may actually very well be present in solution as well given the denaturing conditions used in the spray solutions. For instance, the mass spectrum of cytochrome c sprayed under conditions preserving the native state[9] yield exclusively charge states +7 and +8. Like Konermann,[9] we should be conserving native structures during our IMS analysis.

The prion protein sample in our work consists of residues 90-231 and is a naturally occurring sequence, not an engineered sequence. Its solution structure has been shown by NMR to be quite similar to both bovine and human PrP^C and is much more likely to survive desolvation unharmed than the small model peptides used in the work done in the late 1990s which shows stability in the gas phase of helical structures.[5] These were small polyalanine peptides capped on the c-terminal end by lysine to stabilize the helix dipole. While a similar dipole-stabilized helix-based structure formed in the gas phase cannot completely be ruled out as the unusually stable prion structure observed here, this scenario is highly unlikely for the reasons outlined above and because helical structures tend to have fairly large cross sections[5] in contrast to the fairly compact stable structures observed here.

Supplementary Data:

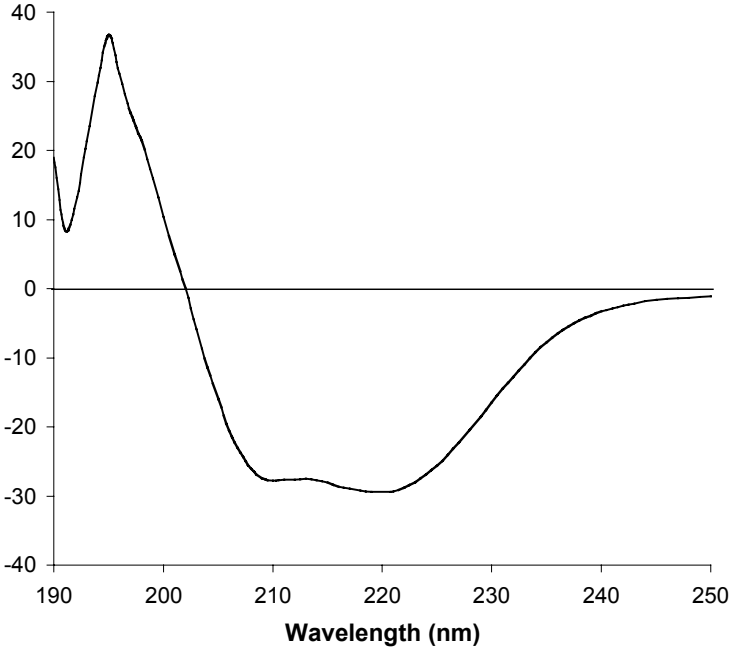

Figure S1. CD spectrum of α-PrP at pH 7.5 after arrival at UCSB showing charcteristic α-helical signatures with minima near 208 and 222 nm. The spectrum shown is the average of 12 scans taken in a 0.1cm path length cell, bandwidth 1nm, 1nm step size, and 5 second averaging time.

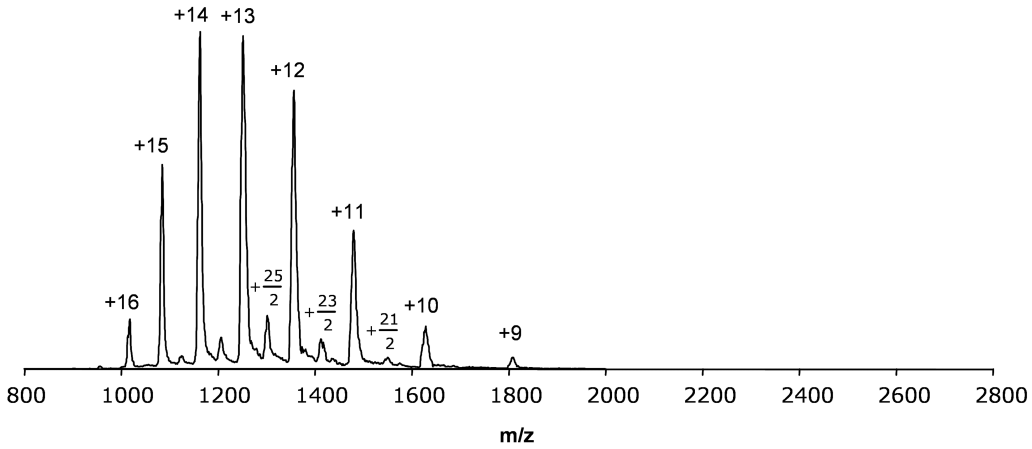

Figure S2. Mass spectrum of α-PrP at pH 2.5. Significantly more oligomer is present (non-integral *z/n* values) compared to the spectrum taken at neutral pH (Figure 1).

Table S1. Cross section summary of α-PrP

	α-PrP Cross Sections (Å^2)*	
Charge State	Acidic pH 2.5	Neutral pH 7.5
7	1474^C	1444^C
	1765^I	1763^I
8	1541^C	1540^C
	1913^I	1878^I
9	2059^I	2038^I
	2238^E	2200^E
10	2211^I	2175^I
	2345^E	2332^E
11	2320^I	2255^I
	2435^E	2436^E
12	2683^E	2648^E
13	2821^E	2778^E
14	2894^E	2891^E
15	2978^E	2996^E

* Cross sections are reproducible to within 2%
C Compact structures
I Intermediate structures
E Extended structures

Materials and Methods:

Sample Preparation

PrP expression and purification was carried out within the Pinheiro group at the University of Warwick in the United Kingdom. Syrian hamster prion protein, residues 90-231, was expressed in *Escherichia coli* 27C7 as previously described,[10] and purified from inclusion bodies. α-PrP was prepared by refolding the purified protein under oxidizing conditions into an α-helical structure.[11] Samples were shipped to UCSB suspended in 10 mM ammonium acetate buffer as frozen aliquots. The secondary structure propensity of the protein was confirmed by CD spectroscopy after arrival at UCSB (Figure S1). Samples for ESI-MS analysis were prepared by diluting the frozen aliquots to 5 μM to 10 μM concentrations with 10 mM ammonium acetate and pH adjusted using acetic acid or ammonium hydroxide.

Ion Mobility Spectrometry and Mass Spectrometry

Experiments were performed on a home built mass spectrometer[12] equipped with a nano electrospray ionization source and a DC drift cell capable of performing ion mobility spectrometry.[13] Ions are generated in the nano electrospray source, transported into the vacuum chamber via an ion funnel, pulled by a weak electric field through the drift cell filled with He buffer gas, and are mass selected using a quadrupole mass analyzer. When operated in mobility mode, ions are trapped in the ion funnel and pulsed into the drift cell. The pulsing triggers a timing sequence that allows detection of the ions as a function of time in an arrival time distribution (ATD). The reduced mobility, K_0, of the mass selected ions is obtained from the ATD using Eq. (1), where *l* is the length of the

$$K_0 = l^2 \frac{273}{760T} \frac{P}{V} \left(\frac{1}{t_A - t_0} \right) \qquad (1)$$

drift cell, T is the temperature (in Kelvin), P is the pressure inside the drift cell (in torr), V is the voltage applied across the cell, t_A is the ion's arrival time taken from the ATD, and t_0 is the amount of time the ion spends outside the drift cell before reaching the detector. t_A is plotted vs. P/V for a series of drift voltages, yielding a straight line with a slope inversely proportional to K_0 and an intercept equal to t_0. Through the use of kinetic theory, the reduced mobility is related to the ion's collision cross section, σ, by Eq. (2) [14]

$$\sigma = \frac{3e}{16N_0} \left(\frac{2\pi}{\mu k_B T} \right)^{1/2} \frac{1}{K_0} \qquad (2)$$

where *e* is the charge of the ion, N_0 is the number density of the buffer gas at STP, μ is the ion-buffer gas reduced mass, and k_B is the Botzmann constant.

Injection Energy Studies

The amount of energy with which the ions are pulsed into the drift cell can be varied, and has an effect on the amount of time it takes the ions to reach a constant drift velocity. At low injection energies, ions are gently pushed into the cell and reach thermal equilibrium with the buffer gas within just a few "cooling" collisions. At low injection energies any solution structure retained by the ions upon transfer to the gas phase is generally preserved. At high injection energies, the ions need more collisions to reach thermal equilibrium, and the larger collision energies can lead to internal excitation and isomerization to a more stable gas phase conformation.

References:

1. Kirkitadze, M. D.; Bitan, G.; Teplow, D. B. *J. Neurosci. Res.* **2002**, *69*, 567-577.
2. Kayed, R.; Head, E.; Thompson, J. L.; McIntire, T. M.; Milton, S. C.; Cotman, C. W.; Glabe, C. G. *Science* **2003**, *300*, 486-489.
3. Glabe, C. G. *Neurobiology of Aging* **2006**, *27*, 570-575.
4. Bernstein, S. L.; Wyttenbach, T.; Baumketner, A.; Shea, J.-E.; Bitan, G.; Teplow, D. B.; Bowers, M. T. *J. Am. Chem. Soc.* **2005**, *127*, 2075-2084.
5. Hudgins, R. R.; Ratner, M. A.; Jarrold, M. F. *J. Am. Chem. Soc.* **1998**, *120*, 12974-12975.
6. Shelimov, K. B.; Clemmer, D. E.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1997**, *119*, 2240-2248.
7. Shelimov, K. B.; Jarrold, M. F. *J. Am. Chem. Soc.* **1997**, *119*, 2987-2994.
8. Valentine, S. J.; Clemmer, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 3558-3566.
9. Liu, J. L.; Konermann, L. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 819-828.
10. Mehlhorn, I.; Groth, D.; Stockel, J.; Moffat, B.; Reilly, D.; Yansura, D.; Willett, W. S.; Baldwin, M.; Fletterick, R.; Cohen, F. E.; Vandlen, R.; Henner, D.; Prusiner, S. B. *Biochemistry* **1996**, *35*, 5528-5537.
11. Sanghera, N.; Pinheiro, T. J. T. *J. Mol. Biol.* **2002**, *315*, 1241-1256.
12. Wyttenbach, T.; Kemper, P. R.; Bowers, M. T. *Int. J. Mass Spectrom.* **2001**, *212*, 13-23.
13. Wyttenbach, T.; Bowers, M., T. *Top. Curr. Chem.* **2003**, *225*, 207-232.
14. Mason, E. A.; McDaniel, E. W. *Transport Properties of Ions in Gases*; Wiley: New York, 1978.