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

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Many devastating neurodegenerative diseases, known as transmissible spongiform encephalopathies (TSEs), are believed to be caused by a misfolded form of the prion protein (PrP) known as PrP^{Sc}.[1] PrP^{Sc} is remarkable in that it appears to be infectious even in the absence of nucleic acid,[1] and its infectivity can survive in soil for time periods on the order of years.[2] The infective agent in prion diseases is resistant to inactivation by standard decontamination procedures[3] and has been shown to survive under conditions simulating those of medical waste incinerators.[4]

The normal cellular form of the prion protein, PrP^{C}, is fully degraded in the presence of proteinase K. In contrast, PrP^{Sc} is resistant to proteinase K digestion and has an exceptionally high thermal stability.[5] According to the "protein-only" model proposed by Prusiner,[1] these disparities are due to conformational differences between the two forms of the protein. Here we used ion mobility spectrometry[6] combined with mass spectrometry (IMS−MS) to examine the size and conformational stability of α-PrP, a truncated form of recombinant Syrian hamster prion protein PrP(90−231) that is predominantly α-helical, nonaggregating, and believed to be structurally similar to PrP^{C}. Our data reveal at least one extremely stable conformation of α-PrP. In addition, the first set of absolute collision cross sections measured for this protein is presented.

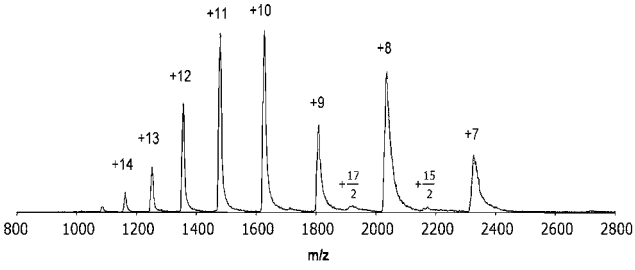

Figure 1. Mass spectrum of α-PrP at pH 7.5. The numbers above the peaks correspond to *z*/*n*, where *z* is the charge and *n* is the oligomer order.

A mass spectrum of α-PrP taken at pH 7.5 is shown in Figure 1. The spectrum contains a bimodal charge state distribution (CSD) indicative of the coexistence of multiple distinct conformational families in solution.[7] The presence of compact conformations results in the low CSD centered near +8, and the presence of more extended conformations results in the distribution centered near +11. IMS−MS was used to examine conformations of the protein in each of the charge states shown in Figure 1. The fundamental principle behind IMS is that ions of different sizes travel at different speeds when pulled by a weak electric field through a cell filled with an inert gas. Ions with compact structures travel through the cell faster than ions with more extended structures. This results in arrival time distributions (ATDs) in which compact ions appear on the left (at shorter arrival times) and extended ions appear on the right (at longer arrival times).

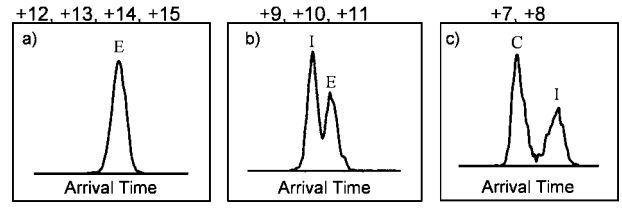

Figure 2. Representative ATDs for each charge state of α-PrP taken at an injection voltage of 50 V. The specific ATDs shown are (a) +13, (b) +10, and (c) +7. The labels are C = compact, I = intermediate, and E = extended.

IMS data also show evidence for multiple distinct conformational families. Figure 2 contains representative ATDs for each charge state examined. For the +7 and +8 charge states, there is a compact family of isomers (labeled C) and an intermediate family of isomers (labeled I). Starting at charge state +9, the compact family is gone, the intermediate family remains, and a new extended family of isomers (labeled E) appears. The intermediate and extended isomers remain through the +11 charge state, but only the extended isomer persists through charge states +12 and above. (See Figure 3 and the Supporting Information for details regarding peak assignment.)

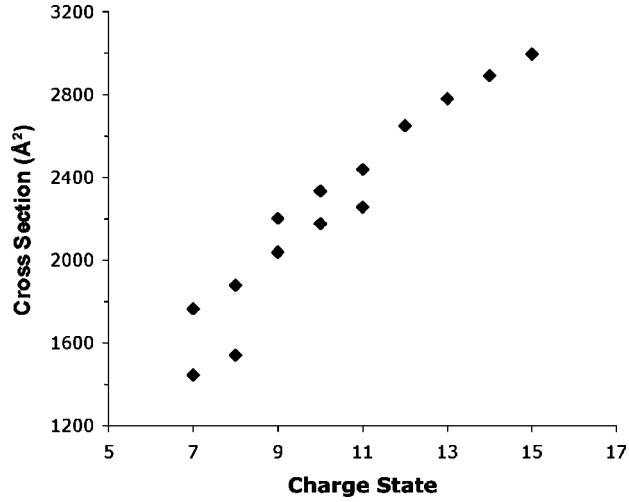

Figure 3. Plot of cross section vs charge state for α-PrP data taken at pH 7.5. The measurements were reproducible to within 2%.

The amount of energy with which ions are injected into the drift cell can be varied and the conformational stability of each charge state investigated as a function of this energy. Under gentle injection conditions (30 V), the more compact families are dominant,

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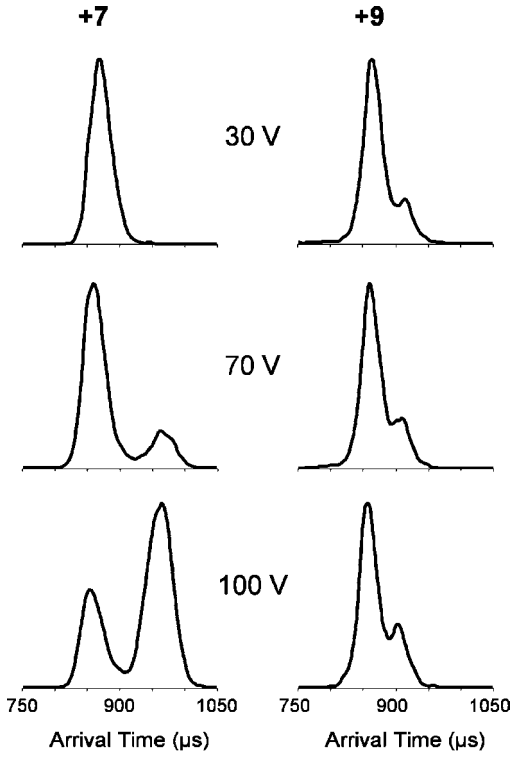

Figure 4. Injection energy dependence of α-PrP conformational stability. The +7 charge state (left) undergoes significant conversion to a more extended structure with increasing injection voltage, as do the +8 and +11 charge states (not shown). The +9 (right) and +10 (not shown) charge states retain their more compact structures up through 100 V.

indicating that these are the conformations most likely present in solution. For charge states +7, +8, and +11, as the injection energy is increased, isomerization to more extended structures takes place, as shown for the +7 charge state in Figure 4. This behavior has been seen previously in many protein and DNA systems.[8] The +9 and +10 charge states are exceptions to this trend. For these two charge states, the more compact solution structure is stable enough that an injection voltage of 100 V is not sufficient to convert a significant amount of the compact structure into the more extended structure. As can be seen in Figure 4, the +9 charge state remains predominantly compact up to an injection voltage of 100 V, with only minimal conversion to a more extended structure.

Jarrold and co-workers have previously shown that small α-helical peptides have increased stability in the gas phase.[9] However, these were small, engineered sequences, and one would not expect such stability to persist in a naturally occurring protein the size of α-PrP. In contrast to the results reported here for the +9 and +10 charge states of α-PrP, model protein systems of a similar size, including cytochrome *c*[10] and myoglobin,[11] typically are easily unfolded with increasing injection energy (see the Supporting Information for a discussion of stable gas-phase structures).

The conformational stability of α-PrP was also studied as a function of temperature. For each charge state with multiple features in its ATD, the more compact structures were more stable than their extended counterparts at low temperatures. As temperature was increased, the compact and intermediate structures were converted to intermediate and extended structures, respectively. The ATDs presented in Figure 5 demonstrate this temperature dependence.

The top panel of Figure 5 displays ATDs for the +8 charge state of α-PrP at three different temperatures (78, 325, and 450 K). At 78 K, the compact and intermediate features had almost equal

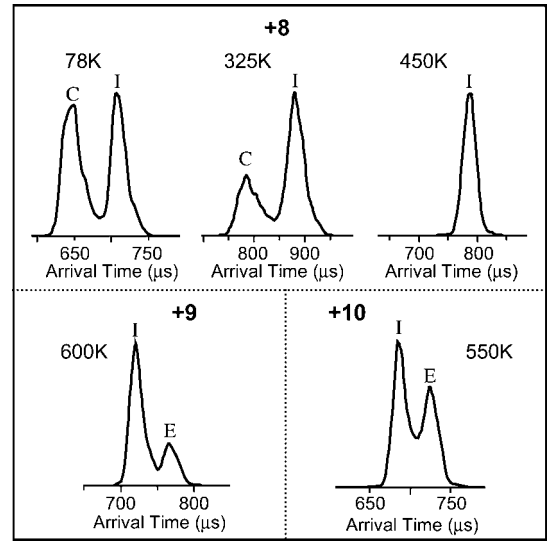

Figure 5. Temperature dependence of α-PrP conformational stability. For every charge state with multiple conformations except +9 and +10, the protein isomerizes to its more extended structure between 425 and 475 K. The +10 charge state isomerizes to its more extended structure between 550 and 575 K, and remarkably, the +9 charge state resists conversion up through 600 K.

intensity. By 325 K there was a definite shift in intensity to favor the intermediate feature. By 450 K all of the compact structure had been lost and converted into the intermediate structure. For the +7, +8, and +11 charge states, conversion of the more compact to the more extended structure was complete between 425 and 475 K. Again, the +9 and +10 charge states proved to be exceptions. The +10 charge state retained a significant amount of intermediate structure up through 550 K but was completely converted to an extended structure by 575 K. The remarkable standout was the +9 charge state. Even at 600 K, the highest temperature for which data was collected, almost all of the intermediate structure was retained.

While protein unfolding in the absence of solvent is expected to occur at higher temperatures than in solution, previous studies of α-synuclein by this group (data not shown) and a gas-phase unfolding study of the Trp-Cage protein[12] have shown that complete or at least significant unfolding generally occurs by 450 K. It is also interesting to note that only the +9 and +10 charge states showed unusually high stability, since on the basis of the primary sequence of α-PrP, +9 and +10 are its most likely charge states in solution at physiological pH. This agreement suggests that we may have been observing a very stable solution structure that persists upon both solvent evaporation and very high temperature.

The extreme resistance of prions to procedures that inactivate conventional pathogens[3] is of great concern for the transmission of prion diseases. Circular dichroism (CD) and FTIR measurements have shown that the α-helical content of the protein decreases during the conversion from PrP^{C} to PrP^{Sc} and that there is a substantial increase in the amount of β-sheet structure present.[13] However, the mechanism by which the conversion proceeds is still unknown, and there may be significant overlap between the two structures. Our data demonstrate that the dominant solution charge state of α-PrP shows notable conformational stability, suggesting that there may be aspects of PrP^{C} structure that are relevant to the extremely stable structures of PrP^{Sc}.

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Supporting Information Available: Materials and Methods, CD data, absolute collision cross sections of α-PrP, the acidic mass spectrum of α-PrP, and details of ATD peak assignments. This material is available free of charge via the Internet at http://pubs.acs.org.

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