BIOANALYTICAL CHEMISTRY

Protein oligomers frozen in time

The size and shape of amyloid-β protein assemblies have been studied using electrospray-ionization ion-mobility mass spectrometry, and the protein tetramers and dodecamers have been identified as an important oligomerization state in the development of neurodegenerative disease.

David E. Clemmer and Stephen J. Valentine

When Fenn and co-workers recorded early electrospray ionization (ESI) mass spectra of biopolymers, they observed what are now characteristic charge-state distributions as well as the formation of non-covalent complexes that persisted into the gas phase[1]. Writing on page 326 of this issue[2], Bernstein *et al.* describe the remarkable differences in the distributions of oligomers formed by different protein isoforms — different forms of the same protein that may be produced by small changes in the encoding gene — of amyloid-β (Aβ) protein using electrospray-ionization ion-mobility mass spectrometry. Their results address the first steps of β-fibril assembly — a phenomenon that may provide a physical explanation for the first steps in the formation of large fibrils, which are found as signatures in patients with neurodegenerative diseases such as Alzheimer's.

Bernstein *et al.* suggest that oligomerization of these proteins proceeds through key oligomer sizes and structures that regulate further growth and assembly. Some polypeptides form relatively small complexes with stable, closed structures that resist further growth. One sequence, the wild-type Aβ42, is dramatically different. The small complexes that are formed are not self-terminating and thus can coalesce, ultimately leading to fibril formation.

The ability to characterize protein structures in the gas phase is quite remarkable in and of itself. In the mid 1990s, MacLafferty and co-workers trapped different charge states of cytochrome *c* ions in a vacuum for extended times (up to 30 minutes)[3]. On exposure to very dilute deuterated solvents, variations in the exchange rates and levels for the different charge states were found. The fact that heteroatom hydrogens within the proteins are protected from deuterium exchange to different degrees requires that the proteins have defined structures, even in the complete absence of solvent. Moreover, some individual charge states exchanged at different rates, such that a single peak in the mass spectrum would split into multiple peaks over time — with differing degrees of deuteration. This result indicates that the nature of populations of conformations in the gas phase is very different than in solution. That is, in the gas phase, the different conformations do not seem to be in equilibrium with one another, even after extended trapping times. This work led to a

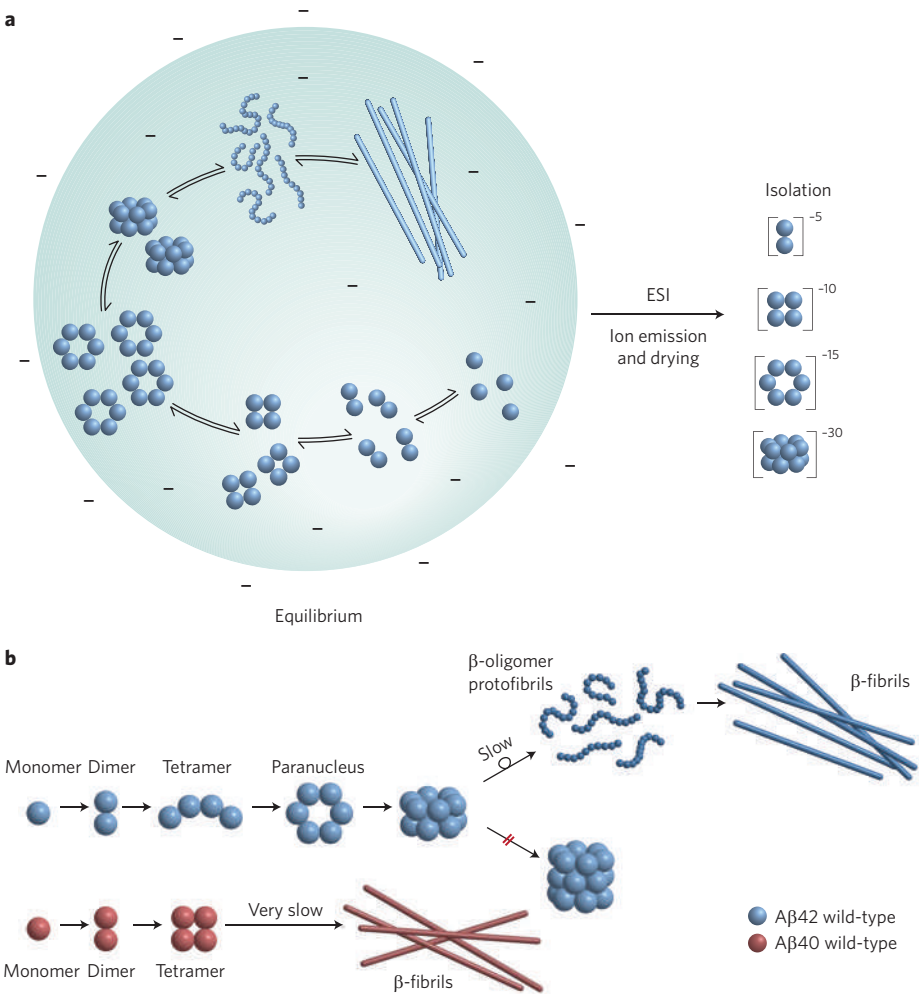

Figure 1 | Electrospray ionization allows protein oligomers in solution to be frozen out and their size and shape to be determined by ion-mobility spectrometry. **a**, Cartoon of an electrospray droplet that contains a distribution of the Aβ42-protein aggregation states that exist in equilibrium in solution. Once electrosprayed into the gas phase the various oligomer species are isolated from one another and the population of states is fixed. Separations of oligomers based on mass-to-charge (which is similar for all the oligomers) can be further refined by shape-to-charge. **b**, All the amyloid-β proteins studied (for example, the wild-type Aβ40) assemble to form dimers, tetramers and ultimately fibrils, but the open geometry of the wild-type Aβ42 tetramer allows it to also form hexamers (paranucleus) and dodecamers.

flurry of interest in the structures of entirely 'naked' proteins[4,5]. Is it possible that different conformational states become trapped as the distribution of ions emerges from the ESI droplets — freezing the distribution in time? To the extent that this is true (Fig. 1a) studies of these structures in the gas phase can provide insight about populations of conformations that are impossible to observe in solution because of the rapid equilibrium between states.

Bernstein *et al.* inject short pulses of the long-lived gas-phase structures that are formed from the ESI droplet into a helium buffer gas and expose the ions to a weak electric field. As the 'packet' of ions drifts through the gas, different species separate according to their mobilities. For example, oligomers with compact conformations undergo fewer collisions than those with extended conformations, and thus will have higher mobilities. Similarly, oligomers with higher charge states experience a greater drift force than those with lower charge states, and thus will also have higher mobilities. Therefore, it is possible to distinguish between the different oligomers based on variations in shape-to-charge even when they have the same mass-to-charge ratio. Moreover, because the interactions with the simple gas are well understood it is possible to calculate ion mobilities for trial geometries and by comparison with experiment, obtain insight into the shapes of the oligomers[6,7].

When this approach is applied to the *in vitro* distributions of oligomers of the Aβ40 and Aβ42 isoforms the results are remarkable. The ion-mobility distribution for the wild-type Aβ42 shows features that are assigned to the dimer, tetramer, hexamer and dodecamer, whereas the Aβ40 isoform forms only the dimer and tetramer (Fig. 1b). Similar studies of other Aβ42 alloforms — in which single amino acid residues have been changed — also show oligomer formation; however, the growth again seems to be restricted at the tetramer. The abrupt absence of larger oligomers for the Aβ40 isoform and other Aβ42 alloforms suggests that the tetrameric form has a key role in the inability of these systems to assemble into larger complexes — and thus is an important species in the development of disease. Bernstein *et al.* propose a compact tetrameric form for these less toxic proteins. It is the closed nature of this structure that resists rapid accumulation of protein units. On the other hand, the wild-type Aβ42 tetramer favours an open conformation that can easily incorporate another dimer to form a hexamer ring, and these rings can stack together to form dodecamers. The authors speculate that over time the dodecamer may rearrange such that further assembly can occur, leading to the large fibrils seen in the advanced stages of neurodegenerative diseases.

The potential understanding of the early steps of fibril formation provided by this study are profound and a number of interesting issues are raised. For example, one can imagine using this insight to develop interventions that are designed to arrest oligomer growth at a specific size. Although the present application of these technologies is tremendously inspiring, there is a clear need to improve the techniques. For example, a method of selecting and trapping the different oligomers based on differences in shape would make it possible to carry out dissociation studies that could address the stability of different oligomer sizes and also corroborate assignments. Furthermore, one imagines that within each of the oligomer sizes there are likely to be populations of stable structures. As the ability to resolve species with this technology improves it will become possible to examine more of the details of how different conformations of assemblies grow. Finally, these results present an extraordinary opportunity for theory. With the additional constraint of the measured mobility it will be interesting to see what calculations can say about the details of structures that terminate or allow fibril growth. ❐

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ORGANIC SYNTHESIS

Scavengers in full flow

Reducing the manual labour associated with chemical synthesis by using continuous-flow reactors that not only make compounds, but also purify them, opens up new avenues to reaction automation and rapid scale-up.

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Synthetic chemistry is a labour-intensive undertaking that has been practised as a craft for most of the twentieth century. One aspect that has not changed since the days of the alchemists, however, is that synthetic transformations have been conducted almost exclusively in batch mode. Prototypical reaction formats involve a vessel — be it a small round-bottomed glass flask or a large tank made of stainless steel — inside which the starting materials and reagents are stirred and often either heated or cooled. Following the reaction — which may be stopped by the addition of a quenching reagent — work-up and purification are necessary to separate the desired product from other materials that remain in the vessel.

Ever more selective and efficient chemical reactions have rendered the bond-forming process more efficient and versatile. Nevertheless, the cost in terms of time and money is influenced more by 'post reaction' operations than the actual transformation itself. During the past decade, however, synthetic chemists have begun to explore the use of continuous-flow reactors as an alternative to traditional batch syntheses. Such approaches can be used to automate reaction screening and multistep syntheses, perform dangerous and difficult reactions safely and efficiently, and scale up reactions rapidly.

With the advent of parallel synthesis in the 1990s — whereby the same type of reaction is performed in separate vessels