VIP Proteins Very Important Paper

Deutsche Ausgabe: DOI: 10.1002/ange.201508995
Internationale Ausgabe: DOI: 10.1002/anie.201508995

Mass Determination of Entire Amyloid Fibrils by Using Mass Spectrometry

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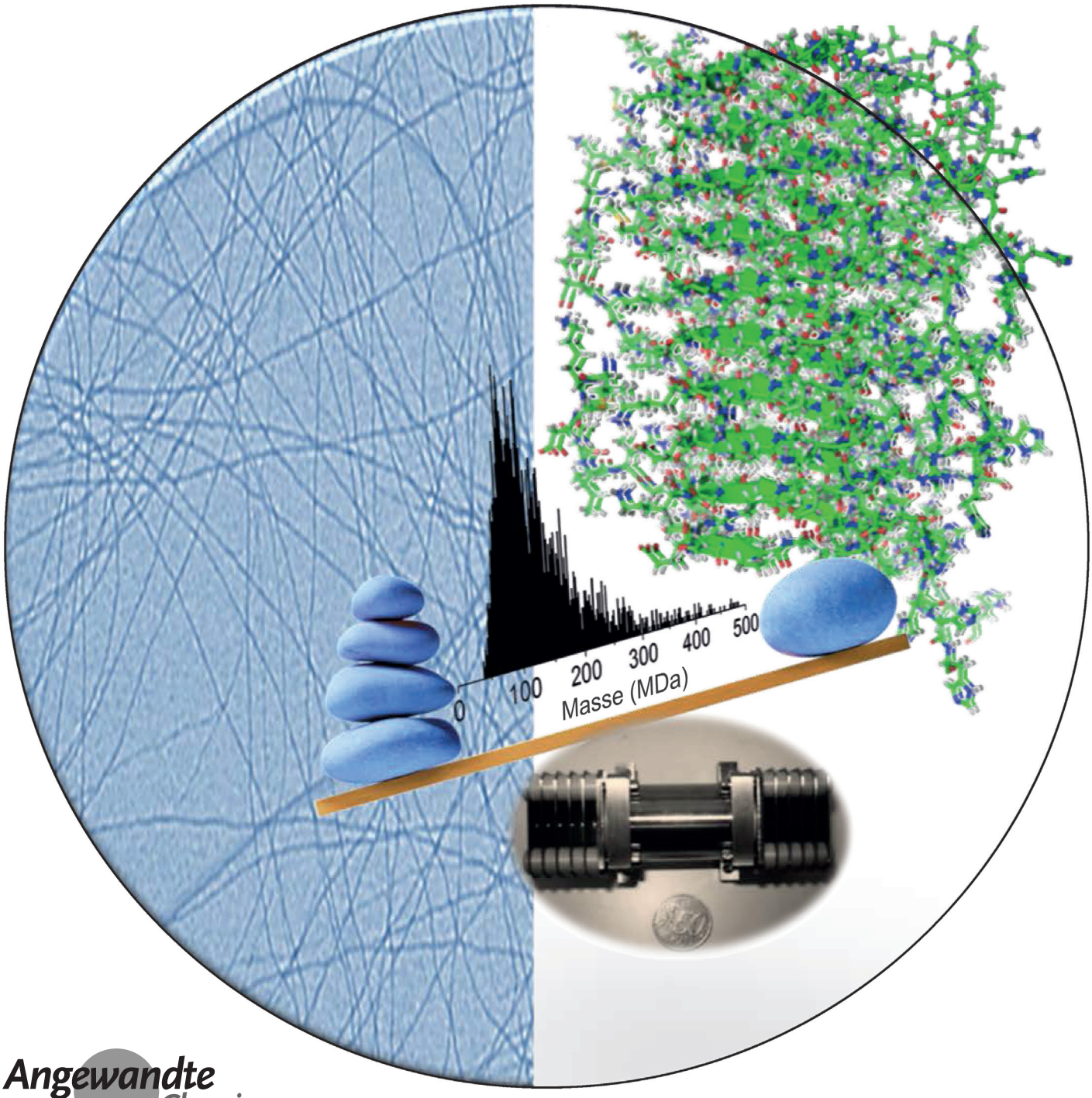

Abstract: *Amyloid fibrils are self-assembled protein structures with important roles in biology (either pathogenic or physiological), and are attracting increasing interest in nanotechnology. However, because of their high aspect ratio and the presence of some polymorphism, that is, the possibility to adopt various structures, their characterization is challenging and basic information such as their mass is unknown. Here we show that charge-detection mass spectrometry, recently developed for large self-assembled systems such as viruses, provides such information in a straightforward manner.*

Amyloid fibrils were first reported in the context of neurodegenerative disorders such as Alzheimer's disease and spongiform encephalopathies, and are related to misfolding of specific proteins, through mutation, cleavage, or other alterations that destabilize their native structure.[1] Functional amyloids were later described within organisms from bacteria up to humans.[2] In these cases, the amyloid fibril formation results from well-controlled processes, which involve specific proteins and fulfil particular physiological functions. The formation of amyloid fibrils allows the transmission of non-Mendelian phenotypes, that is, inherited information, in yeast and fungi.[3] It allows changes in synapses for the establishment of long-term memory to be maintained.[4] The amyloid fibril formation has also been proposed for the storage of peptides within secretory cells,[5] as a template for the polymerization of melanin[6] or as an extracellular matrix for the formation of biofilms by bacteria.[7] Nowadays, amyloid fibrils, as well-defined fibrillary nanostructures, are attracting increasing interest as potential high-performance nanomaterials because of their efficient spontaneous bottom-up self-assembly in mild conditions and their unique structure-related properties (i.e., high aspect ratio, mechanical strength, adaptable functionality).[8] It is possible to form various amyloid fibrils from a single peptide or protein. This polymorphism leads to fibrils of different diameter, twist, and even chirality, and may result from various protofilament associations.[9] Together with the self-propagating abilities of these amyloid structures, this leads to the strain phenomenon described in the prion-related diseases; a strain being associated to specific amyloid structure that self-propagate.[10] However, getting insight into the amyloid fibril structure is quite challenging, and basic information such as their mass (the total amount of proteins within the fibrils) and the number of populations present within a sample are lacking. Such information is of primary importance to investigate strain phenomena within prion propagation and to envisage the use of amyloid fibrils as nanomaterials. Only mass-per-length (MPL) values could be estimated in few cases, by using time-consuming microscopy-based techniques.[11]

Mass spectrometry (MS) has proven to be a powerful tool to investigate protein structure and function.[12] The use of mass spectrometry has also allowed the investigation of the assembly process and the organization of large protein complexes.[13] The recently introduced Orbitrap Exactive EMR mass spectrometer opens the possibility to achieve efficient isolation of native protein complex ions with molecular weights of over a few hundred kilodaltons.[14] Hence, conventional MS tools are limited to the study of the early stages of amyloid-type aggregation phenomena; with a mass-range upper limit not exceeding the order of megadaltons, amyloid fibrils are out of reach.[15] In parallel to recent progress made with nanomechanical mass sensors,[16] the innovative technique of charge detection mass spectrometry (CD-MS) extends this limit far above the megadalton mass range, thus allowing the study of larger systems such as viruses, bioparticles, or self-assembled polymer nano-objects.[17] CD-MS is a single-ion MS technique based on the concept of image current detection. When an ion travels through a conductive tube, the resulting image charge impressed on the tube is equal to the charge carried by the ion. The duration of this induced signal is equal to the time-of-flight of the ion through the tube. By measuring simultaneously velocity and charge of individual ions, and knowing the acceleration voltage, one can compute the mass of every detected ion. This approach was pioneered by Shelton et al.[18] and the coupling to electrospray ionization (ESI) was introduced by Benner and co-workers in 1995 for determining the mass of macro-ions heavier than one megadalton.[19] Herein we describe the application of CD-MS to amyloid fibrils, and show the potential of this method to provide unprecedented insight into amyloid fibril features. The approach is illustrated with amyloid fibrils related either to misfolding in the case of α-lactalbumin or to cell function in the case of the HET-s prion domain.

When incubated at pH 2, α-lactalbumin (αLa), a whey protein, forms twisted amyloid fibrils with lengths from 1 to 2 μm (Figure S1 in the Supporting Information).[20] These fibrils result from the association of filaments (Figure 1 a) and, despite some polymorphism arising from the number of filaments in the interaction (2–5 filaments per fibril), they share common structural features: they are left-handed helical structures with a periodicity along the axis of 85 nm. The apparent fibril diameter varies between 20 nm and 37 nm, depending on the number of filaments involved (Figure 1 a).

The 2D graph (charge vs. mass) shown in Figure 1 c reports mass measurements on around 4500 αLa fibrils; m (the mass) of each macroion is obtained from a combination of both z (the charge) and m/z values. A single slope is

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201508995.

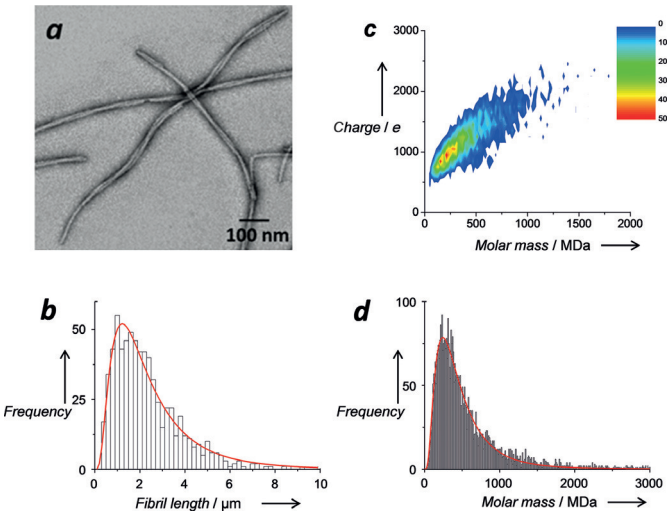

Figure 1. a) TEM image of α-lactalbumin amyloid fibrils. b) Length distribution of amyloid fibrils based on TEM images analysis. c) 2D graph of CD-MS measurements performed on a sample of α-lactalbumin amyloid fibrils. d) Mass distribution drawn from (c). The mass distribution is histogrammed using a given bin size (10 MDa). Each bar represents the number of measured ions whose masses correspond to the mass range of the bin.

observed in the two-dimensional graph, thus indicating that only one population of αLa fibers is detected. The mean charge carried by αLa fibril macro-ions is about 1.9×10^{-16} C (ca. 1180 *e*). The surface charge density of αLa fibrils is around 12 times lower than the surface charge density of individual αLa proteins observed by MS (see Table ST1 in the Supporting Information). The accessible surface area of proteins is reduced in supramolecular assemblies as compared to monomers because of the contact between the monomers. This effect is also observed for sub-megadalton protein assemblies[21] and the tobacco mosaic virus (see Figure S5 and Table ST1).

According to the mass distribution (Figure 1d), the mean fibril mass is 395 MDa, with a polydispersity index (PDI) of 1.62. Based on the mass of single αLa (14178 Da), it is possible to determine that the average number of proteins per fibril is equal to 2.8×10^4. Extensive analysis of TEM images of the sample used for the CD-MS experiments shown in Figure 1c allowed us to determine the length distribution of the αLa fibrils, for which the mean length is 2.01 µm with a polydispersity index (PDI) of 1.65 (Figure 1b). Both calculated PDI values are similar, which tends to indicate that the polydispersity induced by the polymorphism in terms of number of filaments per fibril may be relatively weak. When the obtained mean length is associated with the mean mass measured by CD-MS (395 MDa), this allows the MPL value to be estimated at 197.5 kDa nm^{-1}, meaning that each nanometer of fibril contains about 14 proteins. This result is consistent with fibrils that are constituted from several filaments. Some of these proteins must be buried inside the fibril, that is, inaccessible to the solvent; this hypothesis explains the low charge carried by the fibril macro-ions.

The protein HET-s belongs to the proteins that form functional amyloid fibrils; it is involved in the self-/non self-recognition of filamentous fungi.[22] The fibril formation is due to the self-assembly of a so-called "prion-domain" (HET-s (218–289)). Solid-state NMR spectroscopy has been used to show that its structure inside fibrils forms a β-solenoid with a triangular hydrophobic core.[23] For our experiments, HET-s (218–289) fibrils were prepared at pH 4, where the filaments remain isolated.[24] When observed by AFM (Figure 2a) and TEM (Figure S3a), the population of the fibrils is homogeneous with an apparent diameter of around 3 nm (Figure 2b), which is in agreement with the expected diameter from the high-resolution structure (Figure S3b).

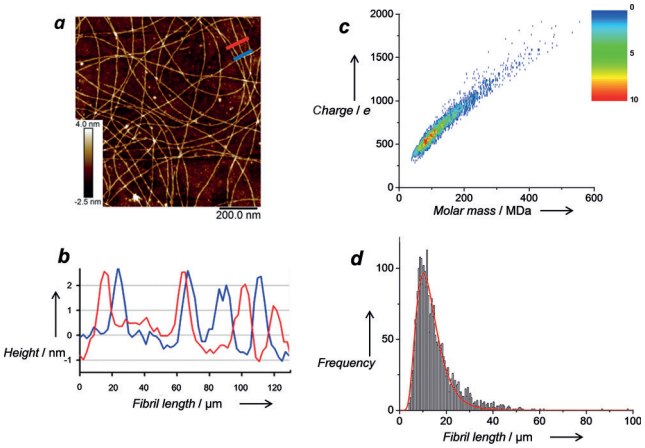

Figure 2. a) AFM image of HET-s fibrils prepared at pH 4; b) Height profiles along the blue and red lines drawn on (a); c) 2D mass–charge graph of HET-s fibrils as obtained by CD-MS. d) Length distribution of HET-s fibrils drawn from the measured mass distribution and the MPL value calculated from the high resolution structure of the fibrils (Figure S3b). The red curve represents the lognormal function fitting the experimental distribution from which the mean length value was extracted.

According to the two-dimensional CD-MS graph (Figure 2c), the mean mass of the HET-s (218–289) fibril is 112 MDa with a polydispersity index of 1.19 (see Figure S4 for the projected mass distribution of the sample). The mean charge on HET-s fibrils is about 1.0×10^{-16} C (ca. 650 *e*; Figure 2c). When the fibril diameter is kept constant, the polydispersity in mass can be attributed to the dispersity in fibril length. Knowing that the molar mass of monomeric HET-s (218–289) protein is 8.65 kDa, the mean aggregation number of the produced HET-s fibrils is 1.3×10^4 proteins per fibril. In parallel, by combining the mass distribution data obtained by CD-MS with the MPL value of 9.2 kDa nm^{-1}, calculated from the high-resolution structure (Figure S3b), we were able to translate the experimental mass distribution into a length distribution (Figure 2d). The mean length is 12.2 µm, and together with the diameter determined by using AFM (3 nm; Figure 2b,d), the values provide an aspect ratio of 4000, which is comparable to those reported for carbon nanotubes.[25] Such an aspect ratio for amyloid fibrils makes them difficult to observe in their entirety by electron microscopy. On the scale of the picture shown in Figure S3a, the fibrils appeared particularly thin and none of them have

both extremities present on the picture; the two highlighted fibril portions are around 4 μm long.

We have shown that the use of CD-MS provides a direct access to the masses of amyloid fibrils. The synergic single-particle approach that combines microscopy and mass spectrometry gives access, for the first time, to crucial features of the samples, namely mass and length distributions. It is our hope that this method can be used for following the growth kinetics of amyloid fibrils in vitro, especially in terms of defining the pathway from oligomers to the final fibrillar structures, and for studying the length distribution of rodlike systems in general. Up to now, this basic information on these assemblies of primary importance for biology questions related to diseases and functions, and for an emerging nanotechnology field could only be estimated by time-consuming approaches based on microscopy. The ability to maintain noncovalent interactions in the gas phase, the small amounts of samples required, and the speed of data acquisition render electrospray CD-MS ideal for monitoring protein aggregation in real time, directly from the reaction solution. In the near future, we can expect the expansion of this methodology to other amyloid fibrils will provide meaningful insight into amyloid fibril properties. Work along these lines is currently underway in our laboratory with amyloid beta fibrils. For instance, a straightforward prediction is that the coupling of CD-MS to hydrogen/deuterium exchange experiments will provide a wealth of information, as it has been the case for other mass-spectrometry methods.[26]

Acknowledgements

We are grateful to the Agence Nationale de la Recherche for supporting this work (Grants ANR-08-BLAN-0110-01 and ANR-11-PDOC-032-01). We would like to thank Xavier Dagany, Christian Clavier, Michel Kerleroux, Marc Barbaire, and Jacques Maurelli for their invaluable technical assistance as well as Arthur Viodé (MSc student) for his contribution to this work. We wish to thank Jean-Luc Pellequer for the gift of tobacco mosaic virus used in this work. We thank Daphna Fenel and Dr. Guy Schoen, from the IBS/UVHCI platform of the Partnership for Structural Biology in Grenoble (PSB/IBS) for the electron microscopy studies.

Keywords: amyloids · fibrils · mass spectrometry · proteins · self-assembly

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 2340 – 2344
Angew. Chem. **2016**, *128*, 2386 – 2390

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Received: September 25, 2015
Revised: December 12, 2015
Published online: December 23, 2015

