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Fibrillar vs Crystalline Full-Length β-2-Microglobulin Studied by High-Resolution Solid-State NMR Spectroscopy

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Elucidating the fine structure of amyloid fibrils as well as understanding their processes of nucleation and growth remains a crucial challenge, linked to our current poor insight into protein misfolding and aggregation diseases such as Alzheimer's and Parkinson's. Since the details of fibril architecture are only just beginning to be understood,[1] obtaining atomic-scale structural information on amyloid fibrils is an essential priority to understand their supramolecular structure and shed light on the processes of fibrillation.

β-2-Microglobulin (β2m, the light chain of the Class I Major Histocompatibility Complex) is a 99-residue protein responsible for dialysis-related amyloidosis[2] and is recognized as a molecular archetype for the study of folding and amyloid transition processes.[3] Various experimental conditions (most commonly low pH) lead *in vitro* to β2m conversion from the native structure into amyloid fibrils,[4,5] which have been shown to be to various extents related to their *in vivo* pathological counterparts.[6]

Several packing models have been proposed for fibril assembly based on biochemical, biophysical, and mutagenesis studies.[4,7,8] For instance cryo-EM analyses of the fibrils suggested that highly unstructured monomeric β2m at pH 2.5 refolds into a globular but non-native structure upon fibrillation, packing into a hierarchical architecture with asymmetrically associated protofilaments.[7]

Solid-state NMR (ssNMR) can in general complement mutagenesis or biophysical studies with structural data at the atomic scale,[9] particularly to describe protein fold and packing in fibrillar species.[10] For β2m fibrils, ssNMR experiments have characterized protofilament-like fibrils formed by the K3 peptide fragment (residues 20−41) of the protein,[11] resulting in a proposed conformation for the peptide and a 3D structure for the fibrils. As there may be fundamental differences in the aggregation potential of isolated peptides compared with their parent protein, a study of full-length β2m fibrils is now of the highest priority.

Here, we report a ssNMR approach that probes the structural features of β2m by comparing spectra of the crystallized protein (representative of the native fold) with those of amyloid fibrils obtained *in vitro*. To test whether amyloids commonly studied at low pH retain their overall structure under more physiological conditions, fibrils grown at pH 2.5 and 7.4 are investigated.

Figure 1 shows regions from a series of 2D $^{13}C-^{13}C$ and $^{15}N-^{13}C$ correlation experiments recorded on a hydrated crystalline precipitate of native [U−^{13}C, ^{15}N]β2m. A DARR recoupling scheme with short mixing times (20 or 30 ms) was used to obtain spectra

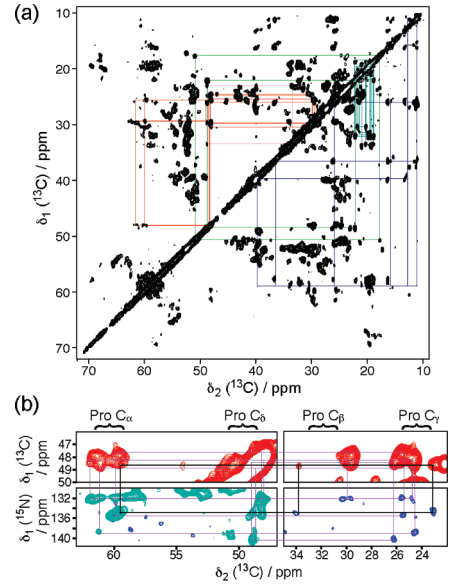

Figure 1. (a) Aliphatic region of a DARR spectrum recorded on crystalline β2m on a 700 MHz spectrometer (20 ms mixing time) highlighting Ala, Ile, Pro, and Val spin systems. (b) Expansions of the 2D DARR (red), NCA (sky blue), and NCACX spectra (blue), showing the proline spin systems (purple, *trans*-Pro; black, *cis*-Pro).

dominated by intraresidue transfers. The $^{13}C-^{13}C$ spectra display good resolution and sensitivity, in which most of the residues are visible. The availability of the corresponding β2m assignments in solution[12] provided the basis for a partial assignment of Cα, Cβ, and N shifts, especially in the well-dispersed regions (e.g., Thr, Ala). Notably, all but one of the Pro residues show a 4−6 ppm chemical shift difference between the β- and γ-C resonances (Figure 1b), which is characteristic of a *trans* Pro conformation.[13] This difference is larger (10.5 ppm) for one proline system, thus corresponding to the only native *cis* Pro (Pro32).

Figure 2a shows a comparison between 2D $^{13}C-^{13}C$ correlation spectra recorded on crystalline β2m precipitate (red) and β2m fibrils (blue) grown at pH 2.5. The presence of signals for many residues with good signal-to-noise in spectra with dipolar transfer schemes indicates that a large fraction of the β2m chain is structured and does not possess large-scale/large-amplitude reorientational dynamics. This is further supported by the absence of signal in the INEPT spectrum shown in Figure 4 in the Supporting Information. On the other hand, the lower spectral resolution compared to the crystalline protein implies differential subunit packing within the fibrils, slow dynamics of the molecules, or dispersion due to sample inhomogeneity.

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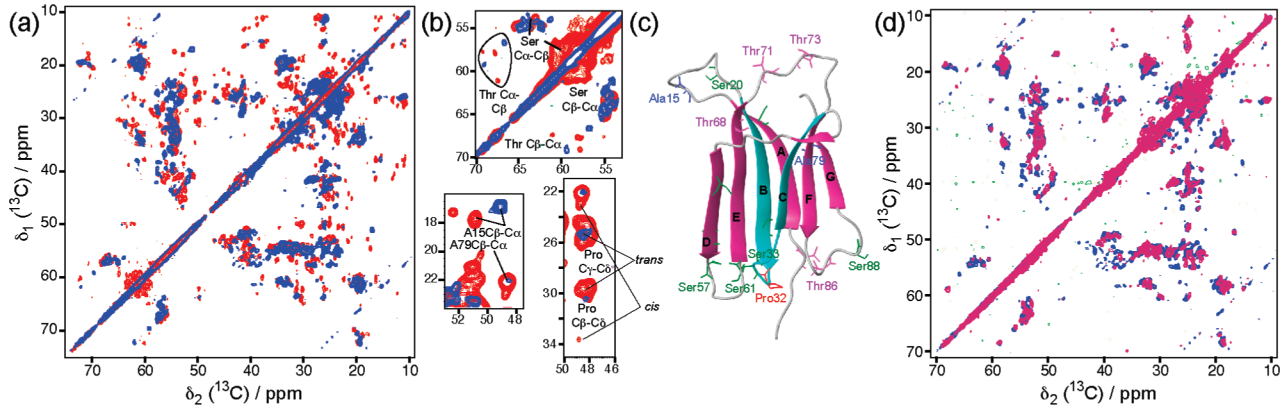

Figure 2. (a−b) Overlay of the aliphatic regions of the DARR spectra recorded on native crystalline β2m (red, 10 kHz MAS on a 700 MHz, mixing time of 20 ms in (a) and 30 ms in (b)) and pH 2.5 β2m fibrils (blue, 30 ms mixing time, 10 kHz MAS on a 900 MHz). (c) Representation of full-length β2m crystal structure (pdb code: 2YXF). Residues referred to in the text are featured (blue, Ala; green, Ser; magenta, Thr; and red, Pro32). The K3 fragment is shown in cyan. (d) Overlay of the aliphatic region of the $^{13}C-^{13}C$ correlation spectra (DARR mixing of 30 ms) recorded on β2m fibrils grown under acidic (pH 2.5, blue, at 10 kHz MAS on a 900 MHz) or neutral (pH 7.4, purple, at 11 kHz MAS on a 1 GHz) conditions. (Further plots of the correlation spectra are provided in the Supporting Information.)

Some distinct changes reflecting structural or dynamics modifications can be observed between the crystal and fibril spectra (Figure 2b). Ala15 is shifted, and signals from Thr appear either absent or shifted in the fibrils. Interestingly, they are all in the loop regions of the native tertiary structure (Figure 2c). Also, the native Ser residues of loops (near-diagonal signals) do not give rise to signals in the fibrils. Conversely, the signals from Ala79 and from Ser residues located in the β-strands of the native fold look conserved in the crystal and fibril spectra. In agreement with previous hypotheses,[14] this suggests that, in the transition to the fibrils, structural modifications mainly occur within the β2m loop regions (Figure 2c) and that most residues appear to participate in a protected β-sheet built from β-strands present in the native structure. This means that β2m fibrils do not match typical fibril architectures, which involve a rigid core and floppy subdomains.[10] Additionally, when considering the Pro resonances, no pattern distinctive of a *cis*-Pro (Figure 2b) is observed in the fibrils, implying a *trans* conformation for all Pro residues. This is remarkable since a Pro32 *trans*−*cis* isomerization is known to be the slow step in β2m folding[15] while the corresponding *cis*−*trans* isomerization is a key event in turning native β2m into an aggregation-prone intermediate species.[16]

Finally, to resolve the issue of the structural similarity between fibrils grown at either acidic or neutral pH,[6] we performed correlation spectra on both fibril species. The excellent superposition of such spectra (Figure 2d) indicates a very similar fine structure of the *in vitro* fibrils, suggesting conservation of the amyloid assembly architecture at nonphysiological pH 2.5 as compared to 7.4. This evidence reinforces previous low-resolution FTIR data[6] and stresses the significance of the acidic pH studies relative to β2m pathological aggregation at physiological pH.

In summary, the present study is an important step toward the characterization of the structure of β2m fibrils. Comparison between microcrystalline and fibrillar samples showed that the secondary structure elements appear maintained in the fibrils and suggests that Pro32 *cis*−*trans* isomerization accompanies fibril formation. The data reported here yield a molecular fingerprint of β2m in its pathological conformation, providing a valuable starting point for further studies of great medical relevance on the binding of natural as well as artificial ligands (e.g., collagene, glycosaminoglycans, and SAP)[17] to β2m fibrils.

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Supporting Information Available: Experimental details and full correlation spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Merlini, G.; Bellotti, V. *N. Engl. J. Med.* **2003**, *349*, 583–96.
(2) Bjorkman, P. J.; Saper, M. A.; Samraoui, B.; Bennett, W. S.; Strominger, J. L.; Wiley, D. C. *Nature* **1987**, *329*, 506–12.
(3) Chatani, E.; Goto, Y. *Biochim. Biophys. Acta* **2005**, *1753*, 64–75.
(4) Kad, N. M.; Myers, S. L.; Smith, D. P.; Smith, D. A.; Radford, S. E.; Thomson, N. H. *J. Mol. Biol.* **2003**, *330*, 785–97.
(5) Yamaguchi, K.; Naiki, H.; Goto, Y. *J. Mol. Biol.* **2006**, *363*, 279–88.
(6) Jahn, T. R.; Tennent, G. A.; Radford, S. E. *J. Biol. Chem.* **2008**, *283*, 17279–86.
(7) White, H. E.; Hodgkinson, J. L.; Jahn, T. R.; Cohen-Krausz, S.; Gosal, W. S.; Muller, S.; Orlova, E. V.; Radford, S. E.; Saibil, H. R. *J. Mol. Biol.* **2009**, *389*, 48–57.
(8) (a) Kad, N. M.; Thomson, N. H.; Smith, D. P.; Smith, D. A.; Radford, S. E. *J. Mol. Biol.* **2001**, *313*, 559–71. (b) Relini, A.; Canale, C.; De Stefano, S.; Rolandi, R.; Giorgetti, S.; Stoppini, M.; Rossi, A.; Fogolari, F.; Corazza, A.; Esposito, G.; Gliozzi, A.; Bellotti, V. *J. Biol. Chem.* **2006**, *281*, 16521–9.
(9) (a) McDermott, A. E. *Curr. Opin. Struct. Biol.* **2004**, *14*, 554–61. (b) Lange, A.; Giller, K.; Hornig, S.; Martin-Eauclaire, M. F.; Pongs, O.; Becker, S.; Baldus, M. *Nature* **2006**, *440*, 959–62. (c) Böckmann, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 6110–3.
(10) (a) Heise, H.; Hoyer, W.; Becker, S.; Andronesi, O. C.; Riedel, D.; Baldus, M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15871–6. (b) Petkova, A. T.; Leapman, R. D.; Guo, Z.; Yau, W. M.; Mattson, M. P.; Tycko, R. *Science* **2005**, *307*, 262–5. (c) van der Wel, P. C.; Lewandowski, J. R.; Griffin, R. G. *J. Am. Chem. Soc.* **2007**, *129*, 5117–30. (d) Heise, H. *ChemBioChem* **2008**, *9*, 179–189. (e) Wasmer, C.; Lange, A.; Van Melckebeke, H.; Siemer, A. B.; Riek, R.; Meier, B. H. *Science* **2008**, *319*, 1523–6. (f) Loquet, A.; Bousset, L.; Gardiennet, C.; Sourigues, Y.; Wasmer, C.; Habenstein, B.; Schutz, A.; Meier, B. H.; Melki, R.; Böckmann, A. *J. Mol. Biol.* **2009**, *394*, 108–18.
(11) Iwata, K.; Fujiwara, T.; Matsuki, Y.; Akutsu, H.; Takahashi, S.; Naiki, H.; Goto, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 18119–24.
(12) Katou, H.; Kanno, T.; Hoshino, M.; Hagihara, Y.; Tanaka, H.; Kawai, T.; Hasegawa, K.; Naiki, H.; Goto, Y. *Protein Sci.* **2002**, *11*, 2218–29.
(13) Sarkar, S. K.; Torchia, D. A.; Kopple, K. D.; Vanderhart, D. L. *J. Am. Chem. Soc.* **1984**, *106*, 3328–3331.
(14) Hoshino, M.; Katou, H.; Hagihara, Y.; Hasegawa, K.; Naiki, H.; Goto, Y. *Nat. Struct. Biol.* **2002**, *9*, 332–6.
(15) Kameda, A.; Morita, E. H.; Sakurai, K.; Naiki, H.; Goto, Y. *Protein Sci.* **2009**, *18*, 1592–601.
(16) (a) Kameda, A.; Hoshino, M.; Higurashi, T.; Takahashi, S.; Naiki, H.; Goto, Y. *J. Mol. Biol.* **2005**, *348*, 383–97. (b) Eakin, C. M.; Berman, A. J.; Miranker, A. D. *Nat. Struct. Mol. Biol.* **2006**, *13*, 202–8. (c) Jahn, T. R.; Parker, M. J.; Homans, S. W.; Radford, S. E. *Nat. Struct. Mol. Biol.* **2006**, *13*, 195–201. (d) Eichner, T.; Radford, S. E. *J. Mol. Biol.* **2009**, *386*, 1312–26.
(17) Myers, S. L.; Jones, S.; Jahn, T. R.; Morten, I. J.; Tennent, G. A.; Hewitt, E. W.; Radford, S. E. *Biochemistry* **2006**, *45*, 2311–21.

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