

An Asymmetric Dimer as the Basic Subunit in Alzheimer's Disease Amyloid β Fibrils **

Juan Miguel Lopez del Amo, Matthias Schmidt, Uwe Fink, Muralidar Dasari, Marcus Fändrich, and Bernd Reif*

Aggregation of monomeric amyloid β peptides (A β) into soluble oligomers and insoluble fibrils is one of the major pathological hallmarks of Alzheimer's disease (AD).^[1] In the past few years, magic angle spinning (MAS) solid-state NMR spectroscopy has enabled considerable progress in the structural characterization of amyloid aggregates, and a number of structural models have been suggested.^[2] Amyloid samples fibrilize in a large variety of morphologies.^[3] Differences in fibril morphology have been attributed to differences in peptide conformation.^[4] These variations in the molecular structures of A β fibrils might be responsible for the variations in the toxicity and deposition patterns of fibrils in AD.^[5] Whereas previous NMR studies implied that in all cases a U-shaped peptide fold forms the fundamental structural unit of the fibril,^[2a,b,6] cryo electron microscopy studies suggested that different A β fibril morphologies can be constructed from a dimeric arrangement of two conformationally distinct peptide molecules in each molecular layer of the protofilament.^[7] To date, however, there are no solid-state NMR data that supports the existence of such a structural subunit in A β fibrils.

Herein, we employed a seeding protocol to obtain a homogeneous fibril sample in which only one particular polymorph is present. In addition to MAS solid-state NMR

spectroscopy, electron microscopy (EM) was employed to characterize and quantify different polymorphs of A β fibrils.^[7a,8] Quantification was based on the amyloid fibril width and crossover distance, both of which were obtained in a straight forward way from negative-stain EM images.^[8a] EM images and the statistical analysis of the fibrils that were employed in the MAS solid-state NMR studies are represented in Figure 1. We found that one polymorph was predominant and was populated with an average crossover distance of 80 nm and a width of approximately 14 nm. This polymorph (polymorph I) made up 76% of the analyzed fibrils. A second polymorph (polymorph II) made up 10% of the analyzed fibrils. Relative to polymorph I, this second polymorph had a similar crossover distance (80 nm), but exhibited a broader width (18 nm). The remaining polymorphs accounted for 14% of the analyzed fibrils. The

[*] J. M. Lopez del Amo, M. Dasari, B. Reif
Helmholtz-Zentrum München (HMGU)
Deutsches Forschungszentrum für Gesundheit und Umwelt
Ingolstädter Landstrasse 1, 85764 Neuherberg (Germany)
E-mail: reif@tum.de

J. M. Lopez del Amo, M. Dasari, B. Reif
Center for Integrated Protein Science Munich (CIPS-M) at
Department Chemie, Technische Universität München (TUM)
Lichtenbergstrasse 4, 85747 Garching (Germany)

J. M. Lopez del Amo, U. Fink, M. Dasari, B. Reif
Leibnizinstitut für Molekulare Pharmakologie (FMP)
Robert-Rössle-Strasse 10, 13125 Berlin (Germany)

M. Schmidt, M. Fändrich
Max-Planck-Forschungsstelle für Enzymologie der Proteinfaltung,
Martin-Luther Universität Halle-Wittenberg (Germany)

M. Fändrich
Martin-Luther Universität Halle-Wittenberg (Germany)

[**] This research was supported by the Leibniz-Gemeinschaft, the Helmholtz-Gemeinschaft and the DFG (Re1435, SFB449, SFB740, and SFB610). We are grateful to the Center for Integrated Protein Science Munich (CIPS-M) for financial support. J.M.L.d.A. acknowledges a postdoctoral stipend of the DFG (Lo1546). M.F. is supported through the Exzellenznetzwerk Biowissenschaften (Sachsen-Anhalt).

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201200965>.

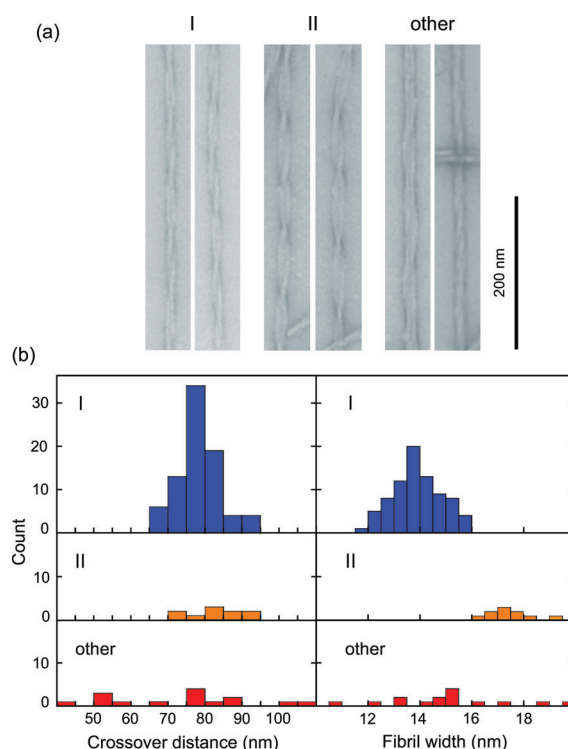


Figure 1. Analysis of fibril morphology. a) Representative negative-stain electron microscopy images of the two dominating fibril polymorphs (I and II) and other polymorphs. b) Statistical analysis of A β fibrils employed in MAS solid-state NMR investigations. Polymorph I accounts for 76% of the fibrils present. Polymorph II and all remaining fibril polymorphs account for 10% and 14%, respectively, of the fibrils present.

The chemical shifts are tabulated in the Supporting Information, Table S1. Residues 1–11 could not be observed by using either CP (Cross Polarization) or INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) NMR experiments. In agreement with previous solid-state NMR experiments, this region of the peptide thus seems to be disordered in the fibrillar state.^[6,12] 2D strip plots, which were extracted from 3D NCACX and NCOCX NMR data and focus on the region around residue S26, unambiguously demonstrate that the fibril sample employed in this study contains two conformers (see the Supporting Information, Figure S4).

To obtain site-specific secondary structure information, we analyzed the secondary chemical shifts,^[13] that is, the differences in chemical shifts between our assigned resonances and the corresponding resonances found in random coil peptides. The results for fibril conformer I and II are shown in Figure 3. Conformer I shows a pattern indicating a β -sheet/

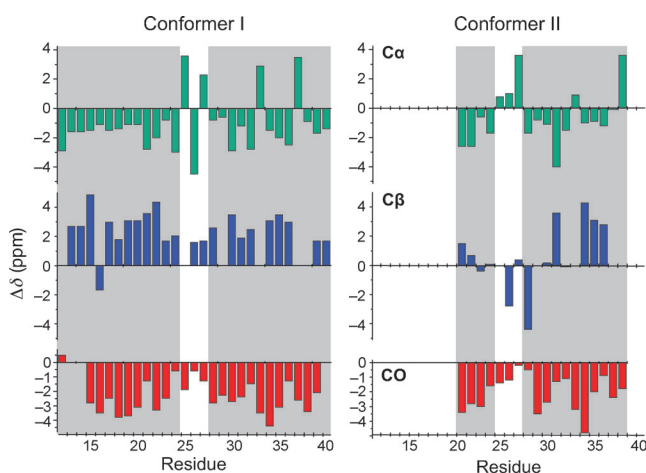


Figure 3. Secondary ^{13}C chemical shift analysis for conformer I and II observed in A β fibrils. $\Delta\delta$ indicates the difference between the herein observed and random coil ^{13}C chemical shift values.

turn/ β -sheet secondary structure, whereas conformer II shows a shorter structured sequence with a pattern indicating only a β -sheet secondary structure. This result is consistent with previous studies in which a pattern similar to that of conformer I is found for A β (1–40) as well as A β (1–42) fibrils.^[6,12] Analysis of the chemical shifts using the program Delta-2D gave a very similar result (see the Supporting Information, Figure S5).^[14] For conformer I, an increased propensity for a PPII (polyproline type II) helix is predicted for the loop region around residue G25, as well as for the region toward the edges of both β sheets. Similarly, in conformer II, the extended β sheet is flanked by residues that have an increased propensity to adopt a PPII helix. PPII motifs are not exclusively found in proline containing peptides and presumably also play an important role in defining the structures of amyloidogenic species.^[15]

In principle, there are two possibilities that can account for the two sets of resonances. In the first possibility, A β folds into different polymorphs, which are approximately equally populated. This possibility seems very unlikely as fibril

seeding would be expected to enrich the sample with a single polymorph. Furthermore, the different polymorphs would then need to have the same nucleation probability, the same fibril stability and the same fibrilization kinetics. Alternatively, in a second possibility, the amyloid fibril unit cell might contain an asymmetric A β dimer, yielding two sets of resonances of equal intensity. The EM analysis shows that one polymorph predominates and therefore suggests the presence of a unique conformer in each molecular layer of the protofilament.^[4] Minor conformers that are populated to only a small percentage will not be observable by solid-state NMR spectroscopy because the NMR signal intensity is proportional to the number of involved molecules. The high reproducibility in the preparation of the fibrils and the observation of a 1:1 ratio in the cross peak intensities associated with the two conformers (see the Supporting Information, Figure S3) strongly supports the presence of an asymmetric dimer in the fibril unit cell.

Our results herein lend support to the idea that A β fibrils can consist of an asymmetric peptide dimer as their fundamental structural unit. Recently, distinct polymorphs of Sup35 and IAPP fibrils have been reported.^[16] Other amyloidogenic peptides that crystallize as asymmetric dimers have also been reported.^[17] In these reports, the different conformers have been associated with different steric zipper interfaces that enable their assembly into composite β -sheet-rich building blocks that make up the protofilaments.^[18] It remains to be determined how the two A β peptides in our preparation are arranged with respect to one another. A more detailed analysis of the conformation of the two A β isoforms, or a characterization of their relative arrangement will require samples that are grown on media containing selectively ^{13}C -enriched nutrients,^[19] or by using deuterated substrates.^[20] Work along this direction is currently in progress.

Received: February 4, 2012

Published online: May 8, 2012

Keywords: Alzheimer's disease · amyloid β peptides · fibrillar aggregates · NMR spectroscopy · structural biology

- [1] J. A. Hardy, D. J. Selkoe, *Science* **2002**, 298, 962–964.
- [2] a) A. T. Petkova, W.-M. Yau, R. Tycko, *Biochemistry* **2006**, 45, 498–512; b) A. K. Paravastu, R. D. Leapman, W.-M. Yau, R. Tycko, *Proc. Natl. Acad. Sci. USA* **2008**, 105, 18349–18354; c) I. Bertini, L. Gonnelli, C. Luchinat, J. Mao, A. Nesi, *J. Am. Chem. Soc.* **2011**, 133, 16013–16022.
- [3] a) H. Abe, H. Nakanishi, *J. Pept. Sci.* **2003**, 9, 177–186; b) H. H. Bauer, U. Aepli, M. Haner, R. Hermann, M. Muller, T. Arvinte, H. P. Merkle, *J. Struct. Biol.* **1995**, 115, 1–15; c) T. P. J. Knowles, J. F. Smith, A. Craig, C. M. Dobson, M. E. Welland, *Phys. Rev. Lett.* **2006**, 96, 238301.
- [4] a) J. Madine, E. Jack, P. G. Stockley, S. E. Radford, L. C. Serpell, D. A. Middleton, *J. Am. Chem. Soc.* **2008**, 130, 14990–15001; b) R. Verel, I. T. Tomka, C. Bertozzi, R. Cadalbert, R. A. Kammerer, M. O. Steinmetz, B. H. Meier, *Angew. Chem.* **2008**, 120, 5926–5929; *Angew. Chem. Int. Ed.* **2008**, 47, 5842–5845; c) J. S. Pedersen, D. E. Otzen, *Protein Sci.* **2008**, 17, 2–10.
- [5] a) A. T. Petkova, R. D. Leapman, Z. H. Guo, W. M. Yau, M. P. Mattson, R. Tycko, *Science* **2005**, 307, 262–265; b) M. Meyer-

- Luehmann, J. Coomaraswamy, T. Bolmont, S. Kaeser, C. Schaefer, E. Kilger, A. Neuenschwander, D. Abramowski, P. Frey, A. L. Jaton, J. M. Vigouret, P. Paganetti, D. M. Walsh, P. M. Mathews, J. Ghiso, M. Staufienbiel, L. C. Walker, M. Jucker, *Science* **2006**, *313*, 1781–1784; c) W. Qiang, W. M. Yau, R. Tycko, *J. Am. Chem. Soc.* **2011**, *133*, 4018–4029.
- [6] T. Lührs, C. Ritter, M. Adrian, D. Riek-Loher, B. Bohrmann, H. Döbeli, D. Schubert, R. Riek, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 17342–17347.
- [7] a) C. Sachse, M. Fandrich, N. Grigorieff, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 7462–7466; b) M. Schmidt, C. Sachse, W. Richter, C. Xu, M. Fandrich, N. Grigorieff, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 19813–19818.
- [8] a) J. Meinhardt, C. Sachse, P. Hortschansky, N. Grigorieff, M. Fandrich, *J. Mol. Biol.* **2009**, *386*, 869–877; b) M. Fandrich, J. Meinhardt, N. Grigorieff, *Prion* **2009**, *3*, 89–93.
- [9] A. T. Petkova, G. Buntkowsky, F. Dyda, R. D. Leapman, W. M. Yau, R. Tycko, *J. Mol. Biol.* **2004**, *335*, 247–260.
- [10] M. Dasari, A. Espargaro, R. Sabate, J. M. Lopez del Amo, U. Fink, G. Grelle, J. Bieschke, S. Ventura, B. Reif, *ChemBioChem* **2011**, *12*, 407–423.
- [11] V. H. Finder, I. Vodopivec, R. M. Nitsch, R. Glockshuber, *J. Mol. Biol.* **2010**, *396*, 9–18.
- [12] A. T. Petkova, Y. Ishii, J. J. Balbach, O. N. Antzutkin, R. D. Leapman, F. Delaglio, R. Tycko, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16742–16747.
- [13] D. S. Wishart, B. D. Sykes, *J. Biomol. NMR* **1994**, *4*, 171–180.
- [14] C. Camilloni, A. De Simone, W. F. Vranken, M. Vendruscolo, *Biochemistry* **2012**, *51*, 2224–2231.
- [15] a) E. W. Blanch, L. A. Morozova-Roche, D. A. E. Cochran, A. J. Doig, L. Hecht, L. D. Barron, *J. Mol. Biol.* **2000**, *301*, 553–563; b) F. Eker, K. Griebenow, R. Schweitzer-Stenner, *Biochemistry* **2004**, *43*, 6893–6898; c) Y. Mansiaux, A. P. Joseph, J.-C. Gelly, A. G. de Brevern, *PLoS One* **2011**, *6*, e18401.
- [16] a) P. C. A. van der Wel, J. R. Lewandowski, R. G. Griffin, *J. Am. Chem. Soc.* **2007**, *129*, 5117–5130; b) J. T. Nielsen, M. Bjerring, M. D. Jeppesen, R. O. Pedersen, J. M. Pedersen, K. L. Hein, T. Vosegaard, T. Skrydstrup, D. E. Otzen, N. C. Nielsen, *Angew. Chem.* **2009**, *121*, 2152–2155; *Angew. Chem. Int. Ed.* **2009**, *48*, 2118–2121.
- [17] M. R. Sawaya, S. Sambashivan, R. Nelson, M. I. Ivanova, S. A. Sievers, M. I. Apostol, M. J. Thompson, M. Balbirnie, J. J. W. Wiltzius, H. T. McFarlane, A. O. Madsen, C. Riek, D. Eisenberg, *Nature* **2007**, *447*, 453–457.
- [18] a) P. C. A. van der Wel, J. R. Lewandowski, R. G. Griffin, *Biochemistry* **2010**, *49*, 9457–9469; b) J. R. Lewandowski, P. C. A. van der Wel, M. Rigney, N. Grigorieff, R. G. Griffin, *J. Am. Chem. Soc.* **2011**, *133*, 14686–14698.
- [19] a) F. Castellani, B.-J. van Rossum, A. Diehl, M. Schubert, K. Rehbein, H. Oschkinat, *Nature* **2002**, *420*, 98–102; b) A. Loquet, K. Giller, S. Becker, A. Lange, *J. Am. Chem. Soc.* **2010**, *132*, 15164–15166.
- [20] a) R. Linser, M. Dasari, M. Hiller, V. Higman, U. Fink, J.-M. Lopez del Amo, L. Handel, B. Kessler, P. Schmieder, D. Oesterheld, H. Oschkinat, B. Reif, *Angew. Chem.* **2011**, *123*, 4601–4605; *Angew. Chem. Int. Ed.* **2011**, *50*, 4508–4512; b) R. Linser, B. Bardiaux, V. Higman, U. Fink, B. Reif, *J. Am. Chem. Soc.* **2011**, *133*, 5905–5912; c) S. Asami, P. Schmieder, B. Reif, *J. Am. Chem. Soc.* **2010**, *132*, 15133–15135.