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1 AUTHOR:

Carl Henrik Görbitz
University of Oslo
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The structure of nanotubes formed by diphenylalanine, the core recognition motif of Alzheimer's β-amyloid polypeptide

Carl Henrik Görbitz*

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Alzheimer's β-amyloid diphenylalanine motif has previously been shown to self-assemble into discrete and extraordinary stiff nanotubes; these nanotubes were initially thought to be distinct from the single crystal structure of diphenylalanine, but it is now shown that the X-ray powder diffraction pattern of the nanotubes is identical to the simulated pattern for the single crystal structure, affording a new foundation for understanding and rationalizing the properties of this remarkable organic material.

Reches and Gazit reported in 2003 self-assembly of diphenylalanine (L-Phe-L-Phe, FF, Fig. 1a) by dilution of a concentrated solution of the dipeptide in 1,1,1,3,3,3-hexafluoro-2-propanol (hf-2-p) with water.[1] Intriguingly, the authors showed that the nanotubes formed could serve as casts for formation of silver nanowires with diameter ~ 20 nm by reduction of ionic silver within the nanotubes by citrate. Further investigations demonstrated that the FF nanotubes have unique mechanical properties placing them among the stiffest biological materials known.[2] Synthesis of peptide-nanotube platinum-nanoparticle composites was subsequently reported by Song *et al.*[3] using the enantiomer D-Phe-D-Phe, which is resistant to degradation by enzymes. The potential of peptide nanotubes as tools in biotechnology is substantial.[4,5] Furthermore, particular interest is focused on the supramolecular aggregation of FF due to its potential role in the formation of amyloid fibers by Alzheimer's β-amyloid polypeptide, where -F19-F20- is a key structural motif.[6] An understanding of the mechanisms involved in formation of amyloid fibers,[7–10] and the ability of such fibers to form transmembrane channels[11,12] is essential for the development of peptide-based drug candidates for treatment of the disease.[13,14]

Several short peptides have a pronounced tendency to form long needles or fibers when being crystallized from aqueous or non-aqueous solutions. Within this group, dipeptides with two hydrophobic residues have been particularly well characterized.[15–18] The single crystal X-ray structure of FF, Fig. 1b, was published in 2001 based on experimental data collected from a needle-shaped specimen with dimensions 550 × 26 × 24 μm grown by fast evaporation of an aqueous solution of the peptide at 80 °C.[15] Based on their results Reches and Gazit concluded, however, that the FF-nanotubes used for production of the wires represented "a completely different molecular arrangement compared with the self-assembled individual tubular structures".[1] From our own experience with hydrophobic dipeptides, we found it puzzling that FF should have another form that Reches and Gazit describe as "semi-crystalline", but which could in fact be a second polymorph. We have never seen any signs of polymorphism for FF and thus decided to scrutinize its structural properties by repeating the procedure described by Reches and Gazit.[1]

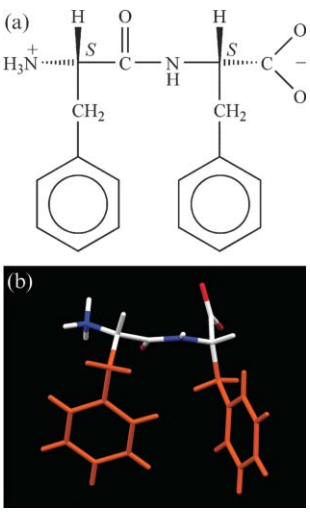

Fig. 1 (a) Schematic structure of FF. (b) The molecular structure of FF from the single crystal structure determination.[15] Side-chain atoms are shown in orange.

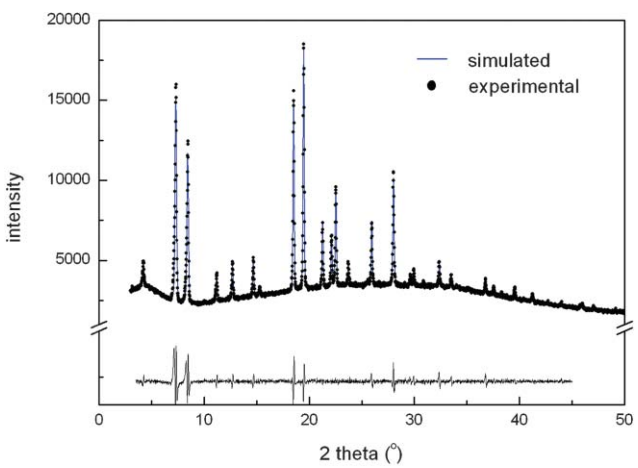

Fig. 2 X-ray powder diffractogram of FF fibers formed as described in the text compared with the simulated diffractogram from the single crystal structure.

Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, N-0315, Oslo, Norway. E-mail: c.h.gorbitz@kjemi.uio.no; Fax: +47 2285 5460; Tel: +47 2285 5441

Accordingly, 25 mg of FF was dissolved in 200 μl hf-2-p at 293 K. Under stirring, this solution was mixed with 20 ml of water, giving a final concentration around 3.5 μM with visual formation of peptide nanotubes occurring within about two seconds. The average fiber diameter appeared to be comparable to or somewhat larger than that observed previously, a broad distribution with average ~ 120 nm.[1] A powder X-ray diffractogram† was collected for a representative sample, and after Pawley refinement of the experimental data the curve was identical to the simulated curve for the single crystal structure, Fig. 2. It has thus been confirmed that explanations for the properties of FF, including the ability to form nanotubes, must be found within the framework of the known single crystal X-ray structure.[15]

By using a smaller volume of water (10 or 5 ml) in the above experiment, fibers with diameters well above 1 μm could be produced. This correlates with the investigations by Song *et al.*,[3] who synthesized their nanotubes by dissolving the peptide in water at 65 °C with subsequent slow cooling, thus omitting hf-2-p.

A tentative model for the construction of a peptide nanotube, arbitrarily chosen to have a 110 nm outer diameter and a 50 nm inner diameter, is presented in Fig. 3a. The exact nature of the inner surface shown in Fig. 3b is not known, but it may be mixed hydrophobic/hydrophilic as shown here, or entirely hydrophobic. The individual narrow channels (van der Waals' diameter 10 Å), Fig. 3c, are hydrophilic in nature and can accommodate guest molecules of some size as well as hydrated metal ions. With respect to formation of amyloid fibers in Alzheimer's disease, it has been observed that interaction between aromatic residues constitutes an essential part of the molecular basis for amyloid formation and stability.[7,8] As is evident from Fig. 3, the aromatic groups generate a striking three-dimensional aromatic stacking arrangement that serves as a glue between the hydrogen-bonded cylinders of peptide main chains and promotes fiber formation. The laminated construction gives exceptional strength[2] for a low-density porous supramolecular network and makes this structure unique among organic materials.

From their investigations of platinum-nanoparticle composites, Song *et al.* concluded that the walls of the peptide nanotube structure could be porous.[3] The model shown in Fig. 3 accounts for this property, but it should be pointed out that the pores run parallel to the main axis of the fiber, and thus do not serve as short-cuts through the wall perpendicular to the axis. The diffusion of Pt-ions must therefore (when crystal defects are excluded) take place from the short end of the fibers, explaining the rather lengthy period of time ("several hours") required for the process to be completed. This is in line with previous observations of I_2-diffusion into the nanoporous structure formed by the dipeptide L-Leu-L-Ser, which reached equilibrium after three days.[18]

The mechanism for formation of hollow fibers or nanotubes is elusive, but in unpublished work on a trifluoroethanol solvate of the dipeptide L-Ile-L-Ile in our laboratory formation of tubular crystals on a much larger scale (needle diameter 0.10 mm, cavity diameter up to 0.08 mm) has been observed visually, so the property of FF in this respect is not completely exceptional.

Reches and Gazit observed that the diameters of the peptide fibers covered a substantial range from 0 to > 300 nm.[1] The investigations by Song *et al.* yielded nanotubes with a 400 to 2000 nm outer diameter and a 300 to 1800 nm inner diameter,[3] which allows free entrance even of macromolecules and gives

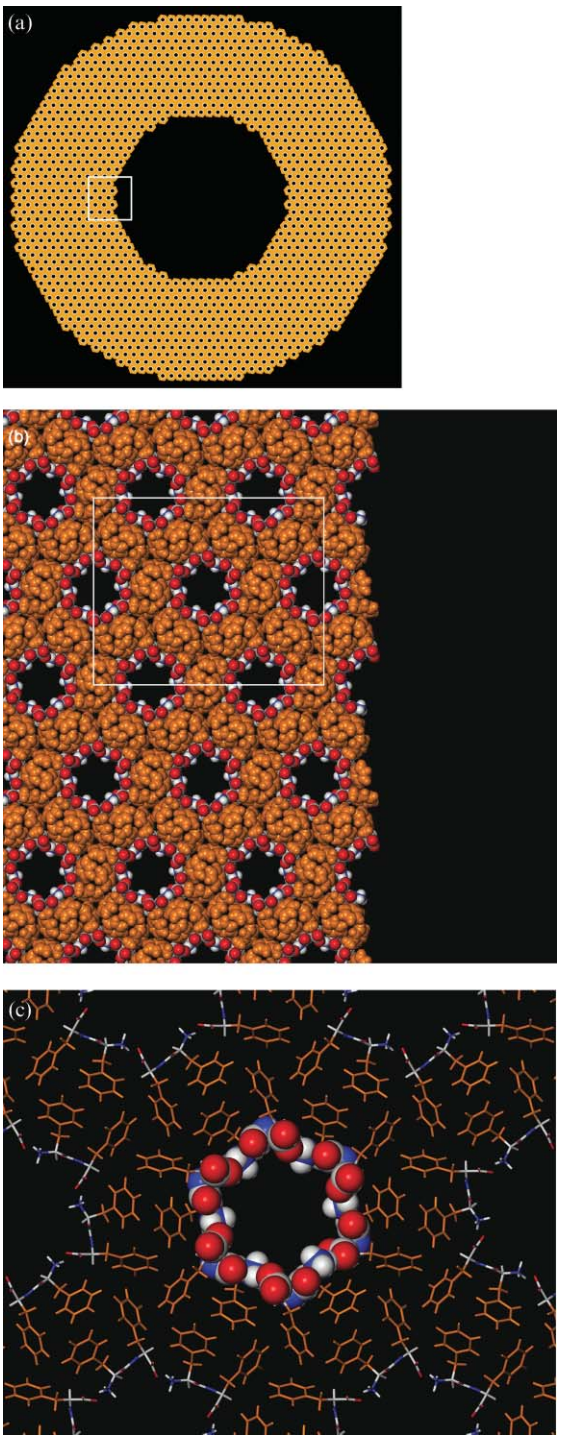

Fig. 3 Model for the construction of hollow FF fibers. Atoms in the peptide main chains are colored according to atom type, while atoms in the phenylalanine side chains are depicted in orange. Shown in (a) is a tube with a 110 nm outer diameter and a 50 nm inner diameter. This is comparable to the average size obtained by Reches and Gazit,[1] but smaller than that obtained by Song *et al.*[3] and in the present investigation. The white square indicates the part enlarged in (b) showing a model of the peptide-channel interface at the inner surface. The rectangle represents the detailed view given in (c) in a capped sticks representation, but with atoms constituting the pore surface in spacefill.

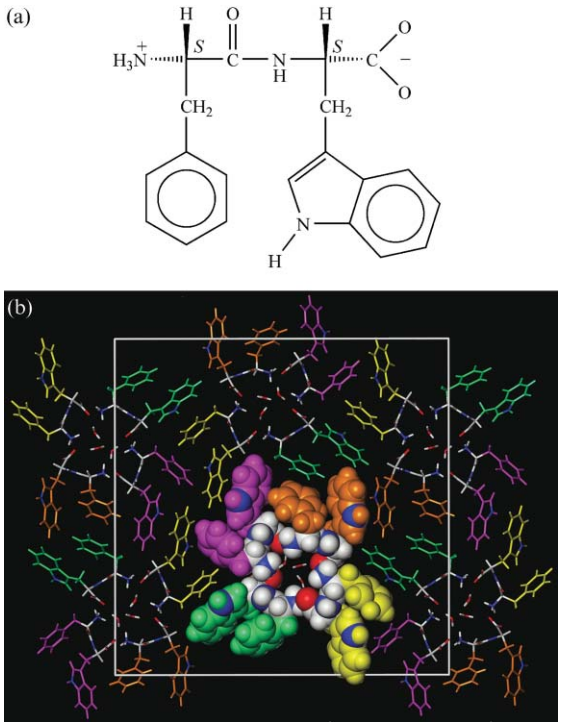

Fig. 4 (a) Schematic structure of FW. (b) Crystal structure and unit cell of FW viewed along the 5.6 Å *a*-axis. Peptide main chains and water molecules are colored by atom type, while C and H atoms in the side chains are colored either in orange, green-blue, yellow or magenta to emphasize the four independent peptide molecules in the asymmetric unit. Atoms surrounding one of the water-filled channels are depicted in space-fill representation.

cavity properties that differ marginally from bulk solvent. It seems unlikely that tubes of such variable (and sometimes very large) diameters can all be responsible for the formation of rather uniform 20 nm metal wires. It is possible that the wires are only produced by a small subset of the nanotubes with specific values for the inner diameters. Alternatively, a completely different mechanism may be in operation in which ions actually reside inside the individual channels, where they would be protected from the citrate oxidizing agent, but in which the oxidation process takes place at the end of the channels. Further investigations are needed to clarify this matter.

Crystallization experiments for other aromatic dipeptides (L-Phe-L-Trp, L-Trp-L-Tyr, L-Trp-L-Phe and L-Trp-L-Trp) were also carried out by Reches and Gazit,[1] who observed nanoscale tubular structures only in the case of L-Phe-L-Trp (FW), Fig. 4a.

The FW experiment has been replicated under conditions similar to those used to produce FF fibers (although with a lower peptide concentration due to the lower solubility in hf-2-p) with equivalent observation of formation of extremely thin fibers (diameter $<$ 300 nm). By using acetonitrile as the precipitating agent in a slow equilibration experiment against a hf-2-p solution of the peptide, fibers with diameter up to 20 μm could be obtained, enough for collection of X-ray single crystal data.† As for FF, the X-ray powder diffraction diagrams for the initial microcrystalline sample and the simulated spectrum of the single crystal structure are identical.

The structure of FW, depicted in Fig. 4b, is relatively complex, with four peptide molecules and three water molecules in the asymmetric unit, but follows the general buildup of other dipeptides in the FF-class[15] in having cylinder-shaped hydrophilic regions with solvent-filled central channels. Hydrophobic side chains emanate from the hydrophilic core, and the structure can be seen as a pseudo-hexagonal close-packing of hydrophobic tubes with a diameter of about 18 Å. The same construction principles and intermolecular interactions govern all structures in the FF class, but the 10 Å diameter channels of FF are by far the largest. Other compounds have channels that are more rectangular in shape with van der Waals' dimensions ranging from 2.5 $\times$ 6.0 Å to 4.0 $\times$ 6.0 Å. It is conceivable that one or more of these dipeptides could also form hollow fibers, but the individual channels are too small to accommodate hydrated metal ions.

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Notes and references

† Siemens D-5000 powder diffractometer with Ge-primary monochromator and MBraun PSD-50M detector, scan range 3–90° 2θ, count 3 s, step 0.016°. Pawley refinement including cell parameters. Siemens SMART 1000 single crystal CCD-diffractometer, $Mo_{K\alpha}$ radiation (λ = 0.71069 Å), data collections on a 0.540 $\times$ 0.020 $\times$ 0.018 mm fiber at 105 K with SMART,[19] data integration and cell refinement with SAINT,[20] absorption correction by SADABS,[21] structure solution by and least-squares refinement on F^2 with SHELXTL.[22] L-Phenylalanyl-L-tryptophan: $C_{20}H_{21}N_3O_3{\cdot}0.75H_2O$, M = 364.91, orthorhombic, $P2_12_12_1$, a = 5.6207(6) Å, b = 35.555(4) Å, c = 35.835(4) Å, Z = 16, $N_{observed}$ = 2726, $R[F^2 > 2\sigma(F^2)]$ = 0.064, w$R(F^2)$ = 0.150, CCDC 299570, details will be reported elsewhere. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b603080g

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