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Amyloids

A Fiberlike Peptide Material Stabilized by Single Intermolecular Hydrogen Bonds**

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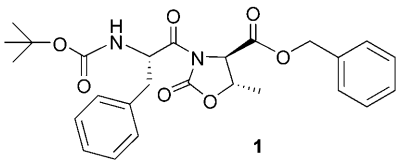
Aggregation and disaggregation are central phenomena in nature.[1] In this context, formation of fibers through self-assembly is of particular interest, as protein fibers are involved in intra- and extracellular functions. Moreover, several diseases such as Alzheimer's or prion diseases are characterized by extracellular protein depositions.[1–4] Recently, it was demonstrated that β-sheet layers are the most stable superstructure, although an exact explanation for the existence of this "dead-end" structure cannot yet be given.[5]

To understand aggregation phenomena, oligopeptides that interfere with[6–8] or mimick [9–14] these processes may be designed and prepared. Indeed, the potential applications of such supramolecular assemblies exceed those of synthetic polymers since the building blocks may introduce biological function in addition to mechanical properties.[15] All reported examples show fiber-forming peptides that are stabilized in the solid state by at least two N–H···O=C hydrogen bonds. Herein, we present a fiberlike material that is stabilized by only single hydrogen bonds between dipeptide units. Such an assembly represents the absolute borderline case of a sheet structure.

The fiberlike material was obtained by aggregation of Boc-L-Phe-D-Oxd-OBn (**1**; Boc = *tert*-butoxycarbonyl; Phe = phenylalanine; Oxd = 4-methyl-5-carboxy oxazolidin-2-one; Bn = benzyl),[16,17] which can be easily synthesized by coupling of Boc-L-Phe-OH with H-D-Oxd-OBn (for details, see the Supporting Information).

Compound **1** was purified by flash chromatography using a 1:1 mixture of cyclohexane and ethyl acetate as the eluting solvent. The formation of a fiberlike white solid was detected after evaporation of the mixture overnight. Moreover, a 20–25 mM solution of **1** in the same solvent mixture forms a gel, which becomes a white solid after slow solvent evaporation (Figure 1a). The molecule also shows good solubility in polar solvents, such as acetonitrile, methanol, diethyl ether, and ethyl acetate, but is not soluble in water. It assembles to form a fiberlike material under a wide variety of solution conditions. This material shows strong birefringence and has defined edges. It is made of bundles of crystalline-shaped filaments clustered and aligned along the main bundle direction (Figure 1b).



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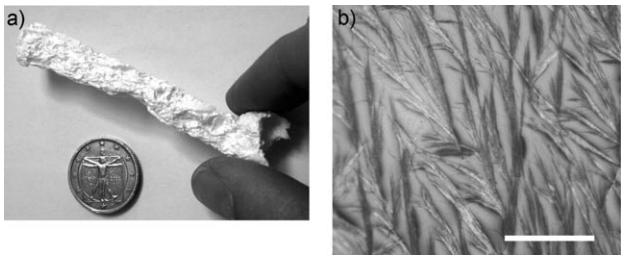

Figure 1. Boc-L-Phe-D-Oxd-OBn (**1**) after slow evaporation of a 1:1 mixture of cyclohexane/ethyl acetate. a) String of pure **1** after slow evaporation of the solvent from a 25 mM solution. b) Optical micrograph obtained through crossed polarizers of fiberlike material. Scale bar: 0.3 mm.

The IR spectra of **1** in dichloromethane and of a 1% mixture of solid **1** with KBr showed very different results. Hydrogen bonding is clearly present in the solid but is distinctly diminished in solution, indicating its intermolecular character. The analysis of the C=O region (1600–1850 cm^{-1}) confirms this conclusion (Figure 2).

A small fragment of **1** was examined by scanning electron microscopy (SEM; Figure 3a). Interestingly, the thickness and width of the mature filaments are almost constant, regardless of the solvent conditions for assembly, whereas their length varies widely even under a single set of experimental conditions. The material is stable. Its overall shape was conserved in the precipitation media for several months and even after it was air-dried. Only strong thermal

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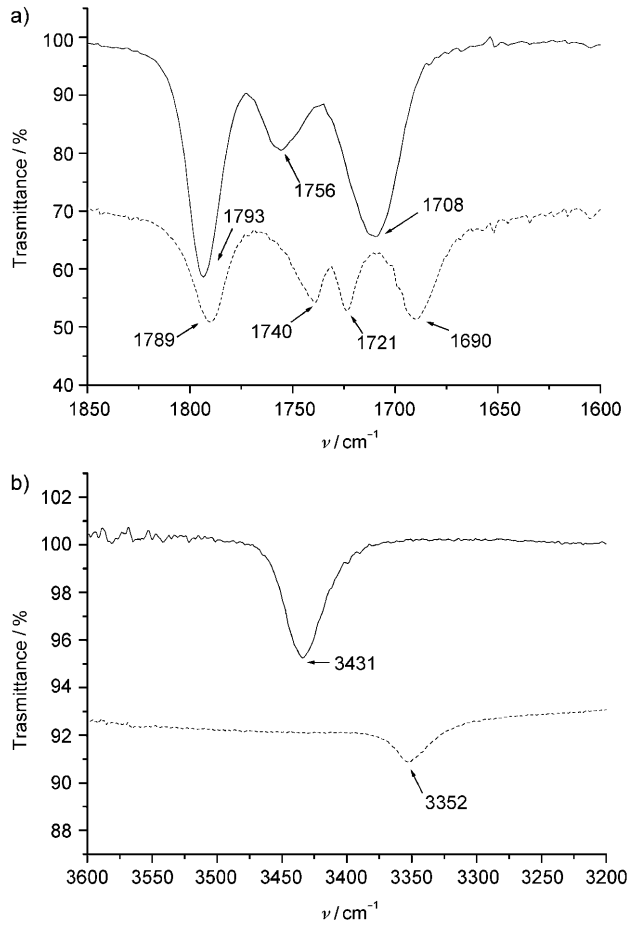

Figure 2. FTIR absorption spectra a) in the NH stretching region (3600–3200 cm^{-1}) and b) in the CO stretching region (1850-1600 cm^{-1}) for 3 mM samples of **1** in pure CH_2Cl_2 (solid line) and as 1% solid mixture with KBr (dashed line) at room temperature.

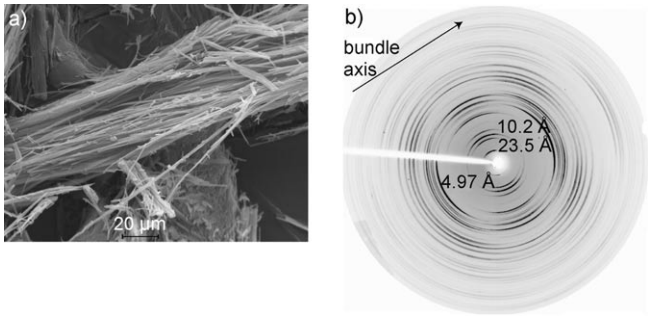

Figure 3. a) SEM image of filaments bundles of **1**. b) X-ray diffraction pattern of a bundle of **1**. The X-ray beam was normal to the bundle axis. The orientation of the bundle axis and the X-ray spacings of the most intense reflections are indicated.

treatment, such as by an electron beam, provoked the collapse of the material.

The highly birefringent fiberlike material was analyzed by X-ray diffraction (XRD) using beams of two different sizes. When a beam size of 200 μm was used, the material showed a strong fiberlike XRD pattern that indicates a preferential orientation of crystalline units along the long axis (Figure 3b). Many strong meridional and equatorial diffraction reflections were observed. The two main equatorial reflections and the strong meridional reflection correspond to lattice distances of 23.5, 10.2, and 4.97 Å, respectively. Indexing of the fiber diffraction reflections (see the Supporting Information) on the basis of a monoclinic unit cell indicates the preferential alignment of the molecule along the crystallographic *b* axis. The use of a narrow X-ray beam (50 μm) or the selection of a small bundle fragment showed diffraction patterns typical of single crystals.

Crystals suitable for X-ray diffraction study were grown by slow evaporation of a solution of **1** in diethyl ether at room temperature (Figure 4).[18] The backbone torsion angles for L-Phe are $\phi = -130.3°$ and $\psi = 154.8°$ and correspond approximately to those in peptide β-strands; the D-Oxd backbone torsion angles are $\phi = 60.6°$ and $\psi = 23.4°$.

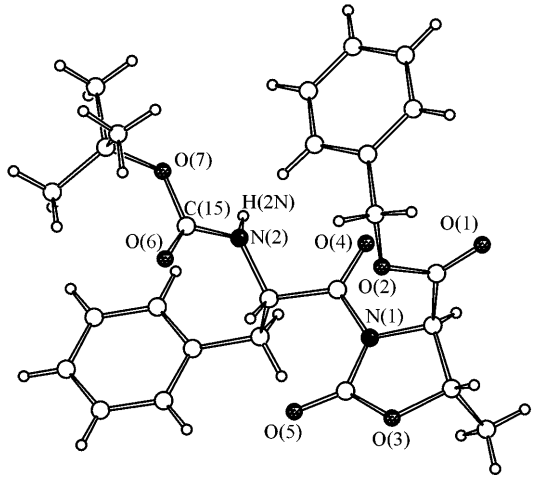

Figure 4. X-ray crystal structure of **1**.

Linear chains are formed in the crystal packing of **1** through only one intermolecular hydrogen bond between the units (Figure 5). This hydrogen bond is between the carbonyl group C(15)O(6) of one molecule and the NH group N(2)H(2N) of the L-Phe residue of an adjacent molecule. The distances of the hydrogen bonds are $d(H\cdots O) = 2.16$ Å and $d(N\cdots O) = 3.00$ Å, and the angle N–H···O is 161°. The peptide units are in a parallel arrangement. Thus, the solid-state structure of **1** could be considered as a borderline case of a parallel β-sheet structure. The phenyl rings of adjacent units are in a displaced-stacked arrangement, but the shift of the phenyl rings is larger than typically found for π–π stacking. Thus, a strong contribution of dispersion energy to the stability arising from this orientation cannot be expected.[19,20]

The structure of **1** differs from that of the peptide analogue Boc-Aib-L-Oxd-OBn with α-aminoisobutyric acid (Aib) instead of L-Phe, which we recently presented.[21] Although the monomers of the Aib peptide are also connected by only one hydrogen bond in the crystal structure, it does not form fibers. The preference of the typical backbone torsion angles of a 3_{10}-helix (βIII-turn) by Aib leads to a nonlinear hydrogen-bonding network and prevents fiber formation.

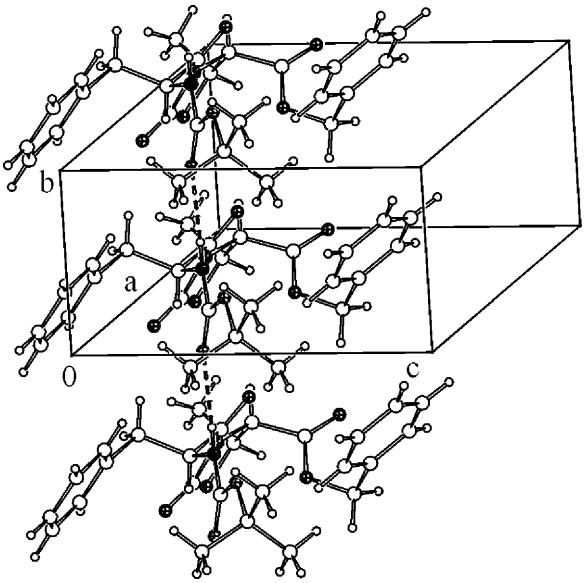

Figure 5. Crystal packing of **1** showing one chain running along the *b* axis connected by a single N–H···O=C interaction between the molecules.

Fibers of **1** were also analyzed by solid-state NMR spectroscopy. A ^{13}C CP MAS NMR spectrum of **1** is shown in the Supporting Information. Narrow NMR lines indicate a very homogeneous preparation. Qualitative structure information can be obtained from these spectra because isotropic chemical shifts correlate with the secondary structure of proteins.[22] In particular, the difference between the chemical shifts of the Cα and Cβ atoms of the amino acids is meaningful as it is independent of external referencing.[23] For L-Phe in **1**, this difference is $\delta = 15.9$ ppm. Values of $\delta = 21.8$, 17.5, and 14.7 ppm are given for this residue in an α-helix, in a random coil, and in a β-sheet structure, respectively.[22] This result confirms that fibers of **1** show a tendency towards a β-sheet structure. As the fibers are only stabilized by a single hydrogen bond, small deviations from the ideal sheet structure are comprehensible. We further analyzed the amplitude of motion of the Cα–H bond vector by measuring the molecular-order parameter of L-Phe in **1**. A value of 0.77 indicates that the molecular site is rather rigid in the fiber, undergoing motions with an amplitude of approximately 30°, which is in agreement with protein fibrils.[24]

A systematic conformational analysis of **1** at the B3LYP/6-311 + G(2d,p) and HF/6-31G* levels of ab initio MO theory shows some conformers of comparable energy with a slight preference of the experimentally found structure (Supporting Information). The calculated backbone torsion angles for the L-Phe residue are $\phi = -114.7°$ and $\psi = 159.9°$ and those for D-Oxd $\phi = 73.9°$ and $\psi = 14.8°$. The calculated difference between the ^{13}C NMR shifts of the Cα and Cβ atoms of L-Phe is $\delta = 14.4$ ppm according to B3LYP/6-311 + G(2d,p). This value corresponds well to the reference value of $\delta = 14.6$ ppm for L-Phe in a sheetlike conformation[22] and the measured value of $\delta = 15.9$ ppm from solid state NMR spectroscopy. The corresponding ^{13}C shift differences calculated for the alternative conformers are distinctly larger and not in agreement with the measured and sheet reference values (see Table S2 in the Supporting Information). Comparing the stabilities of **1** in its X-ray arrangement with its arrangement in the Aib peptide package after optimization of dimers at the HF/6-31G* level confirms the preference of the X-ray structure. In analogous way, the X-ray package found for the Aib peptide is preferred over the alternative arrangement found for **1**.

In conclusion, we have demonstrated that the dipeptide Boc-L-Phe-D-Oxd-OBn with the L-Phe residue in a β-strand conformation spontaneously forms fibers consisting of infinite linear chains, in which the parallel dipeptide units are connected only by a single hydrogen bond. This is the absolute borderline case of a parallel β-sheet structure, since the second amino acid of the dipeptide is not involved in further intermolecular interactions.

Experimental Section

Optical micrographs were obtained by using a Leika Wetzlar microscope equipped with a digital camera. The SEM observations on the fiberlike samples were performed on a Philips 515 instrument operating at 15 kV. The samples were mounted on a mica surface and coated with gold prior to observation. The fiber diffraction pattern of a filaments bundle of **1** was collected at the Elettra in Trieste, Italy (beam-line XRD). The pattern was recorded on a MAR CCD X-ray detector using a wavelength of 0.976 Å and a fiberlike material-detector distance of 130 mm. The images were analyzed by Fit2D software. The powder X-ray diffraction patterns were recorded using an X'Celerator diffractometer (PANalytical) with $Cu_{K\alpha}$ radiation and a Ni filter. The calculated pattern was obtained on the basis of the crystallographic structure of **1** by using the software Mercury 1.4.2. ^{13}C solid-state NMR experiments were carried out on a Bruker Avance 750 spectrometer with a 4 mm spinning module. The strength of the ^{13}C–^{1}H dipolar couplings for determination of the order parameters was measured by using the constant time DIPSHIFT pulse sequence with MREV-8 as homonuclear decoupling sequence at a MAS rate of 5 kHz. Experimental dephasing curves were simulated for one rotor period to extract the dipolar coupling of the respective signal. Order parameters were calculated by dividing the motionally averaged coupling by its rigid limit value. The quantum chemical calculations were performed by employing the Gaussian 03 software package (Gaussian, Inc., Wallingford, CT 2004). For further details on all employed methods, see the Supporting information.

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