β-Sheet Self-Assembly

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Helical-Ribbon Formation by a β-Amino Acid Modified Amyloid β-Peptide Fragment**

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Oligomeric β-peptides can self-assemble into helical structures in certain solvents.[1–3] β-Peptides are also of interest in terms of bioactivity, including resistance to proteolysis[4] as well as antibacterial[5] or antifungal[6] properties. There have been fewer studies on the influence of β-amino acid residues on the self-assembly of mixed oligomeric α/β-peptides.[7,8] Our efforts to investigate the influence of specific residues on the self-assembly of a fragment of the amyloid β (Aβ) peptide have now led us to report on the aqueous self-assembly of βAβAKLVFF, which is modified at the N terminus with two β^2-Ala residues. The Aβ peptide is implicated in amyloid diseases, such as Alzheimer's disease.[9] Fragment KLVFF has been identified as a key sequence involved in β-sheet-fibril formation, and has been targeted for oligomer-disruption therapies.[10,11] We recently studied the self-assembly of the extended variant AAKLVFF in water and methanol. In water, twisted β sheets were observed,[12] whereas in methanol, closed nanotubes were formed; these nanotubes are also based on wrapped β-sheet structures.[13] Herein, we show that substitution of the alanine residues with β-alanine residues leads to helical-ribbon nanostructures. Such structures have been considered theoretically on the basis of the chiral elasticity of membranes, among other models;[14] however, theory suggests that ribbons of finite width are unstable with respect to twisted sheets (helicoids) or spiral ribbons of infinite width.[15] Ribbons have also been observed for β sheets from conventional peptides;[16–19] however, they have not previously been noted for β-amino acid containing peptides.

The peptide βAβAKLVFF was dissolved in water or D_2O to perform cryo-TEM, X-ray diffraction (XRD), circular dichroism spectroscopy, or FTIR measurements.

Cryo-TEM revealed the formation of helical ribbons (Figure 1 a, inset). The average pitch ((70.1 ± 7.7) nm) is much larger than that for the various helical structures observed for β-peptides (ca. 5 Å per turn)[3,20] and thus clearly points to a distinct superstructure. The average diameter is (17.5 ± 3.5) nm. Both left- and right-handed twisted helices are observed; they occasionally intertwine into a double helix (Figure 1). The fact that helices of both handedness are

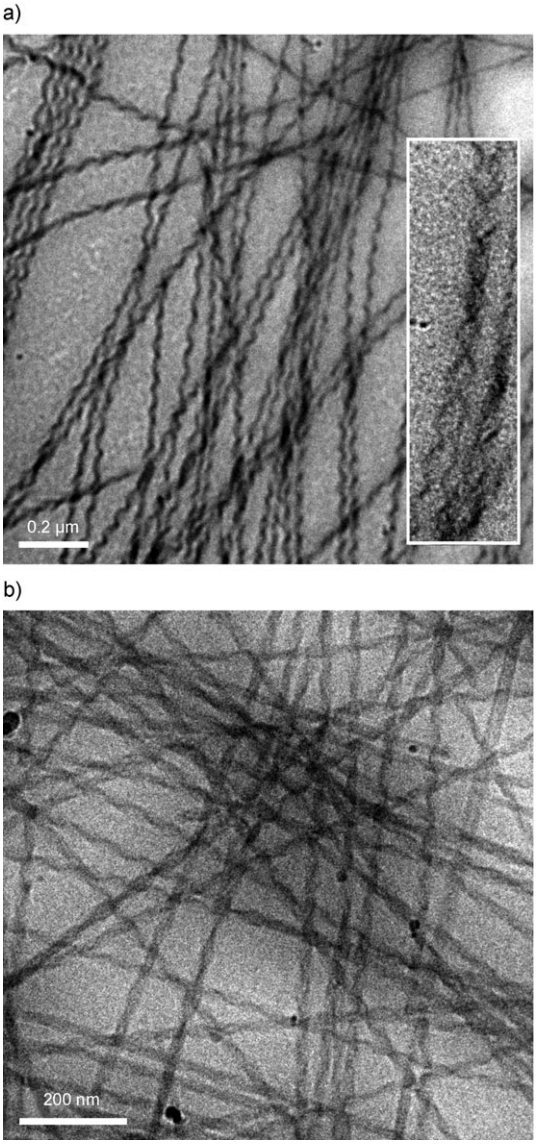

Figure 1. Representative cryo-TEM images at different magnifications. The inset in (a) shows a region from a different micrograph in which twisting into double helices can be observed.

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observed indicates that the helical order does not arise directly from the chirality of the L-amino acids, in contrast to the chiral superstructures formed by polystyrene–poly(isocyanodipeptides), the helicity of which was opposite to that of the molecular building blocks.[21] However, helical coiled micelles of both handedness were observed for triblock copolymers complexed with different multiamines.[22] The helical ribbons observed for βAβAKLVFF may be contrasted with the cylindrical fibrils observed for KLVFF in aqueous solution[23] or the twisted tapes observed for AAKLVFF in water.[12] These two controls clearly indicate the role of the β-amino acids in the formation of the helical nanoribbons.

X-ray diffraction and spectroscopic techniques were used to confirm that the helical-ribbon structures are associated with β-sheet structures. Figure 2a presents an X-ray diffraction pattern obtained for a dried aligned stalk. The perpendicularly arranged equatorial (principal peaks at 27.0, 20.0, and 11.8 Å) and meridional (strong peak at 4.84 ± 0.01 Å) reflections indicate a cross-β structure.[24] The off-meridional reflections at (3.88 ± 0.02) Å at $\pm 35°$ with respect to the meridian result from the helical superstructure. Such reflections were observed previously for twisted β sheets and assigned to the C^{α}–C^{α} spacing.[25–27] Note that in Figure 2a, neither the equatorial nor the meridional reflections are split, whereas these reflections are off-axis for a helical-nanotube structure in which the β strands are tilted with respect to the tube (fiber) axis.[28] The β strands are predominantly arranged perpendicular to the ribbon axis (Figure 2b, I), as in a fibril cross-β structure. The presence of equatorial peaks also at 4.84 Å points to the presence of some ribbons with strands oriented parallel to the ribbon axis (Figure 2b, II). The schematic illustrations in Figure 2b represent single twisted β sheets. In reality, the helical ribbons will have finite thickness, that is, will comprise a finite stack of sheets. It may be estimated by inspection of the cryo-TEM images that the ribbon thickness is 0.3–0.5 times the fibril diameter. This estimate is based on inspection of the dimensions of the "necks" in the twisted helices and implies that the ribbon comprises a stack of 5–8 sheets. The finite ribbon thickness will also influence the width of the equatorial reflections in the XRD pattern; these reflections are observed to be quite broad.

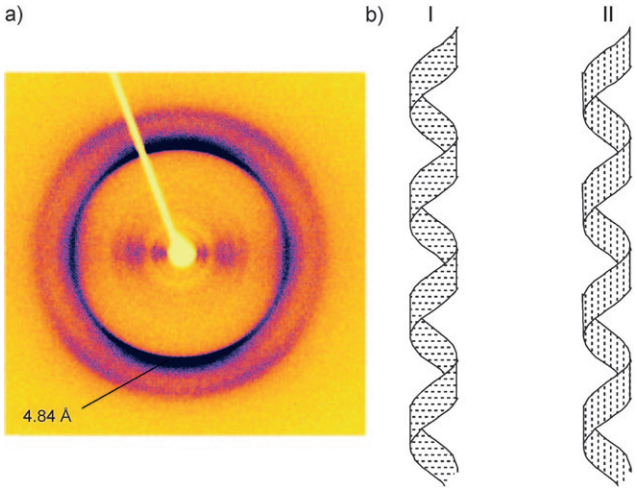

Figure 2. a) X-ray diffraction pattern from an aligned stalk. b) Models for helical-ribbon structures. The dashed lines represent β strands (not drawn to scale). For simplicity, single helical sheets are drawn, although in reality the ribbons comprise stacks of 5–8 sheets.

Circular dichroism spectroscopy supports the formation of β sheets, as a negative maximum was observed at 216 nm for a dried film (Figure 3a). In dilute aqueous solution, the CD spectrum contains a positive maximum at 220 nm. This feature has previously been ascribed to aromatic stacking interactions of the phenylalanine residues in KLVFF-based peptides.[12,23,29] In particular, similar features were observed for AAKLVFF;[12] this result indicates the insensitivity of this technique to the self-assembled superstructure. It is clear that our solution CD spectra do not resemble those reported for 3_{14}-helix monomers or bundles.[30] The solution FTIR spectrum for βAβAKLVFF is quite distinct from that for AAKLVFF (Figure 3b). The latter contains β-sheet features in the amide I region at 1623 and 1674 cm^{-1}. The first peak is absent for βAβAKLVFF, although a peak at 1672 cm^{-1} is still present and accompanied by a broader peak at 1640 cm^{-1}, which may either be a frequency-shifted β-sheet peak or be due to some random-coil secondary structure.[31] The former seems more likely, given that cryo-TEM does not reveal any random-coil-like structures. Further features are present in the amide II region, including a small sharp peak at 1455 cm^{-1}. A full vibrational-mode analysis with allowance

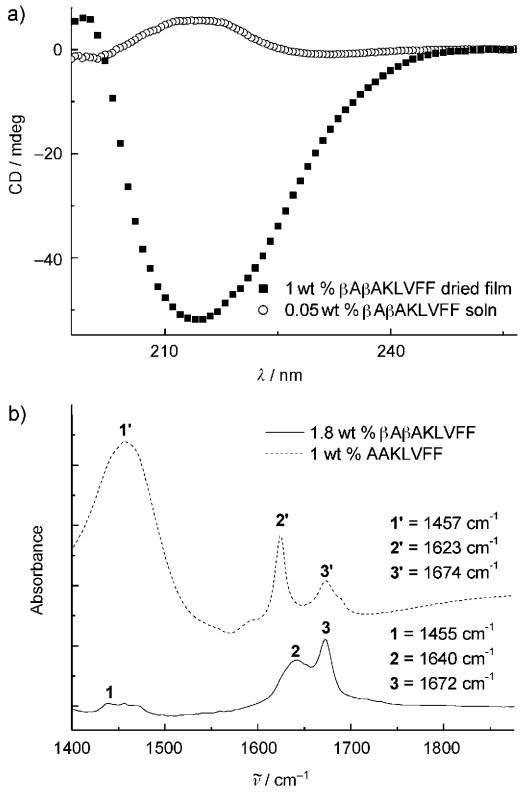

Figure 3. a) Circular dichroism for a solution and a dried film. b) FTIR spectra for solutions of βAβAKLVFF and AAKLVFF.

for transition dipole coupling[32] is required before detailed interpretation of the FTIR spectra is possible.

Further evidence for amyloid formation by βAβAKLVFF was provided by labeling experiments with Congo red dye. In particular, "apple green" birefringence was observed (Figure 4).[9] There was also an enhancement of thioflavin T fluorescence (data not shown).

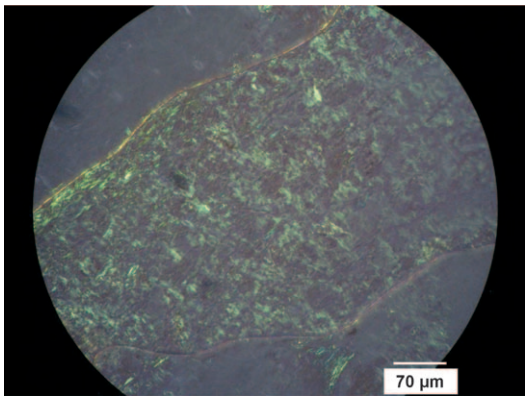

Figure 4. Polarized optical microscope image of βAβAKLVFF (2 wt%) stained with Congo red.

At a higher concentration (6 wt%), βAβAKLVFF forms a nematic phase, as characterized by fluidity and birefringence, and a characteristic texture in the polarized optical microscope image (Figure 4). There is evidence for pre-nematic ordering in the cryo-TEM images, which show the local alignment of ribbons (Figure 1a, top left-hand corner). The nematic phase shows alignment under flow (Figure 5b),

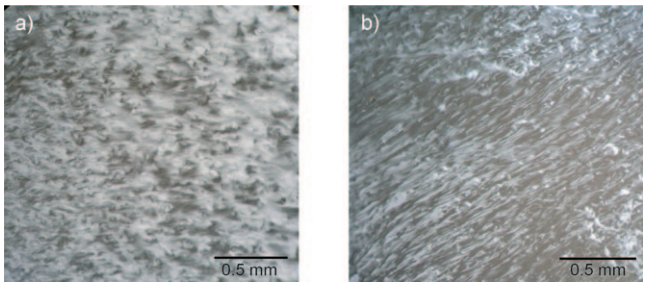

Figure 5. Polarized optical micrographs of a 6 wt% aqueous solution of βAβAKLVFF a) before and b) after shear. In (b), the stripes are aligned along the shear direction.

which was achieved by manual shearing between the glass slide and the coverslip in the microscope. The "banded" texture after shear alignment (Figure 5b) is characteristic of an aligned nematic structure.[33,34]

Our observation of a ribbon β-sheet motif for β-peptides expands the list of known secondary structures[3] for this class of biomolecule. It also highlights our ability to rationally design unanticipated secondary structures on the basis of the sequence modification of model peptides. βAβAKLVFF is expected to be stable against N-terminal aminopeptidases, but not against C-terminal carboxypeptidases or endopeptidases. The introduction of β-amino acids elsewhere in the sequence may be required to further reduce proteolysis. It will be interesting to investigate whether the addition of helical-nanoribbon-forming peptides, such as βAβAKLVFF, disrupts the fibrillization or oligomerization of Aβ peptide or fragments of this peptide, and whether such peptides are useful in the development of therapeutic compounds.

Experimental Section

NH_2-β^2Ala-β^2Ala-Lys-Leu-Val-Phe-Phe-COOH was purchased from CS Bio (Menlo Park, CA) as the trifluoroacetic acid (TFA) salt. Purity is 98.32% based on HPLC (TFA in water/acetonitrile gradient); M_w: expected: 794.98; found: 795.27.

Cryo-TEM, performed at the University of Delaware, is a powerful technique to elucidate the self-assembled structure of amphiphilic molecules in solution.[35] A thin film (ca. 100 nm) of the 1.8 wt% βAβAKLVFF sample in water was transferred to a lacey carbon grid, blotted with filter paper, and plunged into liquid ethane. All samples were prepared by using the environmentally controlled, automated Vitrobot from the FEI Company, Hillsboro, OR (USA). In a typical sample preparation, the sample chamber was maintained at 25°C and 40% relative humidity. Prior to plunging into liquid ethane, the sample was blotted with filter paper twice for 2 s. Following vitrification, the samples were transferred to a Gatan cryoholder precooled to −175°C, before insertion into the electron microscope. Imaging was carried out in the bright-field mode at 120 kV in a Technai T12 electron microscope (FEI Company, Hillsboro, OR). During imaging, the temperature of the sample holder was maintained at −175°C to inhibit sublimation of vitreous water.

The circular dichroism (CD) spectra were recorded on a Chirascan spectropolarimeter (Applied Photophysics, UK). Samples were dissolved in water (0.05 wt%) and loaded into a 1 mm quartz cuvette. Spectra were obtained from 190 to 260 nm with a 0.5 nm step, 1 nm bandwidth, and 1 s collection time per step at 20°C; five readings were taken and averaged.

IR spectra including amide bands were recorded on a Nicolet FTIR Nexus spectrometer equipped with a DTGS detector. Solutions of βAβAKLVFF in D_2O (1.8 wt%) were sandwiched in ring spacers between two CaF_2 plate windows (spacer 0.006 mm). Spectra were scanned 128 times over the range of 4000–400 cm^{-1}.

X-ray diffraction was performed on stalks prepared by drying filaments of the peptide. An aqueous solution (6 wt%) of the peptide was suspended between the ends of wax-coated capillaries and dried. The stalks were mounted (vertically) onto the four-axis goniometer of an R-AXIS-IV X-ray diffractometer (Rigaku) equipped with a rotating anode generator. The XRD data were collected by using a Saturn 992 CCD camera.

A 2 wt% βAβAKLVFF sample was stained with a freshly prepared and filtered Congo red/NaCl solution. The Congo red/NaCl solution contained 0.5 wt% Congo red and 0.2 wt% NaCl diluted in a MeOH/water mixture (80% MeOH, 20% water).[29] A drop of the stained 2 wt% βAβAKLVFF sample was placed onto a glass microscope slide under a cover slip. The sample was then observed with the microscope through crossed polarizers.

Polarized optical microscopy images were obtained with an Olympus BX41 microscope by placing the sample between crossed polarizers. Samples were placed between a glass slide and a coverslip, and the images were captured with a Canon G2 digital camera.

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