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Chirality of Amyloid Suprastructures

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Understanding the chirality of polypeptide suprastructures is an issue of fundamental importance, which has attracted the attention of protein scientists since the first models of protein structures emerged. Thus, it is experimentally well-established that (*S*)-aminoacid[1] β-sheets assume a conformation characterized by a left-handed twist along the sheet.[2] Helical suprastructures resulting from association of several β-sheets should subsequently also display left-handed helicity. This assumption has far-reaching consequences in the understanding of amyloid structures, where it has been both experimentally confirmed and theoretically justified.[3] Here we report that in contrast to the common knowledge, the SAA_{1-12} peptide consistently and exclusively forms amyloid fibers with right-handed helicity at all hierarchical levels from 8 nm to tens of nanometers width.

Amyloids are fibrillar aggregates of proteins with a characteristic cross-β conformation.[4] Over 20 diseases have been identified as amyloid-related.[5] The cross-β structure is minimally defined as a series of β-strands extended perpendicular to the fiber axis and joined by hydrogen bonds parallel to the fiber direction.[4] Several structures, all consistent with the cross-β definition, have been however proposed for specific amyloids, such as β-sheet, β-helix, or other β-folds.[6−8] There is no consensus on whether each or all of these models may be applicable in general or in specific cases.

The mature amyloid fiber consists of several protofilaments, with widths ranging from 8 to 50 nm depending on the number of protofilaments. The morphology of the fiber suprastructure may vary between ribbons and helices even within the same sample.[6,9]

We have examined three disease-related amyloids: $A\beta_{1-40}$, hen lysozyme, and SAA_{1-12}. $A\beta_{1-40}$ is related to the Alzheimer disease. The hen lysozyme protein is highly homologous to human lysozyme which is related to systemic amyloidosis. SAA_{1-12} is a 12 amino acid peptide (RSFFSFLGEAFD) from the N-terminal of the serum amyloid A protein, which is related to secondary systemic amyloidosis (AA). It is commonly accepted that the amyloidogenic core resides in the first 10−15 N-terminal amino acids of the protein.[10,11]

Amyloid fibers were induced to deposit from the three types of peptides[11,12] and characterized by transmission electron microscopy (TEM) and ThT assay; the β-conformation of SAA_{1-12} amyloids was verified by FTIR (see Supporting Information).

Conventionally, amyloid morphology is determined by TEM after negative staining. These images by definition do not contain information on the handedness of fiber helices because TEM micrographs provide projection images that cannot report on the 3-D morphology of objects (Figure 1a,b). TEM micrographs after Pt shadowing may supply 3-D information on handedness only if the grid orientation is known. In contrast, high-resolution scanning electron microscopy (SEM), as well as AFM and STM, is suitable for the determination of helix handedness because it directly provides three-dimensional images (Figure 1c−f). Furthermore, cryo-SEM allows observation at low temperature of biological materials in hydrated form, without the need for fixation or staining (Figure 1g−i). Here we used SEM and cryo-SEM for the determination of fiber handedness. We visualized ~100 fibers from at least three different fiber batches of each peptide.

We found that amyloids of $A\beta_{1-40}$ and of hen lysozyme always and only form left-handed helical fibers (Figure 1d and e, respectively). Surprisingly, amyloids formed by the SAA_{1-12} peptide always and only form right-handed helices (Figure 1f).

To eliminate the possibility that either dehydration or the fixation procedure cause the right-handed chirality of the SAA_{1-12} fibers, we performed cryo-SEM on freeze-dried samples of $A\beta_{1-40}$ and SAA_{1-12} amyloids. The observed chirality of both amyloid types did not change, while the resolution improved (Figure 1g and h, respectively). In order to confirm that the right-handedness of the (all-*S*) SAA_{1-12} amyloids does not result from an artifact induced by external parameters,[13] we synthesized the peptide enantiomer, (all-*R*) SAA_{1-12}. The helicity of (all-*R*) SAA_{1-12} amyloids was found to be exclusively left-handed, the mirror image of the (all-*S*) SAA_{1-12} amyloids (Figure 1i).

All amyloids display both in TEM and SEM a wide range of fiber widths, related to increasing hierarchical levels of aggregation (Figure 2). The TEM clearly shows that the fibers are helical even at the lowest hierarchy (8 nm). The SEM micrographs show that the handedness of the fibers is consistent through all the hierarchy levels of the different amyloid fibers (Figure 2a,d).

From all the above data, we conclude that the opposite handedness of the SAA_{1-12} fibers, relative to the $A\beta_{1-40}$ and to the hen lysozyme fibers, is an intrinsic feature of the peptide structure. This appears to indicate that the cross-β structure of SAA_{1-12} fibers is not formed of β-sheets.

The left-handed chirality observed in the $A\beta_{1-40}$ and hen lysozyme amyloid suprastructures is consistent with the conventional β-sheet structural model (Figure 2c). Indeed, (all-*S*) $A\beta_{1-40}$ amyloids were shown by AFM to consist of left-handed helices, while the enantiomer peptide amyloid fibers are right-handed.[14] This is further confirmed by a model based on ssNMR including the assembly of four β-sheets in a protofilament.[8] Similarly, in agreement with our observations, AFM studies on human wild-type lysozyme showed left-handed helical suprastructure.[15]

In contrast to $A\beta_{1-40}$ and hen lysozyme, the right-handedness observed in (all-*S*) SAA_{1-12} fibers and the left handedness of the enantiomeric (all-*R*) peptide are not consistent with the conventional β-sheet structural model. Although we cannot completely rule out supercoiling as a possibility, we do not deem that it is the probable cause for the right-handed helicity of the fibers. This is because the same handedness was observed at all hierarchical levels, starting with 8 nm fibers and up to 32 nm fibers.

In a recent study, two diastereomeric amyloid populations were shown to coexist.[16] However, the system reached equilibrium at higher temperature with only one fiber handedness, left-handed.

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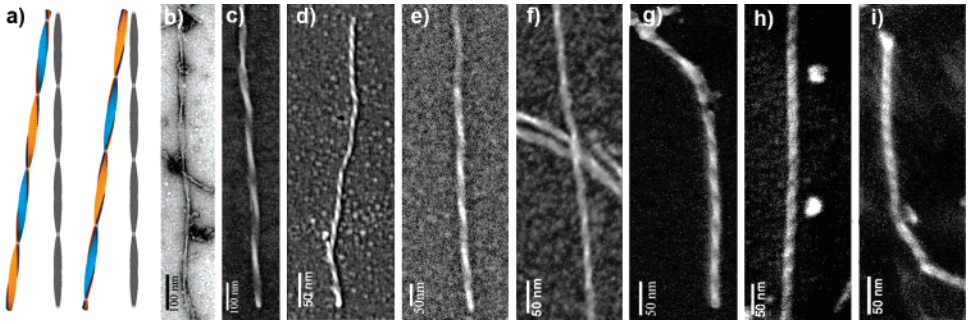

Figure 1. (a) Schematic representation of two helical fibers of opposite handedness (in color) and their projection images (gray). (b) Negative stain TEM and (c) SEM micrographs of hen lysozyme. (d−f) SEM micrographs of (d) Aβ_{1-40}, (e) hen lysozyme, and (f) (all-S) SAA$_{1-12}$. (g−i) Cryo-SEM micrographs of (g) Aβ_{1-40}, (h) (all-*S*) SAA$_{1-12}$, and (i) (all-*R*) SAA$_{1-12}$. The resolution in (b) and (c) is ∼10 nm, and in (d)−(i) is ∼5 nm. The fibers observed in cryo-SEM appear thicker because they are hydrated.

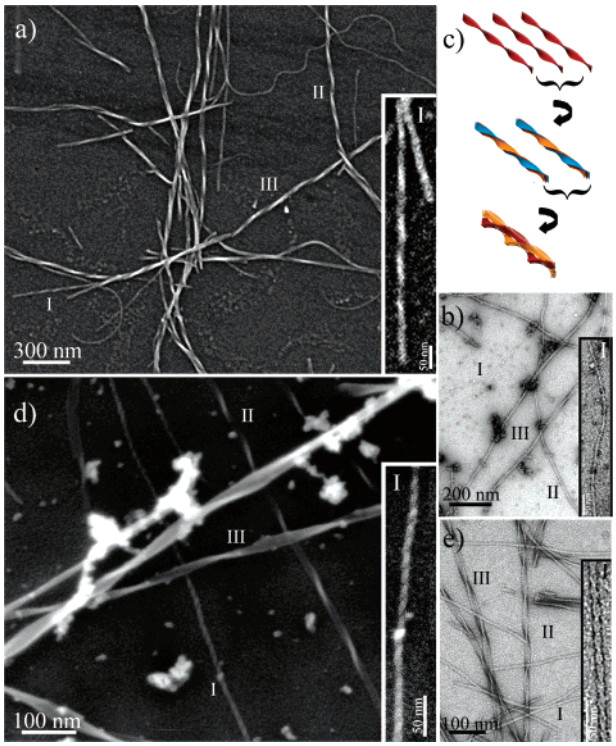

Figure 2. (a) SEM micrograph and (b) negative stain TEM micrograph of hen lysozyme amyloids. (c) Schematic representation of increasing hierarchical levels of β-sheet aggregation. (d) Cryo-SEM micrograph and (e) negative stain TEM micrograph of (all-*S*) SAA$_{1-12}$ amyloid fibers. In a, b, d, e, I, II, and III mark increasing hierarchical levels of fibers. Insets: magnification of fibers I, bar 50 nm.

In contrast, for SAA$_{1-12}$, the fiber helicity is consistently and exclusively right-handed, showing that this is the most stable conformation.

Whatever the answer to the dilemma of the right-handed helicity of SAA$_{1-12}$ amyloid fibers is, its existence shows that the supramolecular chirality of (all-*S*) aggregated amyloid fibers may be opposite, depending on their structural organization. This should not be surprising, when considering that β-helices of (*S*)-aminoacids were found to form both left- and right-handed helical suprastructures.[17] Examples of this same absence of unequivocal correlation between molecular and supramolecular structures are also well-documented for phospholipids, peptide amphiphiles, cellulose, and DNA.[18] Surprisingly, this is not documented and even less understood for amyloid fibers.

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Supporting Information Available: SAA peptide syntheses, amyloid preparation and characterization, EM sample preparation and a complete reference list is available This material is available free of charge via the Internet at http://pubs.acs.org.

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