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X-ray Scattering Study of the Effect of Hydration on the Cross-β Structure of Amyloid Fibrils

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Amyloid fibrils formed from different peptides and proteins are associated with more than 20 diseases.[1] Similar self-assembled structures form in vitro from a broad range of non-disease-related polypeptides. They are generally thought to adopt a common cross-β structure[2] in which the peptide chains assemble into β-sheets, with the constituent β-strands running perpendicular to the fibril axis. The evidence for this structure comes from wide-angle X-ray scattering (WAXS) studies of aligned fibril samples that show the presence of characteristic anisotropic patterns consisting of an axial reflection at 4.7 Å, corresponding to the inter-strand spacing, and an equatorial reflection at typically 8−12 Å, which is usually broader and corresponds to the spacing between two or more β-sheets stacked within the fibril. Samples with such alignment are typically prepared by processes that involve a significant degree of sample dehydration.[3] There is evidence, however, that the structure of β-sheet-containing fibrils can be significantly affected by the level of hydration in the sample.[4] We therefore set out to explore whether evidence for a cross-β pattern similar to that seen in dried samples can be obtained for fibrils in aqueous solution under conditions similar to those in which other biophysical techniques are commonly used to characterize these structures.

We examined the effects of dehydration and concentration on 2D WAXS patterns obtained from two types of amyloid fibrils, one formed by an 11-residue peptide corresponding to residues 105−115 of transthyretin ($TTR_{105-115}$), and the other by lysozyme, a 129-residue protein from hen egg white (HEWL). $TTR_{105-115}$ and HEWL both form well-characterized amyloid fibrils in vitro,[5] and either wild type or mutant forms of both transthyretin[5] and lysozyme[6] are implicated in human forms of amyloid diseases. Fibrils were formed from 20 mg/mL of $TTR_{105-115}$ or 75 mg/mL of HEWL solutions, and aliquots of both fibril suspensions were used for three types of experiment. In the first, the aliquot was dried to form an aligned stalk using conventional methods;[3] in the second, it was concentrated by centrifugation to form a hydrated pellet, and in the third, it was used directly without any further concentration or dilution. These are referred to, respectively, as "stalk", "pellet", and "solution" in Figures 1 and 2. Stalks from HEWL and $TTR_{105-115}$ fibril samples both gave rise to anisotropic 2D WAXS patterns displaying the typical features of a cross-β pattern for stacked sheets (Figure 1). The axially aligned inter-strand reflection is at 4.7 Å, and the equatorial inter-sheet reflection is at 8.8 and ∼10 Å for $TTR_{105-115}$ and HEWL fibrils, respectively. The WAXS patterns from both pellets and solutions of hydrated HEWL and $TTR_{105-115}$ fibrils also show distinct inter-strand and inter-sheet reflections (Figure 1). As expected, these reflections are fainter in the more dilute samples. A broad ring at ∼3.5 Å is also present for all hydrated samples, corresponding to scattering from water (see Supporting Information).

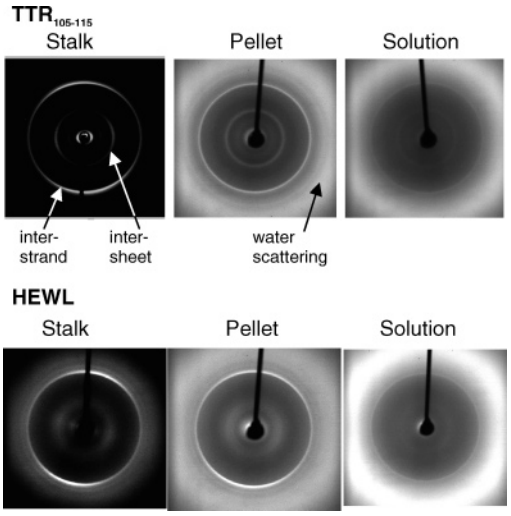

Figure 1. Two-dimensional WAXS patterns obtained from $TTR_{105-115}$ and HEWL fibrils at the three different levels of hydration (see text). The dried stalks are aligned approximately vertically.

To provide a more quantitative comparison between these data, the 2D patterns in Figure 1 were radially integrated over all azimuthal angles with the water background subsequently subtracted (see Supporting Information). The close agreement between the resulting 1D scattering patterns for the dried and hydrated samples of $TTR_{105-115}$ fibrils shown in Figure 2A provides strong evidence that the core structure of these fibrils is independent of the hydration level of the sample. Furthermore, the similarity between the spectra of the pellet and the solution, which differ in fibril concentration by a factor of 20, suggests that this structure also persists in the most dilute solutions used for other biophysical and spectroscopic techniques, such as circular dichroism and fluorescence.

The inter-sheet and inter-strand reflections in the 1D scattering patterns of HEWL fibrils are also essentially unaffected by the level of hydration (Figure 2B). An additional linear background correction was applied to allow a more detailed comparison of the inter-sheet reflections of the different samples (see Supporting Information). The position, width, and normalized intensity of the corrected inter-sheet reflection are similar for all levels of sample hydration, as shown in Figure 2B (inset). This again indicates that dehydration or concentration have no detectable effect on the core fibril structure.

Inter-strand and inter-sheet WAXS reflections representing repeating structures within a fibril only show the anisotropy that is a critical feature of the amyloid cross-β structure when the

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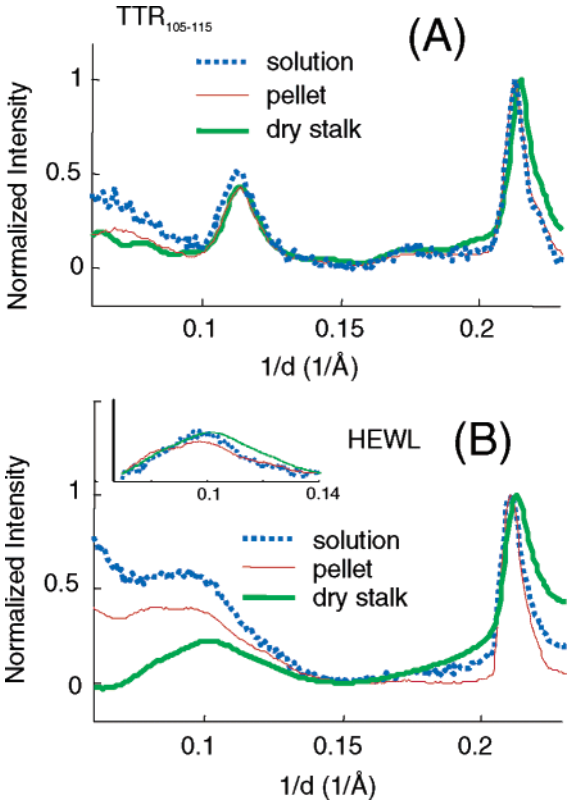

Figure 2. Background-subtracted 1D scattering patterns, normalized relative to the maximum of the 4.7 Å peak, from $TTR_{105-115}$ fibrils (A) and HEWL fibrils (B) in the differently hydrated samples. Inset: inter-sheet reflection from HEWL scattering patterns, with further linear background subtracted.

constituent fibrils in a sample are aligned in a particular direction, as occurs in the formation of dried stalks; in samples that have no overall fibril alignment, such as the solutions in Figure 1, the reflections are isotropic. To explore whether a cross-β structure is characteristic of fibrils in hydrated samples, scattering patterns were acquired from concentrated solutions or pellets in which the fibrils were aligned by flow, either in a purpose built flow cell or in glass capillaries (see Supporting Information). The 2D WAXS patterns of flow-aligned hydrated samples show marked anisotropy, as shown in Figure 3. For both $TTR_{105-115}$ and HEWL fibrils, the inter-strand and inter-sheet reflections show clear axial and equatorial alignment, respectively, just as for the stalks shown in Figure 1. This finding confirms that the orientations of the reflections are also unaffected by hydration and, therefore, that the cross-β structure is indeed present in individual $TTR_{105-115}$ and HEWL amyloid fibrils in hydrated samples.

In summary, we have shown here that it is possible to acquire and analyze X-ray scattering patterns from aligned amyloid fibrils in solution under conditions that are similar to those used for characterization by other biophysical and spectroscopic techniques. Importantly, these data have demonstrated that the cross-β conformation of amyloid fibrils is indeed present in the solution state

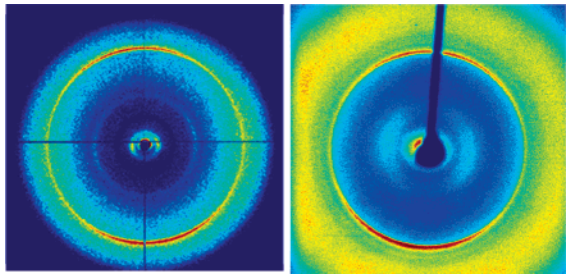

Figure 3. Two-dimensional WAXS patterns from partially aligned hydrated fibril samples. Left: background-subtracted WAXS pattern obtained from a flow-aligned solution of $TTR_{105-115}$ fibrils (flow cell). Right: flow-aligned WAXS pattern from HEWL fibrils (capillary). The flow direction, and therefore the fibril axis, is approximately vertical in each case.

and is not simply a product of the dehydrating conditions typically used to record the X-ray scattering patterns of such species. In the case of fibrils formed by the yeast prion, Sup35, the equatorial WAXS reflection is present in dried but not in hydrated samples;[4] in this case, the structure could contain internal water channels that collapse on drying. In the case of the amyloid fibrils examined here, by contrast, the cross-β structure is present in individual fibrils and is unaffected by the level of sample hydration, suggesting that individual hydrated fibrils contain a stack of two or more sheets whose interfaces are inaccessible to bulk water. Such a conclusion is consistent with models of amyloid fibrils based on data from other structural techniques, such as solid-state NMR spectroscopy,[7] and with the dry interfaces recently observed for peptide sheets in 3D crystals.[8]

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Supporting Information Available: Methods for sample preparation and alignment; details of X-ray data acquisition; scattering patterns without background subtraction; and details of background subtraction and normalization procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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