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ORIGINAL PAPER

# Dehydration stability of amyloid fibrils studied by AFM

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**Abstract** Atomic force microscopy was used to investigate the stability of dehydrated amyloid fibrils formed by human islet polypeptide (IAPP) and A$\beta$(1–42) peptides. IAPP amyloid fibrils were imaged in liquid (hydrated state) and in air (dehydrated). In addition, fibrils dried on the mica surface were rehydrated and re-examined both in liquid and in air (after consecutive redrying). As reported previously, the initial drying process does not result in any major change in the amyloid appearance and the dimensions of the fibrils are preserved. However, when once-dried samples are rehydrated, fibril stability is lost. The fibrils disintegrate into small particles that are attached to the mica surface. This process is further confirmed by studies of the rehydrated samples after drying, on which the morphology of the fibrils is clearly changed. Similar behavior is observed for A$\beta$(1–42) amyloid fibrils, which are apparently stable on first drying, but disintegrate on rehydration. The observed change indicates that dehydration is causing a change in the internal structure of the amyloid fibrils. This has important implications for studies of amyloid fibrils by other techniques. Due to the potential influence of hydration and sample history on amyloid structure, preparation and study of amyloid samples with controlled humidity requires more consideration.

**Keywords** AFM · Peptide · Protein · Aggregation · Amyloid

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## Introduction

Amyloidoses are a group of degenerative diseases in which normally soluble proteins undergo a conformational change accompanied by aggregation and are deposited as amyloid fibrils in the tissues (Rochet and Lansbury 2000). Amyloids are long (~μm) fibrous entities, with diameter in the range of 7–30 nm (Serpell 2000). Amyloid fibrils display a distinctive X-ray diffraction fingerprint that emanates from the cross-$\beta$ structure (Sunde et al. 1997; Geddes et al. 1968; Eanes and Glenner 1968). The exact mechanism by which amyloidogenic proteins affect cells is still under debate. It has recently been proposed that small globular assemblies of amyloid proteins induce cell pathophysiology by forming ion-channel-like structures in the membranes (Quist et al. 2005).

Due to the lack of long-range three-dimensional (3D) order in amyloid fibrils and the fact that it is not possible to make crystals from full-length amyloidogenic peptides, protein crystallography techniques are in general not suitable for studies of amyloid-forming peptides. However, some short amyloid-related peptides have been crystallized and studied using single-crystal diffraction techniques—see below. Instead, structural studies of amyloid have focused on the use of X-ray fiber diffraction (see Makin et al. 2006 for a current review), solid-state nuclear magnetic resonance

(ss-NMR) (Tycko 2004), scanning transmission electron microscopy (Antzutkin 2004; Goldsbury et al. 2005), and site-directed spin labeling (Jayasinghe and Langen 2004). For many amyloids, X-ray fiber diffraction data show unambiguously that the peptide chain is mainly in the $\beta$-conformation and that molecules assemble with a cross-$\beta$ texture. However, to date, no detailed model of the chain organization for a full-length amyloid-forming peptide has been proposed based on the X-ray fiber diffraction data. Using protein crystallography methods, only structures of shorter fragments have been described in detail (Sawaya et al. 2007; Eakin et al. 2006; Diaz-Avalos et al. 2003). In addition, structures of a few amyloid precursor proteins (APPs) have been solved using X-ray crystallography (Elam et al. 2003; Eakin et al. 2006). Crystallization conditions favor crystal formation rather than fibril formation and there is no evidence to suggest that the crystal contacts observed in the crystal packing of amyloid precursor proteins are relevant to the arrangement of proteins within the amyloid fibrils. Indeed, it is likely that most proteins have to undergo some degree of unfolding to enable assembly into amyloid fibrils (Gosal et al. 2005).

In most of the published fiber X-ray diffraction, electron microscopy, and ss-NMR studies of amyloid fibrils, it is assumed that sample dehydration does not result in serious destabilization of the molecular organization and that the structure is preserved. This is confirmed by AFM studies of hydrated (in liquid) and dehydrated (in air) amyloid fibrils (Arimon et al. 2005; Mesquida et al. 2007).

Using atomic force microscopy (AFM), we have investigated the change in the structure of amyloid fibrils induced by repeated dehydration and rehydration. We focus on two, biologically relevant full-length amyloid-forming peptides: IAPP and Alzheimer's A$\beta$(1–42). Human islet amyloid polypeptide (IAPP, also known as amylin) is co-secreted with insulin from the secretory granules in the $\beta$-cells of the pancreatic islets (Clark et al. 1989). IAPP is a 37-residue peptide that is produced by cleavage from an IAPP precursor protein (Goldsbury et al. 2000). The native 3D structure of full-length IAPP is unknown, although in aqueous buffer it forms a stable random-coil conformation indicating that it may be a natively unfolded protein (Higham et al. 2000). In type 2 diabetes, IAPP is deposited as amyloid fibrils in the extracellular spaces of the pancreatic islets. IAPP amyloid fibrils are similar in appearance to fibrils found in other amyloid diseases (Makin and Serpell 2004; Goldsbury et al. 2000), such as those formed by the A$\beta$(1–42) peptide. A$\beta$(1–42) is one of the two closely related peptides found in amyloid deposits of Alzheimer's disease patients. It is cleaved from a larger, transmembrane protein known as amyloid precursor protein (APP) (Selkoe 1991). We show that dehydration permanently destabilizes amyloid fibrils formed by these two peptides and results in formation of small and soluble protein aggregates.

## Materials and methods

IAPP (1–37) was obtained from Peninsula/Bachem: $NH_3^+$-KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-$NH_2$. Peptides were dissolved in water at a concentration of 1 mg/ml and stored for a few days, during which time the amyloid fibril formation process took place. A$\beta$(1–42) was obtained from Bachem (Bachem Holding AG, Bubendorf, Switzerland). Peptides were dissolved in MQ-$H_2O$ at concentration of 0.25 mg/ml and stored for a few days, during which time the fibril formation process took place. Both peptides form fibrils that are stable in MQ-$H_2O$ over long periods of time and are also stable on dilution with MQ-$H_2O$ water. For AFM sample preparation, the stock solution was diluted to 10–20 μg/ml with MQ-$H_2O$, and 10 μl of solution was placed onto a freshly cleaved mica surface. After ≈2 min incubation, the sample was rinsed several times with MQ-$H_2O$. Tapping-mode AFM images were obtained with a Digital Instruments Multimode IIIa AFM (Veeco Instruments Inc./Digital Instruments, Santa Barbara, CA, USA) equipped with an E-scanner either in liquid or in air. For imaging in liquid, care was taken not to dry the sample before use and images were collected using silicon nitride cantilevers (Veeco, USA) with nominal spring constant of 0.12 N/m and resonance frequency of 14–26 kHz. Imaging was carried out in MQ-$H_2O$. For imaging in air, excess MQ-$H_2O$ from the washing step was removed with filter paper and the samples were left to dry at room temperature in ambient conditions for a time period >4 h. Vacuum drying ($p = 10^{-4}$ Pa) was also tested, for which the same effect as for ambient condition drying was observed. Rehydrated samples were made by placing a drop of water onto a dried sample surface and they were investigated by AFM in liquid or by AFM in air after a successive dehydration step as described above. For imaging in air, tapping-mode silicon nitride probes (Nanosensors, Germany) with nominal spring constant of 34–59 N/m and resonance frequencies of 306–366 kHz were used. The AFM was operated at a frequency of 90% of the free resonance frequency of the cantilever. Both height and phase images were collected (512 × 512 pixels) at scan speeds of 1–1.5 Hz. The images were flattened and analysed using Gwyddion AFM software (http://gwyddion.net/).

## Results and discussion

An overview of the sample preparation and history is shown in Fig. 1. In the first part of the study, we examined IAPP amyloid fibrils formed from dissolved peptide and that were hydrated at all times. For these samples AFM topographs were collected in liquid. The samples were then dried and the morphology of the amyloid fibrils was

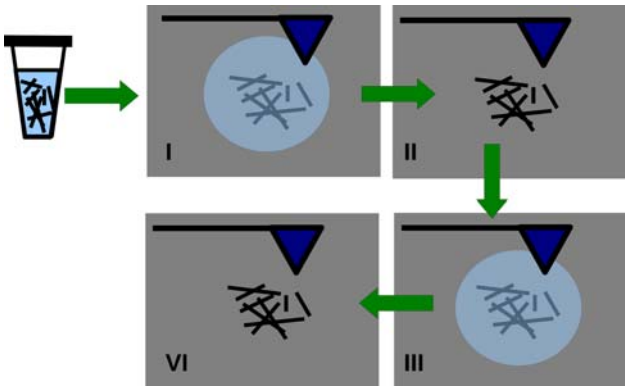


**Fig. 1** Sample preparation strategy. Four type of samples prepared in series were examined in this study: hydrated (I), dried (II), rehydrated (III), and then dried again (IV)

examined in air (Fig. 1, situation II). To investigate amyloid stability, samples were then rehydrated and again examined by liquid AFM (III). Finally, to compare with the first drying step, samples were dried again and examined in air (IV).

Figure 2 shows IAPP amyloid fibrils deposited on mica and imaged in liquid. The appearance and measured heights of the fibrils ($6.0 \pm 1.0$ nm) are similar to those reported previously for the IAPP amyloid fibrils studied with AFM (Goldsbury et al. 1999) and cryo-electron microscopy (Makin and Serpell 2002). Due to the tip-convolution problem, the sharpness of the tip is known to influence the apparent width of structures in AFM images. A locally high concentration of fibrils, as seen in certain areas of Fig. 2d, will make the fibrils difficult to resolve, resulting in the appearance of thicker areas, most likely consisting of more than one fibril. Previously, polymorphism of IAPP fibrils has been shown (Goldsbury et al. 1999), with two fibril populations with heights of 2.4 and 6.8 nm. Our fibrils were prepared at much higher peptide concentration (1 mg/ml, compared with 50 μg/ml) and no indication of polymorphism was detected. In experiments in liquid (Fig. 2) no helical twist was resolved and therefore the measured height represents a surface average.

Mica surface is charged and hydrophilic and in some cases interactions with the mica surface can cause changes in biological specimens. Amyloid fibrils examined here are stable on the mica surface under liquid environment and under tapping-mode AFM imaging conditions as shown by repeated image collection from the same sample area (data not shown).

To examine changes in fibril structure upon drying, IAPP fibrils deposited on mica were dried at room temperature in air (time >4 h). Under these conditions, samples are not totally dehydrated. Due to the hydrophilic nature of the mica surface, there is always a water layer a few nanometers thick associated with the surface. The drying process, however, removes some water associated with the amyloid fibrils, as shown by data obtained after successive rehydration.

Typical AFM topographs from dried samples collected in air are shown in Fig. 3a. Generally straight fibrils with well-defined edges and some evidence of a regular helical twist can be seen (Fig. 3a, b). Amyloid fibrils appear to

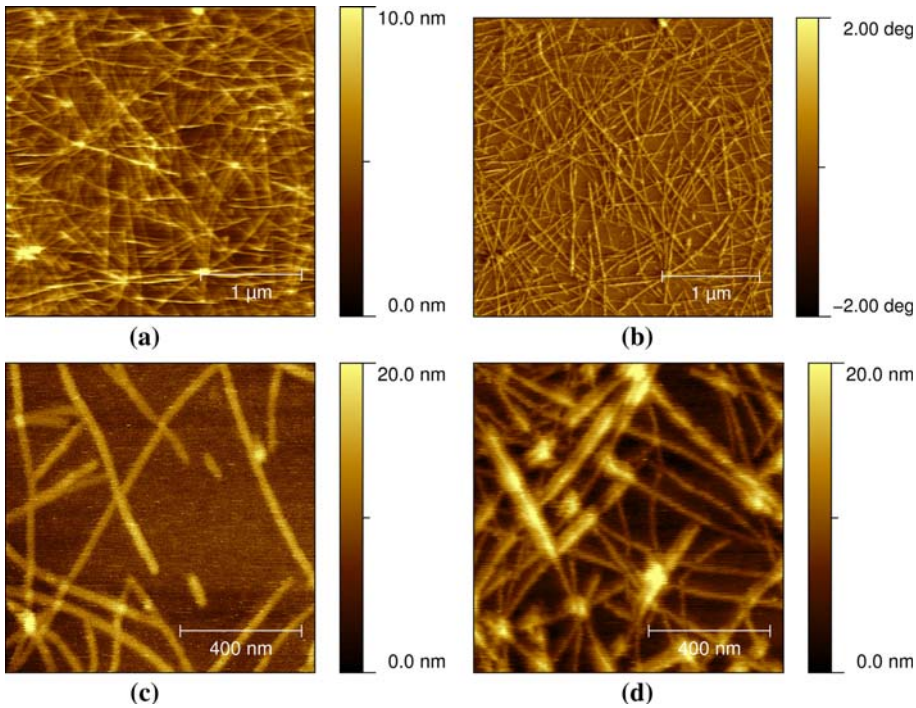


**Fig. 2** Tapping-mode AFM topographs recorded for always-hydrated IAPP amyloid fibrils. **a** Height topograph of a low-magnification, high-coverage sample; **b** phase image corresponding to (**a**); **c**, **d** high-magnification height topographs showing different shape of fibrils

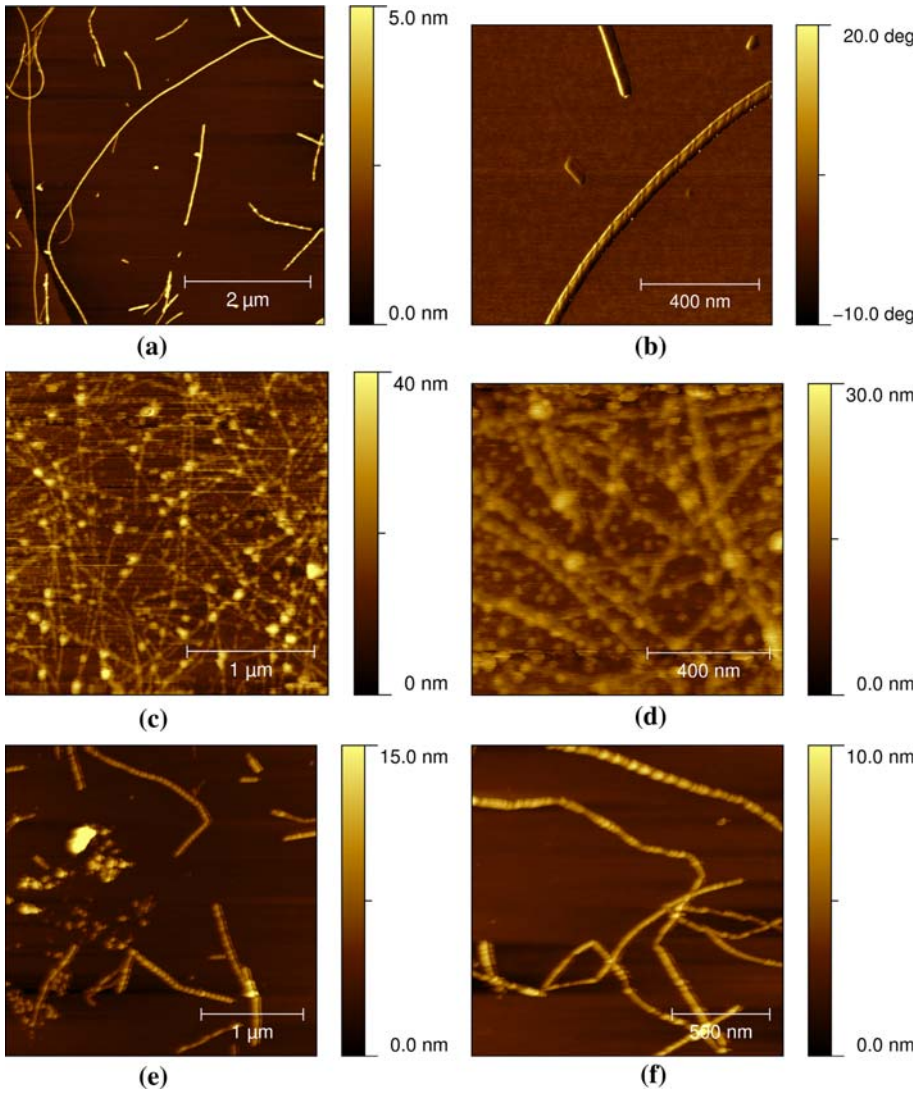


**Fig. 3** Tapping-mode AFM topographs recorded in air after first drying of always-hydrated fibrils shown in Fig. 2; **a** low-magnification height and **b** high-magnification phase image. **c**, **d** Liquid AFM after rehydration of a once-dried sample showing disordered fibrils and spherical protein aggregates; **e**, **f** after consecutive redrying, showing change in appearance of the fibrils compared with topographs shown in (**a**)

remain intact after the first drying step, and there is no evidence that forces due to moving water menisci or other effects cause damage to fibrils attached to the mica surface. Recorded average height ($4.5 \pm 1.0$ nm) is comparable with values recorded in liquid. The difference between the average height of the fibrils measured in liquid and in air can have two origins. First, it is possible that dehydration results in shrunk or flattened fibrils. However, the imaging condition between air and liquid environments are quite different, and different interaction between the sample and AFM tip can result in different apparent heights. More work is needed to establish if drying also results in significant change in fibril dimensions.

To investigate IAPP amyloid stability upon gentle dehydration described above, we attempted to image rehydrated samples in liquid. This proved challenging. This was mainly due to small spherical particles that dissociated from the sample surface on rehydration. These particles attached to the AFM tip, making normal operation of the microscope impossible after only a few minutes of data collection in liquid. Low- and high-magnification images of the rehydrated samples are shown in Fig. 3c and d. Large amounts of spherical protein aggregates of different sizes were observed.

To confirm that the observed particles come from disintegration of IAPP amyloid fibrils, samples were again dried (first the excess liquid was drained with filter paper) and fibrils were imaged in air. In this case, soluble protein particles did not affect image collection. Typical images collected in air are shown in Fig. 3e and f. Dramatic change in fibril appearance can be observed. Fibers no longer have straight and well-defined edges, but instead appear to be composed of small ill-defined particles. In addition these particles are observed to be attached to the mica by themselves and in clusters.

Collected data show that drying resulted in denaturation of IAPP amyloid "native" structure. This change, however, was not detected in studies of dried samples and only

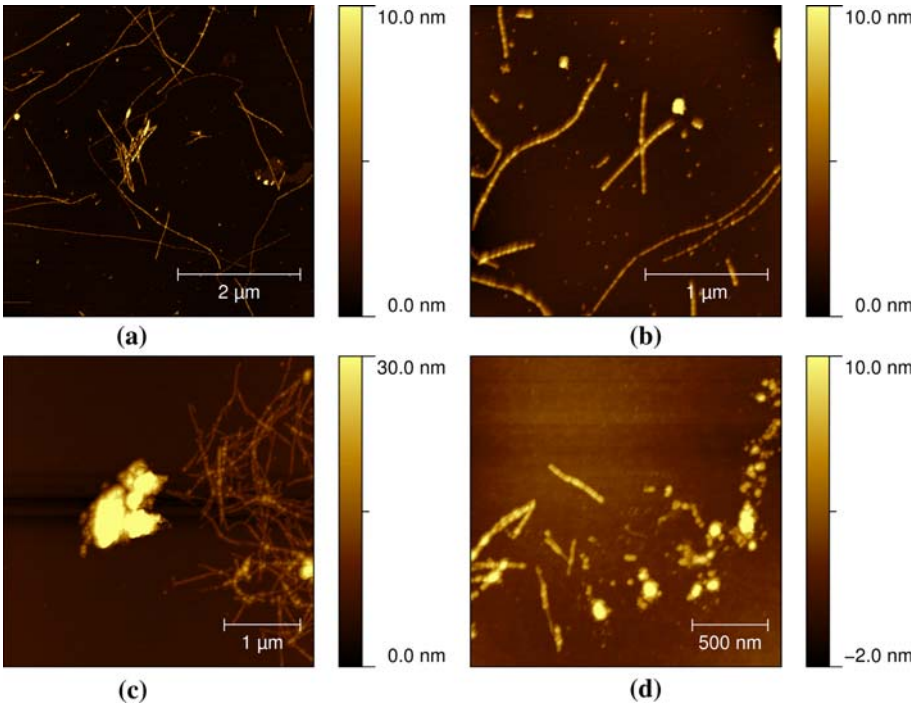


**Fig. 4** Tapping-mode AFM topographs of A$\beta$(1–42) amyloid fibrils imaged in air after drying of always-hydrated sample (**a**, **b**) and tapping-mode AFM topographs of A$\beta$(1–42) amyloid fibrils imaged in air after rehydration and redrying (situation IV, Fig. 1) (**c**, **d**)

became apparent after consecutive rehydration of fibrils adsorbed onto a mica surface.

To investigate if this is a general phenomena or a feature specific to IAPP amyloid fibrils, we investigated stability of fibrils formed by A$\beta$(1–42) peptide. Figure 4a, b show topographs of A$\beta$(1–42) fibrils collected in air after the first dehydration step (situation II, Fig. 1). Fibers that appeared similar to those reported previously were observed (Arimon et al. 2005; Nichols et al. 2005; Moreno-Herrero et al. 2004; Crowther and Wischik 1985; Antzutkin 2004). For some fibrils a helical twist could be clearly resolved. To investigate the stability of the A$\beta$(1–42) fibrils, the sample was first dried in air and then rehydrated. Changes in the structure of the fibrils were investigated using AFM in air after consecutive drying, in a similar way to the procedures used for IAPP samples (situation IV, Fig. 1). In a similar way to IAPP peptide, the appearance of the fibrils has been affected by rehydration. They have lost the characteristic morphology and appear to be composed of many small particles. Unstructured aggregates of various sizes can also be observed (Fig. 4c, d).

Our observations indicate that the amyloid fibrils studied here are permanently and irreversibly destabilized on gentle dehydration. This is likely due to the fact that water molecules play an important role in stabilizing the "native" amyloid structure, and drying causes disruption of the structure. This disruption could be a result of loss of some interactions stabilizing the "native" structure, change in the relative strength of different interactions, large volume change or other effects. Our observations have been made using in vitro prepared amyloid fibrils and it is possible that ex vivo amyloid could behave differently. However, since in vitro prepared amyloid fibrils are used in the majority of structural studies, our findings are still significant. This observation has important consequences for future structural studies of amyloids. Hydration of the sample from which structural data is collected should be controlled to prevent structural changes due to loss of water. Our results show that this drying process might result in permanent change in the structure, resulting in loss of long-range order and consequently loss of detail on X-ray diffraction patterns. Therefore more effort should be put into collecting diffraction data from oriented hydrated samples, perhaps using the method described by Kendall and Stubbs (2006) or a sample cell similar to that described by McDonald et al. (2008). Two studies in which amyloid fibrils have been investigated under hydrated conditions have provided some contradictory data. Kishimoto et al. (2004) suggested significant differences between fiber diffraction patterns recorded for dried and hydrated samples of yeast prion Sup35 amyloid fibrils and fibrils made from shorter fragments of the same protein. Squires et al. (2006), on the other hand, observed no significant difference between diffraction patterns recorded from hydrated and dried samples of amyloid fibrils made from $TTR_{105–115}$ and 129-residue hen-egg white lysozyme. In light of the results presented herein, this type of experiment requires more attention.

## Conclusions

We show that IAPP and A$\beta$(1–42) amyloid fibrils are destabilized on dehydration and that a change in structure

is evident for rehydrated samples. The structure formed by dehydrated IAPP amyloid fibrils has recently been investigated in some detail using X-ray fiber diffraction and cryo-electron microscopy (Makin and Serpell 2004). Collecting diffraction data from semihydrated samples should provide more insight into the structure of IAPP amyloids and the nature of the transition on drying. To address the stability question for large assemblies of amyloid fibrils, fiber diffraction experiments could be performed when the samples are repeatedly de- and rehydrated and the amount of moisture within the sample is controlled.

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