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Q-Sense | [Application Note] 14

Protein fibrillation studied with QCM-D

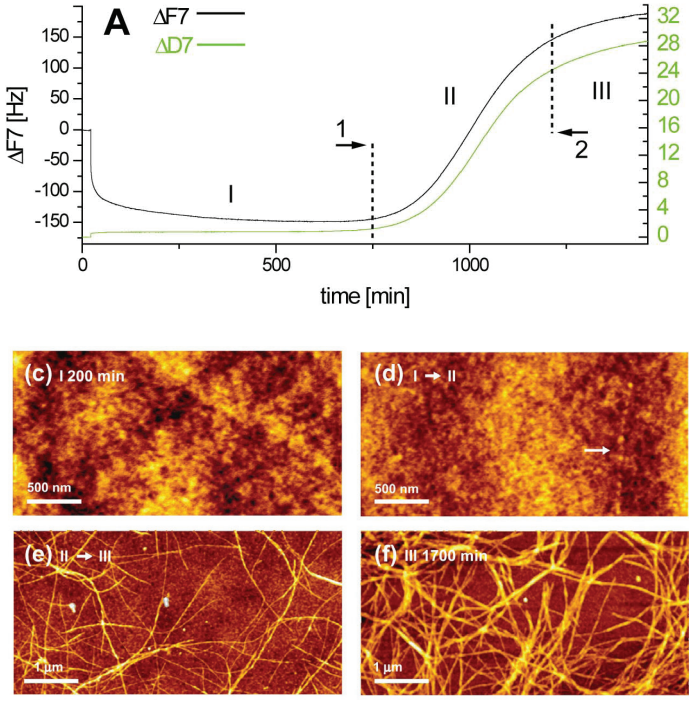
The ability of certain polypeptides to aggregate into long, thin fibrils called amyloid structures is associated with multiple protein folding disorders and is also a major problem in biotechnological and pharmaceutical applications. Here, QCM-D has been used to monitor the changes in thickness and viscoelastic properties of multi-layer amyloid deposition in situ for the first time. This provides novel insights into the kinetics of protein fibrillation which other techniques cannot provide.

Introduction

Quartz Crystal Microbalance with Dissipation (QCM-D) is a surface sensitive technique, which provides real-time information on mass and structure of thin films. The mass of the adsorbed layer is sensed as a change in the resonance frequency of the sensor movement (Δf) and the viscoelastic properties are deduced from the damping of the sensor movement, called dissipation (ΔD).

Approach

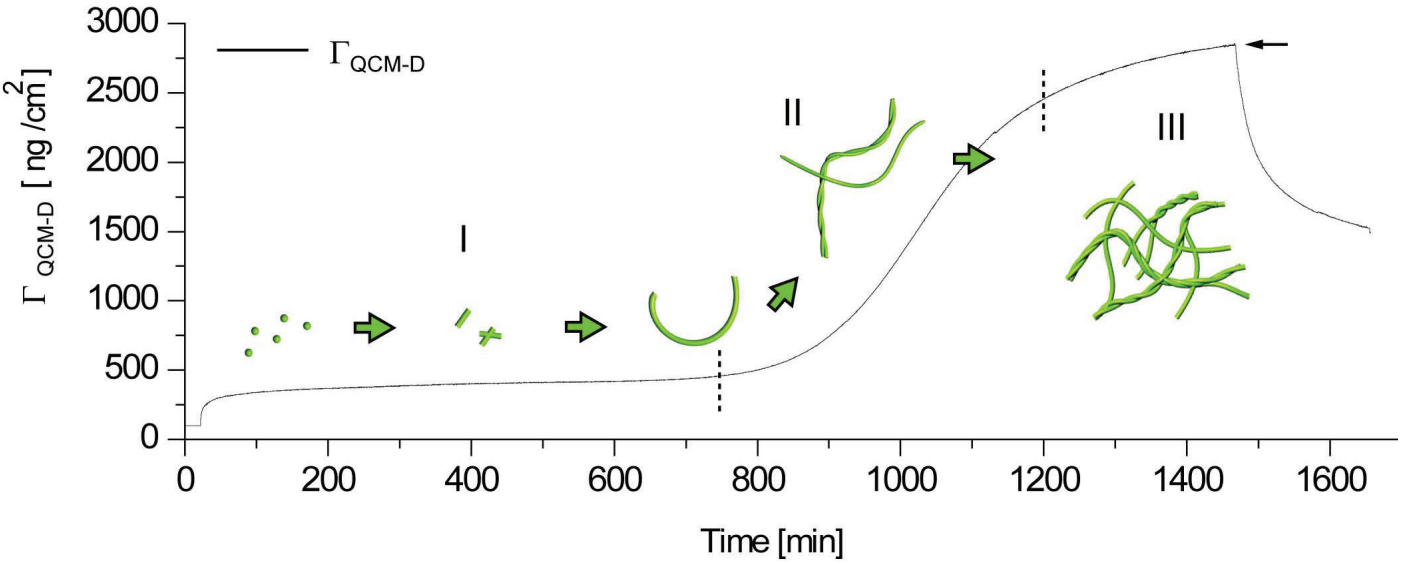
To study the interfacial fibrillation of peptides, a solution of glucagon monomers was injected into the QCM-D chamber onto tantalum sensors simultaneously with incubation on ex situ sensors for AFM studies.



[Figure 1]: QCM-D (A) and AFM (B-E) data from a typical experiment on glucagon fibrillation. Arrow 1 and 2 correlate with arrows in AFM images.

Results and discussion

The QCM-D glucagon adsorption response could be divided into three characteristic phases, I, II and III, as shown in Figure 1A. In phase I, glucagon was injected into the chamber and monomers adsorbed to the surface. This was observed as a decrease in frequency, meaning an increase in mass at the surface. From the simultaneous low ΔD increase, it was concluded that the formed layer was rigid, since a high ΔD as the opposite, signifies a very viscoelastic structure. The adsorption was further confirmed by the corresponding AFM image (Figure 1B). After a lag time, the adsorbed layer transformed radically and became more viscoelastic as reflected by the significant increase in ΔD. This behavior was ascribed to glucagon fibrillation at the surface. This conclusion was also verified by AFM (arrow in Figure 1C). The process of fibrillation continued throughout phase II (Figure 1A) and micrometer long fibrils were observed (Figure 1D, E). What can be noted is that the frequency also increased from phase II and even reached a value above the base line which would give a negative surface mass density with a simple Sauerbrey model, clearly at odds with the AFM images. The data was however analyzed using an extended viscoelastic model in QTools*. This evaluation is only possible due to the simultaneous measurement of frequency and dissipation, unique to Q-Sense instruments. The analysis gave a detailed quantitative description of the changes in the surface mass density and viscoelastic properties of the forming layer. Figure 2 shows the modeled surface mass density. Phase I showed a low surface mass density of a rigid multilayer formation. After a characteristic lag time, the onset of fibrillation and maturing of fibrils passed a transition point, when the layer became dramatically more viscoelastic, and the fibrillation entered phase II. This phase was characterized by a decrease in shear modulus and shear viscosity (not shown) and a large increase in surface mass density. This mass increase could not be seen from the frequency signal alone, but became evident when modeling using both frequency and dissipation and consideration was taken to the frequency dependence of the layer. As illustrated in the figure, the properties were attributed to the fibrils gradually forming a



[Figure 2]: Voigt modeled surface mass density throughout the characteristic regimes of glucagon fibrillation, along with an illustration of dynamic interfacial fibrillation. (Arrow shows end-of-experiment flushing).

dense network of fibrils growing from multiple points. In their continuous growth, these fibrils formed a still more viscoelastic network which increased in both height and density of the layer. Finally, the process progressed into a stable phase III as a result of the finite amount of monomers available, terminating the fibril formation. The process could be continued with a secondary injection of fresh monomers, but without a second lag time.

*) QTools is a viscoelastic modeling and curve fitting software provided by Q-Sense.

Conclusions

This work demonstrates for the first time the ability to monitor the temporal development and viscoelastic changes of multilayer amyloid deposition in situ. Thanks to simultaneous modeling of frequency and dissipation, the structure of the growing layer could be quantified. The QCM-D could also be used to measure the growth kinetics of single protein fibrils and to study amyloid growth inhibitors, which has been done in a different study (Welland et al, 2007). All together these studies show that the QCM-D is a helpful tool in the understanding and prevention of protein folding disorders. The approach can also be used to get better insight into protein aggregation associated with manufacturing of protein phar-maceutics.

References:

1. Quartz crystal microbalance studies of multilayer glucagon fibrillation at the solid-liquid interface. Biophysical Journal, 93, 2162-2169, 2007. M.B. Hovgaard, M. Dong, D.E. Otzen and F. Besenbacher.

2. Kinetics and thermodynamics of amyloid formation from direct measurements of fluctuations in fibril mass. PNAS, 104, 10016-10021, 2007. T.P.J. Knowles, W. Shu, G.L. Devlin, S. Meehan, S. Auer, C.M. Dobson, M.E. Welland.

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