Solution Piezoresponse Force Microscopy of Lysozyme and Insulin Amyloid Fibrils

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Statement of Purpose: Coupling between electrical and mechanical phenomena is a universal feature of virtually all biological systems. Despite the tremendous interest in the role and possible biological significance of piezoelectricity, these issues remain largely unresolved. Insufficient information about electromechanical phenomena in biological systems is a result of the lack of characterization techniques capable of providing such information on the nanometer scale. Piezoresponse Force Microscopy (PFM) has demonstrated potential for imaging structure of connective and calcified tissues with sub-10 nm resolution [1]. Recently, we demonstrated electromechanical imaging in liquids [2]. It has also been shown that imaging is possible both in distilled water and in electrolyte solutions up to 10^{-4} M. The ability to map electromechanical properties in aqueous media opens the way to characterization of biological systems in native-like conditions.

Here we have used solution PFM imaging to characterize one-dimensional aggregates of protein molecules. Amyloid fibrils, insoluble protein aggregates characterized by a stacked β-sheet structure with strands arranged perpendicular to the fibril axis, have various pathogenic and non-pathogenic roles and recently have received attention for possible nanobiotechnology applications [3]. We demonstrated the first evidence of piezoelectricity of an individual amyloid fibril using solution PFM.

Methods: PFM utilizes the inverse piezoeffect to image local polarization orientation. In PFM, a local oscillatory electric field is generated by applying an *ac* voltage to a conducting tip in contact with a sample, and the deformation due to the piezoelectric effect is detected. The imaging paradigm in PFM is complementary to conventional atomic force microscopy (AFM) and scanning tunneling microscopy (STM): while AFM is sensitive to tip-surface forces through the mechanical motion of the cantilever (mechanical detection) and STM is sensitive to tip-bias induced current (current detection), PFM detects bias-induced surface displacement (electromechanical detection).

A Veeco (Santa Barbara, CA) Dimension Scanning Probe Microscope (SPM) set up for PFM operation was used with NanoScope version 7.00b14 software (Veeco). A custom-modified fluid tip holder (Veeco) allowed the tip to be directly biased in liquid. Measurements were performed using Au-coated Si tips (MikroMasch, Wilsonville, OR). Lysozyme and insulin were purchased from Sigma. Lysozyme and insulin amyloid fibrils were prepared by overnight heating in an acidic solution (pH=2.0) followed by multiple washings with HPLC-grade water [4, 5]. Suspensions of lysozyme or insulin fibrils were placed onto freshly cleaved mica substrates for imaging.

Results/Discussion: Both lysozyme and insulin fibrils showed strong electromechanical coupling. Two PFM scans with applied *ac* biases of 10 V (Fig. 1a,c) and 2 V (Fig. 1b,d) of the same lysozyme fibril bundle in distilled water show similar topography (Fig. 1a,b) whereas the piezoresponse amplitude (Fig. 1c,d) disappears with decreased driving voltage, consistent with a piezoelectric effect. It should also be noted that PFM image provides better resolution and reveals more detail of the fibril structure (Fig. 1c).

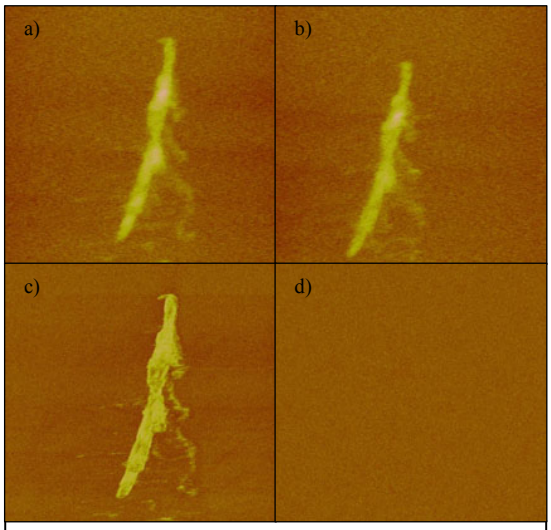

Figure 1. PFM images of a lysozyme fibril bundle. Scan size is 2.03 μm^2. The top are topographic images, and the bottom are piezoresponse amplitude images. Note that the amplitude feature disappears with decrease in applied ac bias from 10 V (c) to 2 V (d) while the topographic images reveal that the fibril bundle remains present.

In the future, we plan to use solution PFM for characterization of electromechanical response of live cells. It can be expected that electromechanical mapping of individual cells will help to understand complex electromechanical responses related to their physiological activity and will possibly serve as the basis for novel diagnostic methods and therapeutic interventions.

Conclusions: This first evidence of piezoelectricity of an amyloid fibril bundle in aqueous solution introduces the ability to map electromechanical properties of biological systems in native-like conditions at the nanoscale.

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