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Substrate-Facilitated Assembly of Elastin-Like Peptides: Studies by Variable-Temperature in Situ Atomic Force Microscopy

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Understanding how proteins assemble to form functional complexes is fundamental to efforts in chemistry, structural biology, biochemistry, and biophysics. Recent initiatives range from designing model proteins to study the role of specific secondary structure motifs on protein−protein interactions and their relationship to diseases such as Alzheimer's and Parkinson's, to the rational design of protein-based nanostructures.[1,2] Of particular interest are proteins that can undergo reversible disorder−order phase transitions, such as coacervation.[3−10]

Derived from exons 20, 21, 23, and 24 of human elastin, the elastin peptide (EP) series of proteins are known to coacervate, forming fibrillar aggregates with a high degree of β-structure (Table 1).[3,9,11,12] It is currently believed that overall protein hydropathy and the arrangement of hydrophobic domains underpin the self-assembly of these proteins and thus the reversible coacervation phase transition. To investigate the initial stages of coacervation, three EP peptides, EP I, EP II, and EP IV, prepared as reported previously,[3] were studied by in situ variable-temperature atomic force microscopy (Table 2).[13,14]

To investigate the role of hydrophobic stabilization on coacervation, two model substrates were chosen. Freshly cleaved mica presents an atomically flat, nominally charged surface while the similarly structured (0001) face of highly oriented pyrolytic graphite (HOPG) is highly hydrophobic.

In situ tapping mode AFM (TMAFM) performed in protein-free coacervation buffer (pH 7.5, 50 mM Tris, 1.5 M NaCl, 1 mM $CaCl_2$) on samples prepared by deposition of ~10 μl of a 5 or 10 μM peptide solution revealed that, on mica, all the EP peptides adsorbed as discrete aggregates (Figure 1a).[15] However, adsorption of the EP peptides onto the (0001) plane of HOPG resulted in the immediate formation of a ~1.2 nm thick irregularly shaped film (Figure 1b). Over the course of several hours, this thin film gradually expanded to cover the entire surface of the HOPG substrate and was subsequently populated by extended fibrils oriented at 60° to each other. The rod-shaped EP II aggregates were typically ~1.2 nm tall with a deconvoluted width of ~3 nm.

A significant increase in the rate of fibril formation was noted when the experiments were performed at temperatures above the reported EP II solution coacervation value ($T_{coacervation}$) of ~28 °C (Figure 2). Remarkably, while nucleation and growth were accelerated at the higher temperatures, the oriented fibril arrangement was retained. Extended in situ imaging revealed that fibril growth occurred by elongation at the fibril ends, suggesting that fibril growth initiated from a single nucleation site. We observed neither fibril bifurcation nor fibril diffusion, which would further support a model of independent nucleation and growth, with strong surface stabilization.

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Table 1. Sequence Details for Exons 20, 21, 23, 24; Repeating Hydrophobic Domain Sequence (−PGVGVA−) Is Shown in Bold

exons	sequence
20	FPGFGVGVGGIPGVAGVPGVGGVPGVGGVPGVGI
21,23	PEAQAAAAAKAAKYGVGTPAAAAAAKAAAKAAQF
24	GLV**PGVGVAPGVGVAPGVGVAPGVGVAPGV GVAPGVGVAPGVGVA**PAIGP

Table 2. EP Series Label and Corresponding Exon Sequence

EP series label	exon sequence
EP I	20−21−23−24
EP II	20-(21−23−24)$_2$
EP IV	20-(21−23−24)$_4$

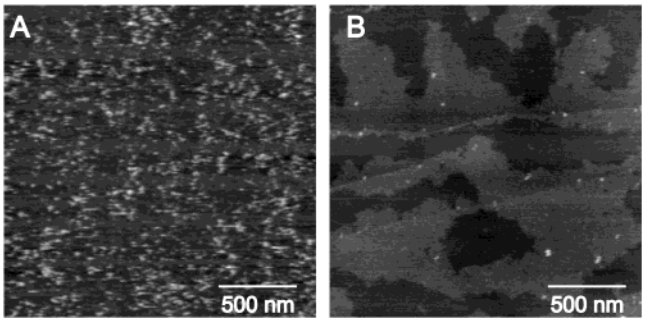

Figure 1. Discrete aggregates of EP II on (a) mica and (b) HOPG. Images acquired in buffer solution at ambient temperature. Image size: 2 μm × 2 μm.

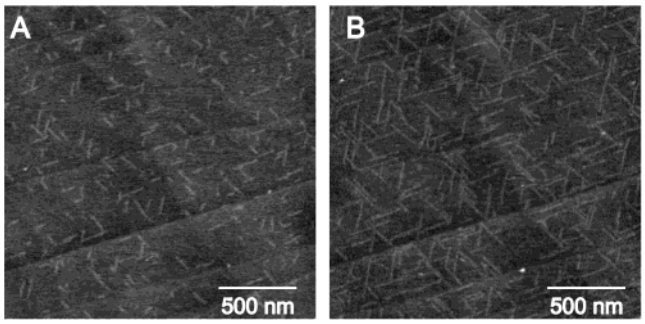

Figure 2. In situ TMAFM image acquired in buffer of EP II assembly on HOPG at 37 °C. The elapsed time between (a) and (b) is ~3 h. Image size: 2 μm × 2 μm.

Studies of the shorter, less hydrophobic EP I peptide ($T_{coacervation}$ ≈ 41 °C) revealed similar growth phenomena with rapid dense fibril formation initiating on top of an apparently amorphous thin underlayer (Figure 3). Again, the fibrils grew as individual entities with inter-fibril angles of 60° and 120°.

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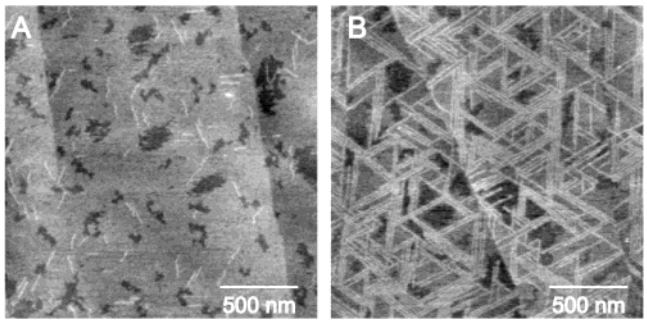

Figure 3. In situ TMAFM images of the rapid nucleation and growth of EP I fibrils on HOPG at 50 °C. Note that images (a) and (b) were not obtained in the same area; however, the general trend was the same. Elapsed time between (a) and (b): ~2 h. Image size: 2 μm × 2 μm.

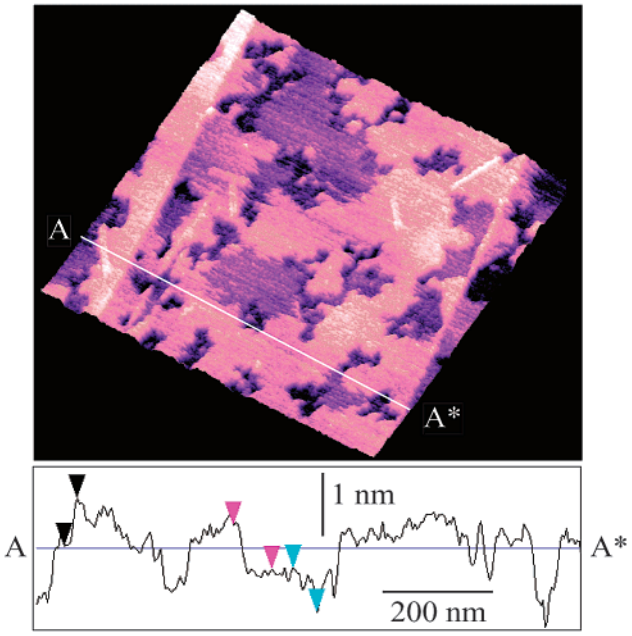

Figure 4. In situ TMAFM image revealing EP I fiber aggregates assembled on the underlying EP I film. Image acquired at 37 °C. Image sizes: 1 μm × 1 μm. Section analysis confirms the presence of a multilayered structure. Arrows are omitted from the AFM image for clarity purposes.

Close inspection of these fibrils revealed a curious structural feature. In contrast to the EP II system, we found that the EP I fibrils grew only on the upper layer of a bilayer film comprising two distinct ~0.8-nm thick layers (Figure 4). Experiments performed using the more hydrophobic EP IV peptide yielded similar results although the propensity for solution association appeared to be qualitatively higher. In all cases, we were unable to resolve any periodicity along the fibril backbone. Furthermore, there was no evidence of substrate-induced alignment for any of the EP peptides when mature EP fibrils formed by ex situ coacervation adsorbed onto either mica or HOPG.

While epitaxial alignment of proteins on HOPG and other inorganic substrates has been reported, the mechanism of protein association and formation at these interfaces remains unclear.[16,17] In these previous studies, the protein fibrils formed directly at the HOPG surface, a phenomena that we also observed for insulin fibril formation on HOPG (data not shown). This contrasts with the results from our present study wherein an intermediate peptide layer facilities both fibril formation and alignment.

While the presence of ordered fibrils on top of an underlying film suggests that the underlayer is acting as a nucleation template akin to the Stranski−Krastanov island-layer crystal growth model, the question of ordering within the first layer remains unanswered. The lack of fibril formation on mica, and the propensity for ordered growth of the EP peptide films on HOPG suggest that hydrophobic peptide−substrate interactions facilitate organization of the peptides at the HOPG−solution interface.

We propose that these forces conspire to arrange the hydrophobic EP peptide aggregates into an energetically favorable hexagonally close-packed arrangement at the HOPG surface. Such an ordered first layer would thus act as a template for subsequent fibril growth. This model is consistent with a hydrophobic coacervation mechanism and the view that specific alignment of individual hydrophobic domains is necessary for β-sheet/β-spiral formation.[18] Studies of other EP peptides and proteins with similar structural motifs are ongoing to address the utility of this model for protein nanostructure fabrication.

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