Disassembly and assembly of peptide fibrils via local mechanical perturbation

Introduction

MUC1Q11 is a 35 residues peptide known to form fibrils, which exhibit potential for cancer vaccine development. Using time-dependent and high resolution atomic force microscopy (AFM) imaging, this presentation reveals that MUC1Q11 forms fibrils on mica, following a nucleation process along the crystal axis of mica(0001) surfaces. Under 10 to 20 μM concentration and room temperature, individual fibrils as long as 1 micron could form within 2 hrs, covering 15% of mica surfaces. This natural growth process could be significantly altered using an AFM probe to cut the newly formed fibrils into short fragments, because these newly exposed sites became new nuclei to initiate growth of fibrils, leading to pine-needle like branches, and much higher coverages e.g. 45% in 30 min. This poster reveal the impact of local mechanical perturbation and the protein assemblies formed accordingly.

Methods

MUC1Q11 was synthesized on solid support.

Purity of the peptide was verified by HPLC and Mass Spectroscopy.

MUC1Q11 comprise two distinct units linked via a spacer:

Lyophilized peptide was dissolved in 1/6xPBS buffer (pH 7.4) with 200 μM or 400 μM final concentration and was incubated for 1 to 5 days at room temperature.

Fibril formation were studied with Atomic Force Microscope (AFM) imaging. Samples were deposited on freshly cleaved mica surface and were scanned in aqueous condition using tapping mode imaging using Olympus Biolever A.

Mechanical perturbation was carried out by:

1. Contact mode AFM imaging using set point equivalent with 1.0 nN Force vertical force.
2. AFM force measurements imposing 2.0 - 4.0 nN vertical force to the sample.
3. Low set point high free amplitude (ratio 0.6) AC mode scanning.

Fibrils grow from cutting points resulting multiple new elongating fibrils.

Before cutting the fibrils

0 min

5 min

12 min

18 min

24 min

400 nm

100 nm

Mechanical cutting is the reason of the growth of new fibrils as it can be seen on the time-laps images and zoom out image.

Before cutting the fibrils

5 min

24 min

Zoom-out image, 30 min

New fibrils exhibit individual growth behaviors

Before cutting the fibrils

5 min

15 min

25 min

Linear growth speed vary between 4.12 nm/min and 4.47 nm/min.

Fibrils exhibit variable individual growth behaviors

Before cutting the fibrils

15 min

20 min

25 min

30 min

35 min

Linear growth speed vary between 7.93 nm/min and 12.89 nm/min.

Conclusions

• Using an atomic force microscopy, we have applied a local force to break MUC1Q11 fibrils to smaller fragments where the cutting sites became new nuclei to initiate growth.
• The growth of the new fibrils follow the direction of the short fragments along the primary axis of the surface. The location, surface coverage or density of the new fibrils are dictated by the cutting density and time of growth.
• We have demonstrated high degree of control over the assembly and surface coverage, e.g. we were able to attain much higher coverages than the naturally formed fibrils on surfaces. Pine-needle like assembly is formed following our cut.
• Using local mechanics to impact fibril protein assembly at nanometer level provide a new platform to engineer new hierarchical structures of proteins, and therefore offer great promise to understand and control self-assembly of protein fibrils, and to produce new biomaterials, as well as to improve protein aggregate based immunotherapy.

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