

P17 – Determination of Physical Properties of a Single Peptide by AFM Experiments

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By stretching a polymer in solution using single molecule techniques it is possible to infer about its physical properties. In particular, AFM stretching experiments allow for a full characterization of the elasto-mechanical properties of the sample under study, taking into account both statical and dynamical regimes [1]. In the presented work, single molecule AFM force spectroscopy experiments have been used to determine mechanical properties of a polymer obtained starting from the Exon 28 (Ex28) of the human elastin gene. Elastin is a protein with important mechanical properties and, in particular, it shows quasi ideal elastic behavior associated to the presence of many hydrophobic unstructured domains (such as Ex28) into the protein structure. A typical result of an AFM length clamp stretching experiment is a force versus distance curve directly showing the response of the polymer to the external constant velocity stretching, from the tip-sample contact point up to the rupture of the bond between the probe and the sample (resulting in a steep peak). This experiment, performed in liquid environment, can give information on the mechanical properties of the molecule. The main contribution in the force exerted by the polymer on the AFM tip is due to the presence of a thermal bath driving the system towards a maximum entropy configuration. This entropic-only chain behaviour [2] can be described, switching to a mathematical description, in terms of the worm-like chain (WLC) model [3]. The Ex28 coded polymer has been used as a starting point to obtain bio-materials with specialized elasto-mechanical functions. In particular, a mutated polypeptide based on the EX28 sequence has been synthesized (named EX28K) with the aim of obtaining a new polymer with the same mechanical and physical properties of the native molecule but with increased aggregation properties, induced by a cross-linking reaction. AFM stretching experiments have been used to verify the mechanical properties of the engineered proteins at a single molecule level. The obtained results allowed us not only to answer this question, but also to give some insight into the first aggregation steps of the polymer towards the formation of reticulated structures.

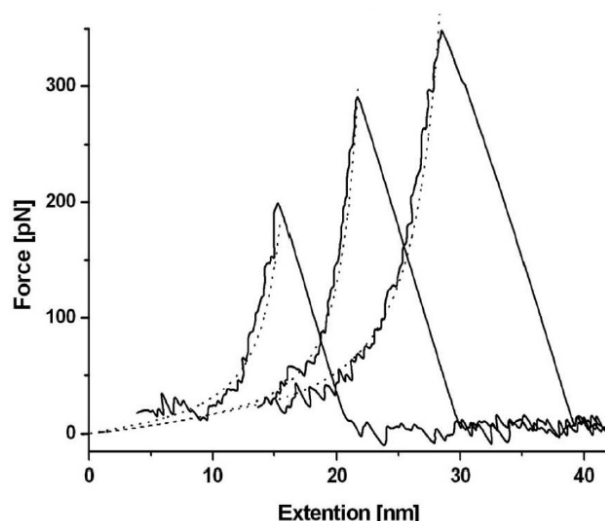


Figure 1: single molecule AFM stretching experiments on EX28K coded polymer after crosslinking

[1] R.A.Harris, J.E.Hearst; J. Chem. Phys. 44, 2595 (1966)

[2] C.Bustamante, J.F.Marko, E.D.Siggia, S.Smith; Science 265, 1599 – 1600 (1994)

[3] N.Saitô, K.Takahashi, Y.Yunoki; J. Phys. Soc. Jpn. 22, 219-226 (1967)

Determination of physical properties of a single peptide by AFM experiments

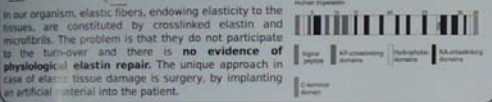
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Elastomeric proteins

Elastomeric proteins have the function to confer rubber-like elasticity, undergoing high deformation without rupture and returning to their original state upon removal of the stress. They are broadly diffused both in the vegetal (e.g. gluten) and animal (e.g. resilin in insects or abductin in bivalve molluscs) kingdoms and they share some common features:

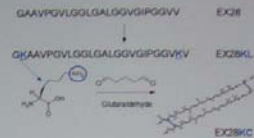
- highly flexible monomer chains
- network of crosslinks
- sequence similarity \Rightarrow high content of Glycyl residues
- presence of repeated sequences

Elastin is a protein with important mechanical properties and, in particular, it shows quasi ideal elastic behavior associated to the presence of many hydrophobic unstructured domains into the protein structure. From a genetic point of view, human tropoelastin gene has a **cassette-like organization** with exons alternatively encoding for functional (elastic/cross-linking) and structural (multiconformations/ α -helix) autonomous domains



Protein inspired bio-materials

Elastomeric proteins like elastin can suggest how to obtain bio-materials with peculiar mechanical properties starting from a natural sequence. We tested this approach with the peptide coded by exon 28 of the human tropoelastin gene, EX28.



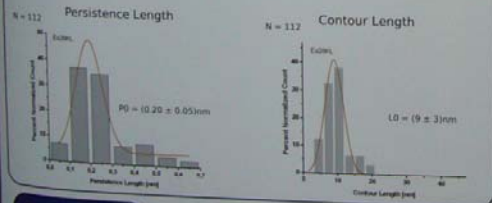
EX28 sequence was modified by introducing two lysine residues able to cross-link each other after a reaction with Glutaraldehyde. In order to verify the mechanical properties of the obtained peptides, we performed **single molecule atomic force microscopy** experiments. A single EX28KC coded protein (Linear or Cross-linked) molecule attached to a gold substrate is pulled with the cantilever and the response force curve is recorded as a function of the elongation.



EX28KL

Worm-Like chain model
 An elastic polymer in solution behaves like an entropic spring. The force exerted by such a polymer pulled in solution can be described using the worm-like chain (WLC) model.

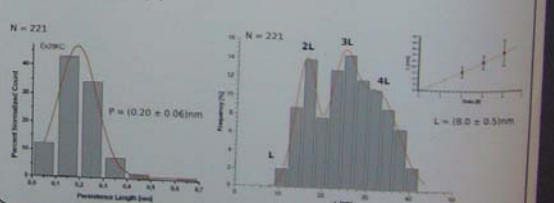
$$F = \frac{k_B T}{P} \left[\frac{1}{4} \left(1 - \frac{x}{L} \right)^{-2} - \frac{1}{4} + \frac{x}{L} \right]$$



EX28KC

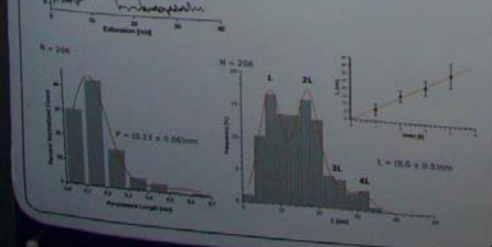
By pulling a system made by two identical EX28KC strands connected by cross-linking we expect to find a contour length that is close to the length of the single EX28KC strand. If the two peptides are strongly correlated each other (they behave like a single filament) we expect the same value also for the persistence length P but this parameter can decrease down to $P/2$ if the monomers are completely uncorrelated.

$L = L_0$? $P/2 < P < P_0$
 $L = 9 \text{ nm}$? $0.1 \text{ nm} < P < 0.2 \text{ nm}$



EX28KC + NaCl

The histogram for the contour length of EX28KC show many peaks for multiple values of the same base length. We interpreted this big tendency to aggregate and we are never able to see single EX28KC complexes. By rising the ionic strength of the solution we expect to disturb the intra molecular bonds, revealing shorter strands.



Conclusions

EX28KC goes towards beta conformation. In the case of EX28KL, $L = 9 \text{ nm}$, comparable with the length of the extended molecule, indirect measurements suggest that EX28KL assumes mainly unstructured conformations. EX28KC has shown to prefer the beta conformation. A beta strand with the EX28KC sequence has a more compact shape, with a full length of 7.93 nm , very close to the 6.0 nm that we found.

For EX28KC, $P = 0.2 \text{ nm}$, exactly as for EX28KL, indicating that the two strands behave in a strongly correlated way (two beta strands tightly packed). After adding NaCl, we measured a value of $P = 0.13 \text{ nm}$ nearer to $P/2$, indicating that the salt in solution not only interferes with the inter-dimers bonds but also it lowers the monomer-monomer interaction.

AFM stretching experiments have been used to verify the mechanical properties of the engineered proteins at a single molecule level. The obtained results allowed us not only to answer this question, but also to **give some insight into the first aggregation steps** of the polymer towards the formation of reticulated structures.