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Effect of chemical cross-linking on the mechanical properties of elastomeric peptides studied by single molecule force spectroscopy

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ABSTRACT

Mechanical properties of animal tissues are mainly provided by the assembly of single elastomeric proteins into a complex network of filaments. Even if the overall elastic properties of such a reticulated structure depend on the mechanical characteristics of the constituents, it is not the only aspect to be considered. In addition, the aggregation mechanism has to be clarified to attain a full knowledge of the molecular basis of the elastic properties of natural nanostructured materials. This aim is even more crucial in the process of rational design of biomaterials with selected mechanical properties, in which not only the mechanics of single molecules but also of their assemblies has to be cared of. In this study, this aspect was approached by means of single molecule stretching experiments. In particular, the effect of chemical cross-linking on the mechanical properties of a naturally inspired elastomeric peptide was investigated. Accordingly, we observed that, in order to preserve the elastic properties of the single filament, the two strands of the dimer have to interact with each other. The results thus confirm that the influence of the aggregation process on the mechanical properties of a molecular assembly cannot be neglected.

1. Introduction

Elastomeric proteins play an important mechanical role within animal organisms, providing the passive elasticity to tissues, undergoing several mechanical stress-relaxation cycles during their physiological functions (Shewry et al., 2003). Even if these proteins are present in many different species, where they evolved to a defined structure to perform specialized functions, they share common structural motifs. As a matter of fact, they have a modular structure, with several elastic domains, that guarantees the observed reliability and stability of the mechanical performance (Tatham, 2000) and, in addition, they are allowed to self-assemble, forming a complex structured network of elastomeric monomers working in parallel (Lee et al., 2001). Therefore, to have a full understanding of the molecular origin of tissue elasticity, it becomes crucial to adopt a multi-scale approach (Li and Cao , 2010), taking into account not only single molecule mechanical characteristics (Li, 2007), but also the cooperative effect in the organized tissue (Lv et al., 2010). This knowledge would be fundamental in the context of rational design of biomolecules with tailored mechanical properties (Li, 2007) and learning from natural proteins will help highlighting the key aspects of such a complex phenomenon. The present paper lies within this field, taking into account the role of the cross-linking between two natural inspired elastomeric peptides onto the bending stiffness of the molecule.

Among all, elastin can be considered as a good representative of an elastomeric protein, whose molecular and supramolecular structures have been widely investigated (Pepe et al., 2005; Tamburro et al., 2005). In addition, the gene of the human tropoelastin, the soluble precursor of elastin, has a cassette-like structure allowing for a reductionist approach in which individual exons encode for autonomously folded structural models with specialized function. Among the studied exons, exon 28 (EX28) has been demonstrated to encode for a peptide exhibiting almost all the structural properties of elastin (Bochicchio et al., 2007).

By stretching a polymer in solution using single molecule techniques, it is possible to infer about its physical properties. In particular, atomic force microscopy (AFM) stretching experiments allow for a full characterization of the elasto-mechanical properties of the sample at the single molecule level (Puchner and Gaub, 2009; Kellermayer, 2005). This technology was applied to the study of a modified EX28 sequence in which two lysine were inserted. This mutation leads to a peptide, EX28K, that can be induced to cross-link with a chemical reaction with glutaraldehyde, thus providing a model elastomeric system to test the effect of the reticulation process from a molecular point of view.

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