EFFECT OF CHARGE ON MECHANICAL PROPERTIES OF ELASTOMERIC PROTEINS

Ravi Kappiyoor[*], Ganesh Balasubramanian[**], Daniel M. Dudek[*] & Ishwar K. Puri[*]

[*]*Department of Engineering Science and Mechanics, Virginia Tech, Blacksburg, VA 24061, USA*

[**]*Eduard-Zintl-Institut für Anorganische und Physikalische Chemie, Technische Universität Darmstadt, 64287 Darmstadt, Germany*

Summary Nature achieves mechanical stability by making use of what are termed 'elastomeric proteins'. Several such proteins are found in nature. Unfortunately, it has not been possible to replicate these properties in synthetic rubbers due to our lack of a fundamental understanding of the mechanisms used by the proteins. We present a novel insight into the role of charge interactions on the mechanical properties of resilin and other such proteins.

INTRODUCTION

Elastomeric proteins are characterized as having low stiffness, high extensibility and high resilience [1]. A more fundamental understanding of the mechanisms used by these proteins to achieve these remarkable properties would allow us to create far superior rubbers than those we possess today. Herein, we present the results of a study detailing the role of charge interactions on the mechanical properties of resilin, a hydrophilic protein found primarily in insect cuticles and used for various tasks (e.g. structural stability of fruit fly wings, which beat at ~200 Hz).

The amino acid composition of resilin varies widely between insects. However, all resilins are elastomeric proteins. The mechanical properties of the materials appear to arise primarily from two main similarities that characterize all resilins: (i) they are all hydrophilic, and (ii) a sequence of four amino acids – tyrosine, glycine, alanine and proline – is found to repeat in each resilin. In order to investigate the underlying role these two characteristics play in determining the mechanical properties of resilin, we perform atomistic simulations [3] of tensile tests on four amino acid sequences (motifs) found in resilin, two from fruit fly resilin (YGAPGGGNGGRPSDT and YGAPGGGDGNGGRPSSS in FASTA format) as well as two from mosquito resilin (YGAPAQTPSSQ and YGAPAPSRPSQQ), henceforth called natural motifs. We then perform additional simulations on similar simulations with: (i) the charge on each atom set to half of its natural value (called the half-charge counterpart), (ii) the charge on each atom set to zero (called the zero-charge counterpart), and (iii) all polar amino acids replaced by a corresponding nonpolar amino acid (called the nonpolar counterpart). Further details of simulation methodology can be found in [2].

RESULTS FROM ATOMISTIC SIMULATIONS

The results of our simulations have previously been reported [2]. A few key findings are reproduced here in Figure 1, which shows that the natural motifs have lower stiffness and higher extensibility than their half-charge, zero-charge, and nonpolar counterparts. The coulombic interaction energies are vastly different between the different simulations. Coulombic interactions contributed an average of ~2000 kJ mol^{-1} to the tensile tests of the natural motifs, but only ~1000 kJ mol^{-1}, ~400 kJ mol^{-1} and ~0 kJ mol^{-1}, respectively, for the nonpolar, half-

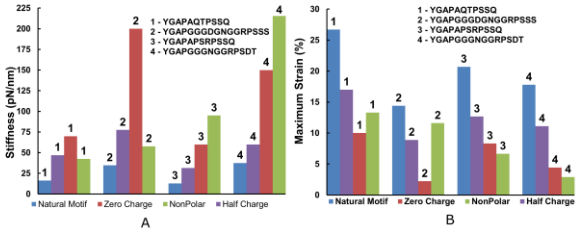

Figure 1. (A) The ratio of maximum displacement observed to maximum force applied (called stiffness) of the four motifs tested. In each case, it is evident that the natural motif has a lower stiffness than any of the counterparts. (B) The ratio of maximum deformation observed to the initial length of the motif (called strain) of the four motifs tested. The natural motif is seen to possess a much higher extensibility than any of the counterparts. Figure shows results presented in [2].

charge and zero-charge counterparts. This increase in coulombic interactions is due to the presence of adsorbed water molecules. The amino acids can interact with either each other or with the solvent. If they interact with each other, the interaction energy between the constituents makes it harder to pull different constituents apart. If, however, they interact with the solvent, there is less energy between the amino acids themselves, making it easier to pull them apart, which is why the natural motifs appear to have a much lower stiffness.

Semi-empirical density functional theory calculations employed on a linear YGAP repeating amino acid sequence of resilin reveal that the protein is more stable in water (polar solvent) than when in chloroform (nonpolar solvent) or in vacuum. A higher dielectric constant (in a polar solvent) produces stronger charge localization on the atoms of the motif facilitating hydrophilic behavior and enhanced hydrogen bonding with the solvent. Larger force constants for the molecular bonds of resilin when in water than when in chloroform improve its capability to store potential energy and retard local failure in the protein structure for a longer time. Thus, dielectric constants of the solvents can influence the elastomechanical behavior of resilin.

OUTLOOK

The results seem to indicate that hydrophobic proteins are stiffer than hydrophilic proteins. However, there are elastomeric proteins that possess similar properties to resilin but are hydrophobic, an example being elastin [4]. In order to investigate the effect of electric charge on the mechanical properties of these proteins, we perform similar simulations to those above in different solvents with varying dielectric constants. Specifically, we use the protein titin [5], which is composed of both hydrophobic and hydrophilic segments. We perform three separate simulations in each solvent: (i) a tensile test of just the hydrophilic segment (called the unstructured configuration due to the lack of secondary structure in this segment), (ii) a tensile test of the hydrophobic segment as found naturally (called the structured configuration due to the high degree of secondary structure found in this segment), and (iii) a tensile test of the hydrophobic segment with all structure removed (called the structure-removed configuration). The solvents used for the simulation are chloroform (a nonpolar solvent), dimethyl sulfoxide (an aprotic polar solvent), and water (a protic polar solvent). We also perform density functional theory calculations on both the initial and final states of all three configurations in order to determine any differences in key thermodynamic properties.

REFERENCES

1. Gosline, J., et al., *Elastic Proteins: Biological Roles and Mechanical Properties.* Philosophical Transactions of the Royal Society B Biological Sciences, 2002. **357**(1418): p. 121-132.
2. Kappiyoor, R., et al., *Elastomechanical properties of resilin.* Soft Matter, 2011. **7**(22): p. 11006-11009.
3. Hess, B., et al., *GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation.* Journal of Chemical Theory and Computation, 2008. **4**(3): p. 435-447.
4. Ashby, M.F., et al., *The Mechanical Properties of Natural Materials. I. Material Property Charts.* Proceedings of the Royal Society of London A, 1995. **450**(1938): p. 123-140.
5. Müller, S., et al., *Rigid Conformation of an Immunoglobulin Domain Tandem Repeat in the A-band of the Elastic Muscle Protein Titin.* Journal of Molecular Biology, 2007. **371**(2): p. 469-480.