Elastomechanical properties of resilin†

Ravi Kappiyoor, Ganesh Balasubramanian, Daniel M. Dudek and Ishwar K. Puri*

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We report the results of the first investigation on the effect of polarity on the elastic properties of an elastomeric protein resilin found in different insects. We simulate tensile tests on four naturally occurring resilin motifs. We repeat simulations on reduced-polarity counterparts of these motifs by setting the charge on each atom to zero, setting the charge on each atom to one-half of the natural value, as well as by replacing all of the polar amino acids with non-polar substitutes. The results highlight a strong correlation between polarity and extensibility. This is due to the presence of adsorbed water molecules that assist deformation energy transfer from the polar motif, allowing it to stretch further before it undergoes failure. These results unambiguously characterize the influence of charge on the mechanical properties of proteins, thus creating pathways for future formulations of novel bioinspired elastomeric rubbers.

Results and discussion

The force versus displacement curve for the YGAPAQTPSSQ motif found in mosquito resilin shown in Fig. 2(a). The motif has low stiffness and is capable of withstanding high strains since the force required to deform the motif is minimal (a maximum...
experiences a maximum force of 17 pN, at which point it has deformed 26.7%. The zero-charge counterpart experiences a maximum force of 21 pN. The half-charge counterpart experiences a maximum force of 13 pN, at which point it has deformed 18.5%. The nonpolar counterpart experiences a maximum force of 26 pN. The zero-charge, half-charge and nonpolar counterparts are presented in Table 1.

In order to determine an underlying cause for the dependence of the elasticity of the motifs of resilin on the polarity of its side chains, we calculate the average contribution to the potential energy due to the charge interactions. The interactions due to the charges on the polar molecules contributes ~2000 kJ mol$^{-1}$ to the average potential energy over the entire simulation. The zero-charge, half-charge and nonpolar counterparts are ~1000 kJ mol$^{-1}$, ~400 kJ mol$^{-1}$ and 0 kJ mol$^{-1}$, respectively. The differences in the energies due to charge interactions occur due to the affinity of the polar amino acid side chains to polar water molecules. As the motif is stretched, the bond lengths of the motif also elongate. This permits more water molecules to come in contact with the motif and subsequently interact with the polar side chains, thereby enhancing the potential energy due to the interaction amongst the charged sites. In order to differentiate the charge interactions between the interchain interactions and the surrounding water molecules, we used a radial distribution function to determine if interlocking occurs between side chains for any of these motifs. These functions indicate that the naturally occurring motifs and their zero-charge and nonpolar counterparts have similar interchain interactions.

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**Fig. 1** Homology model of resilin. Cylinders represent alpha helices and arrows represent beta sheets (some representative cases labeled). An accurate model of resilin would be expected to have little to no secondary structure (i.e., no alpha helices or beta sheets). However, there is a significant amount in this structure (obtained using the templates protease 1KAP and hydrolase 1GO8), making it inapplicable for correct quantitative measurements.

**Fig. 2** Force versus displacement curve for (A) naturally occurring YGAPAQTTPSSQ, (B) the zero-charge counterpart (i.e., the same motif with all of the charges set to zero), (C) the half-charge counterpart (i.e., the same motif with all of the charges set to half their natural value) and (D) the nonpolar counterpart (i.e., a motif in which all of the polar amino acids, except the first Y, are replaced with a random nonpolar motif). The force data shown represents a moving average over the previous 100 force values obtained from the simulation. The naturally occurring motif experiences a maximum force of 13 pN, at which point it has deformed 26.7%. The zero-charge counterpart experiences a maximum force of 21 pN, and at which point it has deformed 18.5%. The half-charge counterpart experiences a maximum force of 13 pN, at which point it has deformed 18.5%.

In order to understand the influence of charges on the mechanical properties of resilin, we simulate (i) YGAPAQTTPSSQ with all charges on each side chain set to zero (we refer to this as the zero-charge counterpart), (ii) the same motif with all charges set to half their natural value (referred to as the half-charge counterpart) and (iii) YGAPAGWPVVG, where all of the polar amino acids (excepting the first tyrosine (Y)) are replaced with nonpolar amino acids (which we refer to as the nonpolar counterpart). The force versus displacement curves obtained for the above two cases are presented in Fig. 2(b) and (c), respectively. Fig. 2(b) shows that the force required to deform the same motif (for which results are shown in Fig. 2(a)) by even 0.1 nm when the charges are removed is 14 pN, which is 1 pN more than the maximum force observed when charges are present. When simulated for longer times, the motif breaks at a deformation of only 0.3 nm, or a strain of 10%, with a force of slightly over 20 pN (supporting online material presents a video of this motif in order to demonstrate what is meant by failure of the motif). Fig. 2(c) shows a similar high initial stiffness for the half-charge counterpart, followed by a similar early failure at a deformation of 0.51 nm, or a strain of 17% with a force of 24 pN. Fig. 2(d) shows that the nonpolar motif also exhibits a noticeably higher stiffness than the original motif, requiring more than 15 pN to stretch 0.4 nm for a strain of 13.33%, when it undergoes failure. Results obtained from simulations for other naturally occurring motifs and their zero-charge, half-charge and nonpolar counterparts are presented in Table 1.

The results presented in Table 1 and graphically represented in Fig. 3(a) and (b) show that all naturally occurring motifs have a lower stiffness (defined as the maximum force observed divided by the maximum displacement observed) and higher extensibility. Since the only difference between the naturally occurring motifs, the zero-charge, half-charge and nonpolar counterparts is the polarity of the amino acid constituents (since the length differences between naturally occurring motifs and its counterparts are at most 1%), we hypothesize that the charges on the amino acids influence the elastic properties of resilins, with increased polarity indicating a higher extensibility and lower stiffness. Such a behavior is also apparent through comparisons between the different naturally occurring motifs. The two strands occurring in fruit fly resilin, though polar, contain a higher percentage of nonpolar amino acids than the strands found in mosquitoes, which contributes to their relatively higher stiffness and lower extensibility.

In order to determine an underlying cause for the dependence of the elasticity of the motifs of resilin on the polarity of its side chains, we calculate the average contribution to the potential energy due to the charge interactions. The interactions due to the charges on the polar molecules contributes ~2000 kJ mol$^{-1}$ to the average potential energy over the entire simulation. The naturally occurring motif while the corresponding contributions in the nonpolar, half-charge and zero-charge counterparts are ~1000 kJ mol$^{-1}$, ~400 kJ mol$^{-1}$ and 0 kJ mol$^{-1}$, respectively. The differences in the energies due to charge interactions occur due to the affinity of the polar amino acid side chains to polar water molecules. As the motif is stretched, the bond lengths of the motif also elongate. This permits more water molecules to come in contact with the motifs and subsequently interact with the polar side chains, thereby enhancing the potential energy due to the interaction amongst the charged sites. In order to differentiate the charge interactions between the interchain interactions and the surrounding water molecules, we used a radial distribution function to determine if interlocking occurs between side chains for any of these motifs. These functions indicate that the naturally occurring motifs and their zero-charge and nonpolar counterparts have similar interchain interactions.
The total energies of the naturally occurring, nonpolar motif, half-charge motif, and zero-charge motif are similar, around $2.75 \times 10^5$ kJ mol$^{-1}$. Of these, a naturally occurring motif has the smallest potential energy. The contributions of charge interactions to the potential energy explain only a part of this difference. The remainder is attributed to how the deformation energies of the motifs are released, since naturally occurring motifs are capable of releasing more of their deformation energy to surrounding water molecules. Since the hydrophilic naturally occurring motifs are able to adsorb water, it is possible that these motifs more readily transfer their deformation energy, improving their elasticity. The nonpolar, half-charge and zero-charge counterpart motifs are less hydrophilic so that their larger stiffness can be attributed to the inability of their hydrophobic side chains to interact with water and transfer energy. Therefore, we examine the hydrogen bonds between the protein and water over the course of the simulation.

The average hydrogen bonds per time step (with each time step being 2 fs) are listed in Table 1. The naturally occurring motifs form more hydrogen bonds with the surrounding water molecules than do their counterparts, reinforcing the role of water as an energy-absorbing layer on the hydrophilic naturally occurring motif. Table 1 also shows that there is some hydrogen bonding of water with the nonpolar motifs but the zero-charge motifs exhibit almost no such bonding. While in the zero-charge motif each charge was set to zero, the carboxyl and amino groups found in each amino acid on a nonpolar motif retain their polarity, thereby inducing a stronger interaction with water. It appears

<table>
<thead>
<tr>
<th>Motif</th>
<th>Type</th>
<th>Max. Force (pN)</th>
<th>Max. Displacement (nm)</th>
<th>Strain (%)</th>
<th>Avg. No. of Hydrogen Bonds Formed</th>
<th>Stiffness (pN nm$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td>YGAPAQTPSSQ</td>
<td>Natural</td>
<td>13</td>
<td>0.8 (unbroken)</td>
<td>26.7</td>
<td>47.195</td>
<td>16.25</td>
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<tr>
<td>YGAPAQTPSSQ</td>
<td>Zero-charge</td>
<td>21</td>
<td>0.3</td>
<td>10</td>
<td>2.951</td>
<td>70</td>
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<tr>
<td>YGAPAQTPSSQ</td>
<td>Half-charge</td>
<td>24</td>
<td>0.51</td>
<td>17</td>
<td>16.561</td>
<td>47.05</td>
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<tr>
<td>YGAPAGWPVVG</td>
<td>Nonpolar</td>
<td>17</td>
<td>0.4</td>
<td>13.3</td>
<td>30.745</td>
<td>42.5</td>
</tr>
<tr>
<td>YGAPGGGDGNNGGRPSSS</td>
<td>Natural</td>
<td>22.5</td>
<td>0.65</td>
<td>14.4</td>
<td>51.176</td>
<td>34.62</td>
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<tr>
<td>YGAPGGGDGNNGGRPSSS</td>
<td>Zero-charge</td>
<td>20</td>
<td>0.1</td>
<td>2.22</td>
<td>6.122</td>
<td>200</td>
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<tr>
<td>YGAPGGGDGNNGGRPSSS</td>
<td>Half-charge</td>
<td>31</td>
<td>0.4</td>
<td>8.19</td>
<td>20.976</td>
<td>77.5</td>
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<tr>
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<td>30</td>
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<td>11.6</td>
<td>37.902</td>
<td>57.69</td>
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<tr>
<td>YGAPAPSRPSSQ</td>
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<td>8</td>
<td>0.62</td>
<td>20.7</td>
<td>43.000</td>
<td>12.9</td>
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<tr>
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<td>15</td>
<td>0.25</td>
<td>8.33</td>
<td>4.195</td>
<td>60</td>
</tr>
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<td>YGAPAPSRPSSQ</td>
<td>Half-charge</td>
<td>12</td>
<td>0.38</td>
<td>12.67</td>
<td>13.073</td>
<td>31.58</td>
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<td>YGAPAPWCWPWI</td>
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<td>19</td>
<td>0.2</td>
<td>6.67</td>
<td>34.756</td>
<td>95</td>
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<tr>
<td>YGAPGGNNGGRPSDT</td>
<td>Natural</td>
<td>30</td>
<td>0.8 (unbroken)</td>
<td>17.8</td>
<td>51.244</td>
<td>37.5</td>
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<tr>
<td>YGAPGGNNGGRPSDT</td>
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<td>30</td>
<td>0.2</td>
<td>4.44</td>
<td>5.707</td>
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<td>30</td>
<td>0.5</td>
<td>11.11</td>
<td>20.585</td>
<td>60</td>
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<tr>
<td>YGAPGGGCGAGAPWWW</td>
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<td>28</td>
<td>0.13</td>
<td>2.89</td>
<td>36.317</td>
<td>213.4</td>
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Fig. 3 (A) The stiffness, defined as the ratio of the maximum force observed before failure to the maximum displacement observed before failure, of each naturally occurring motif (represented by triangles), the zero-charge counterparts (represented by filled squares), and the non-polar motifs (represented by empty squares). The natural motifs have a lower stiffness than their zero-charge, half-charge and nonpolar counterparts. (B) The maximum strains observed, defined as the ratio of the deformation observed before failure (or, if the motif did not undergo failure, the maximum deformation observed) to the initial length of the motif, for the natural motifs (represented by triangles), their zero-charge (represented by filled squares), and non-polar counterparts (represented by empty squares). The natural motifs have a higher extensibility than any of the counterpart sets of motifs.
that the hydrogen bonds formed on the backbone of the protein do not help the stiffness or the extensibility as significantly as the hydrogen bonds formed on the side chains. This is evidenced by a significantly higher stiffness and lower maximum displacement of the nonpolar motifs.

Methods

We use four different motifs, two commonly found in the fruit fly resilin (YGAPGGGNGGRPSDT and YGAPGGGDNGGPRPSSS) and the other two in mosquito resilin (YGAPAQTPSSQ and YGAPAPRPSQQ). Additional simulations involving these motifs are conducted by (i) setting all the charges of every atom in the entire motif to zero (which, although nonphysical and unrealistic, can aid our understanding of the role that polarity has on the mechanical properties of resilin), (ii) setting all the charges of every atom in the entire motif to half of their natural value, and (iii) replacing each of the polar amino acids (except for the initial tyrosine (Y)) in the same motifs with a random nonpolar amino acid. A structure for these motifs is created using the freely available AmberTools program tLeap.58

Our molecular dynamics (MD) simulations, using the GROMACS package56 for all the structures thus prepared, undergo the same set of procedures through a leapfrog time-integration scheme with a time step of 0.002 picoseconds (ps), i.e.: (i) energy minimization (no time marching during this simulation step), (ii) a canonical NVT (constant volume and constant temperature) ensemble based simulation performed over $2 \times 10^7$ time steps with temperature controlled at 310 K by rescaling the molecular velocities every 1 ps, (iii) two NPT (constant pressure and constant temperature) ensemble based simulations, each performed over $2 \times 10^7$ time steps, the first by velocity rescaling for thermostat and a Berendsen barostat to control the pressure at 1 bar, and the second using a Nose-Hoover thermostat and a Parrinello-Rahman barostat, both with coupling times of 1 ps, (iv) an NVE (constant volume and constant energy) equilibration, during which the motif is allowed to move freely, for $5 \times 10^7$ time steps, (v) followed finally by a pulling simulation that holds the N-terminus of the motif (the amide group attached to the alpha carbon of the first amino acid) fixed while pulling on the C-terminus (the carboxyl group attached to the alpha carbon of the final amino acid) at a constant strain rate of 0.1 nm/ns. Since the literature provides no evidence of the strain-rate-dependency of resilin, we chose 0.1 nm ns$^{-1}$ to provide good resolution within a reasonable time. We used the OPLS AA force field57 (which was used for similar studies of protein mechanical strengths58) for our simulations with Lorentz-Berthelot mixing rules employed for cross-interactions. The simulations were run in bulk water (using the SPC potential for water), with periodic boundary conditions. Coulombic interactions had a cutoff distance of 1.4 nm, while van der Waals interactions used a switch cutoff scheme with an inner and outer cutoff of 1.4 and 1.5 nm, respectively. Long range charge interactions were obtained using a Particle Mesh Ewald (PME) summation.

Conclusions

While several different resilins occur in different insects, all of them share two essential similarities. First, most resilins have the same repeating set of four amino acids (YGAP), and, second, they are all polar.10 Our objective was to determine if the polarity of the side chains plays a role in the mechanical properties of resilin. The results unambiguously reveal the strong correlation between the polarity of resilin and its superior mechanical properties. A larger percentage of polar amino acids within a motif provides both a higher resistance to fracture and lower stiffness. This dependence of the elastic behavior on the polarity is explained through the interactions between the amino acid side chains and water. The enhanced potential energy and hydrogen bonding due to charge interactions between the polar amino acid side chains and surrounding water molecules result in the improved mechanical properties of the hydrophilic naturally occurring motifs.

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Notes and references