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Electrostatic charges instigate 'concertina-like' mechanisms of molecular toughening in MaSp1 (spider silk) proteins

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ABSTRACT

According to a recent article authored by Ortega-Jimenez and Dudley [1], the capture success of spiders is in part due to electrostatic charges on the surfaces of insects that macroscopically deform the spider web and increase the chances of insect-web contact. In this brief communication, we further show that electrostatic charges instigate a molecular 'concertina-like' mechanism of deformation in MaSp1 protein, which effectively begins the toughening-up of dragline silk threads prior to insect-web contact.

1. Introduction

Spiders can produce up to 7 types of silks for different applications. Dragline silk has a major role in the structural performance of the web and 1.5 times more force is required to break radial threads within the web compared to spiral threads [2]. Dragline silk is known to have high toughness [3] and its modulus and strength values have been compared to some of the strongest man-made fibres like Kevlar [4]. Moreover, it can extend 5 times its original length. The structural superiority of dragline silk originates from its core composition of major ampullate spidroin proteins (MaSp1 and MaSp2). MaSp 1 is more dominant in spider silk dragline silk than MaSp2, with a ratio of approximately 3:2 or higher, depending on the species [5]. MaSp2 has a high proline content, which introduces steric constraints for hydrogen bond formation that inhibit the formation of α-helix and β-sheet structures [6]. These proteins are rich in glycine and alanine and display a modular structure consisting of a long repeating protein sequence that is flanked by non-repetitive conserved N- and C-terminal regions [5,7–9]. The repetitive group can be a sequence of 150–500 amino acids in length (Molecular weight ca. 250–320 kDa) that are basic repeat units, which can be subdivided into shorter repetitive amino acid motifs [3,10,11]. The resulting secondary structure exhibits confined regions of large-scale hydrogen bonding between protein strands. This secondary structure contains motifs, which can be further divided into four categories; GPGXX motifs that form elastic β-turn spirals, alanine-rich β-sheet stacks (A_n or $(GA)_n$), GGX, and spacers [5,10,12,13]. Dragline silk MaSp1 protein contains GGX motifs wherein X is typically of alanine, tyrosine, leucine or glutamine units. These motifs form amorphous 3_1 helical segments of protein that can elongate considerably under applied force. Moreover, alanine rich A_n and $(GA)_n$ domains form β-sheet nanocrystals, which are responsible for the high strength of the protein strand. Antiparallel β-sheet crystals have crosslinking domains which create a network of hydrogen bonds, embedded in a semi-amorphous glycine rich matrix. The crystalline segments have dimensions of a few nanometres and constitute at least 10–15% of the silk volume [3,14,15]. At 6 monomer units, these β-sheets are mechanically stable and there is no significant mechanical improvement beyond 8 monomer units [16–18]. Shear-induced elongation and alignment during spinning increases the extrusion rate of silk. Stiffer, stronger, but less extensible fibres are produced through the consequent formation of aligned β-sheet crystals [19]. These characteristics of MaSp1 self-assembly are further affected by changes in the pH and in the ion exchange concentrations [14].

Detailed atomic level simulation is indispensable for complicated procedures like protein folding and for providing molecular mechanistic insights where such information cannot easily be obtained through experimentation [20,21]. Most computational studies focus on the size effects and energy interactions between crystalline segments in spider silk proteins [22–24], the size of which is a critical determinant to failure in dragline silk [16]. To date, the effect of charge on the molecular mechanics of MaSp1 has not been reported. In light of the recent findings of [1], which show that web deformation is influenced by electrostatically charged insects, it seems appropriate to research how electrostatic charges affect molecular mechanisms of deformation. In this article we report on the deformation mechanics of two adjacent MaSp1 proteins under the influence of non-contacting electrostatic charges. Our

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research considers the interrelations of 'harder' A_n units with respect to the 'softer' GGX units as a function of an electrostatic pulling force. We first model the effects of size and spacing of A_n units on the folding characteristics to an energetic equilibrium from an extruded state [25]. We subsequently consider the mechanism of elongation and shear between two such folded MaSp1 strands from their energetically stable folded states while under the influence of an electrostatically charged pull. Our findings indicate that silk toughens at the molecular level prior to insect-web contact thus preparing the web for the high impact energies of insect capture.

2. Results and Discussion

We begin by simulating the folding of two adjacent and aligned MaSp1 molecules. This state is modelled in order to monitor the entire possible scenario of deformation for these proteins that occurs under the influence of an electrostatic pull. A_n segments of 8 and 12 were modelled in two conformations (full A_n strands located central to the MaSp1 strand and half A_n segments located equidistant from the centre leaving an amorphous central region). Protein strands containing 2 shorter crystalline segments fold more radically than protein strands with a single crystalline segment situated in the centre of the strand, Fig. 1. In this figure we monitor the distance between the N and C termini of the MaSp1 protein. We also monitor the folding behaviour of both crystalline and amorphous regions as a function of time. Proteins with 2 crystalline A_n regions (2×4 and 2×6), fold to under ca. 13% of their initial lengths, while the proteins with central crystalline A_n regions fold less, to ca. 20% of their original lengths. Rigid crystalline regions stabilise at shorter time scales as they have lower free energies and thus reduced mobility as compared to the amorphous segments. In the amorphous regions, longer chains require more time to stabilise and they fold more extensively. Another key factor affecting the folding mechanism is the location of the crystalline regions along the strand. The amorphous regions at the centres of 2×4 (Fig. 1b) and 2×6 (Fig. 1d) proteins fold considerably more than the amorphous regions at the heads and tails of these proteins. This can be attributed to the mechanical stability of the two rigid poly-Ala sections, which constrain the amorphous material between, thus forcing more extensive folding (and over longer times) to reach energetic stability. The amorphous regions at the terminal sites however, have more freedom to fold to their preferred configurations since they are not constrained from both ends. Hence the amorphous segments at the termini do not fold as radically as the amorphous centre. At the macromolecular level, the entire protein will fold, but folding between the crystalline and amorphous segments is highly disproportionate. The amorphous regions being more malleable than the crystalline regions fold more readily. This in turn reduces the 'folding responsibility' of the poly-Ala segments in taking the molecule to a state of equilibrium. The changes in distance between the terminal atoms of the crystalline and amorphous regions during the folding process are shown in Fig. 1. Proteins with two crystalline segments moreover take more time to reach steady state since the central amorphous regions are constrained in movement by two rigid poly-Ala segments, Table 1. In this table, ΔE_T values indicate the stability of folded protein molecules. MaSp1 strands containing longer β-sheets are more stable than MaSp1 strands with shorter β-sheets. Concurrently, protein strands containing 2 short segments of poly-Ala are energetically more stable in their folded conformations than strands with single poly-Ala segment containing the same number of monomeric alanine units.

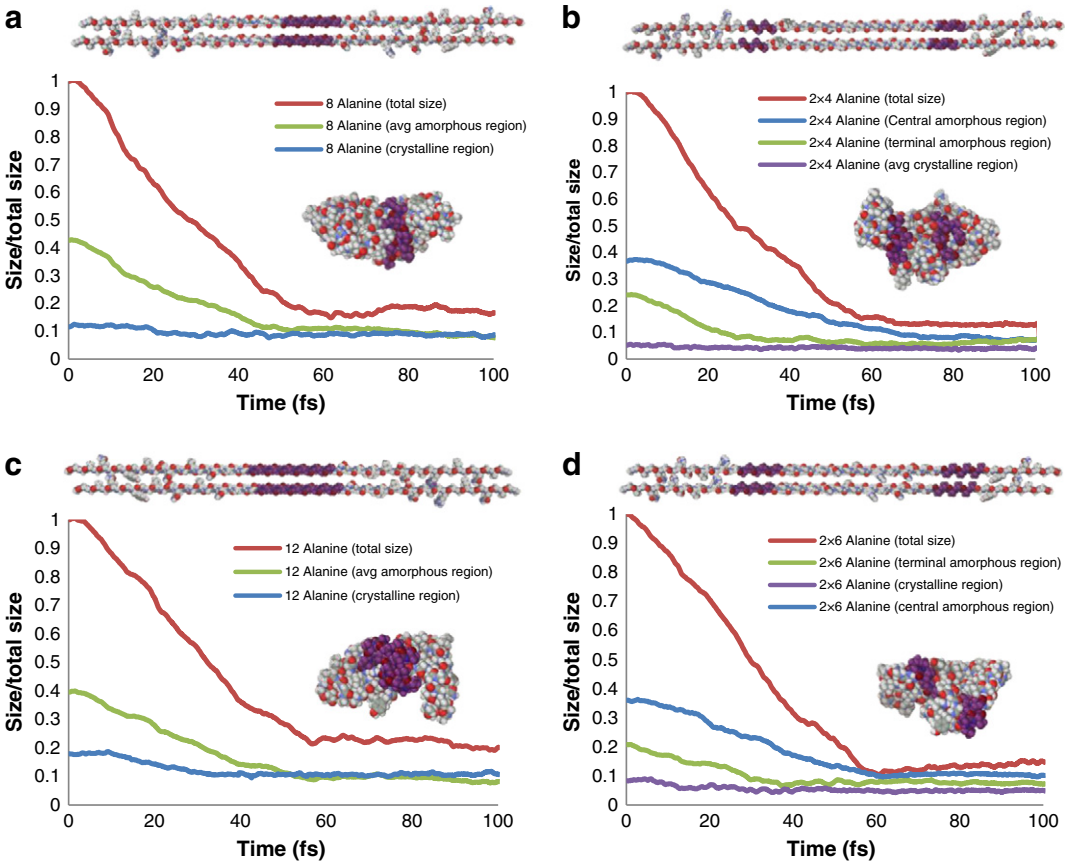

Fig. 1. Variations in the distances between terminal atoms for different regions of MaSp1 strands during the folding process; for silk proteins with (a) an 8 alanine group, (b) 2×4 alanine groups, (c) a 12 alanine group and (d) 2×6 alanine groups. The extended and folded conformations are shown in each case.