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On the roles of the alanine and serine in the β-sheet structure of fibroin

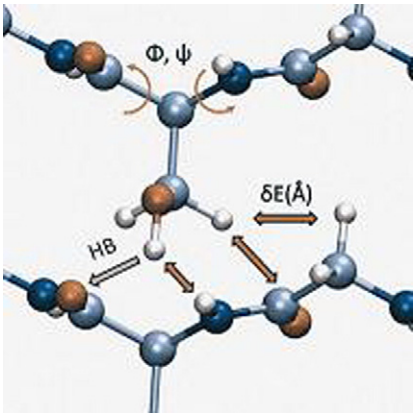
Juan Francisco Carrascoza Mayen *, Alexandru Lupan, Ciprian Cosar, Attila-Zsolt Kun, Radu Silaghi-Dumitrescu

Department of Chemistry and Chemical Engineering, "Babes-Bolyai" University, 11 Arany Janos Str, Cluj-Napoca RO-400028, Romania

HIGHLIGHTS

- Semiempirical MP6, DFT calculations and molecular dynamics calculations on fibroin type models are provided.
- These results show how alanine and serine impact the rigidity of β-sheet structures.
- Alanine adds stability to the rigidity of the sheet, allowing it to maintain a properly pleated structure even in a single β-sheet.
- The role of the serine is proposed to involve modulation of the hydrophobicity.

GRAPHICAL ABSTRACT



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ABSTRACT

In its silk II form, fibroin is almost exclusively formed from layers of β-sheets, rich in glycine, alanine and serine. Reported here are computational results on fibroin models at semi-empirical, DFT levels of theory and molecular dynamics (MD) for $(Gly)_{10}$, $(Gly–Ala)_5$ and $(Gly–Ser)_5$ decapeptides. While alanine and serine introduce steric repulsions, the alanine side-chain adds to the rigidity of the sheet, allowing it to maintain a properly pleated structure even in a single β-sheet, and thus avoiding two alternative conformations which would interfere with the formation of the multi-layer pleated-sheet structure. The role of the serine is proposed to involve modulation of the hydrophobicity in order to construct the supramolecular assembly as opposed to random precipitation due to hydrophobicity.

1. Introduction

Fibroin is a protein rich in glycine, alanine and serine. The "Silk I" conformation of fibroin is known to be relatively flexible, and to rely significantly on intramolecular hydrogen bonds, somewhat reminiscent of collagen, with repeated β-turn motifs [1–3]. This conformation would prevail while fibroin is still within the gland of the worm. By contrast, the "silk II" conformer is more stable, and is known to rely on inter-strand hydrogen bonds between β-strands organized in multiple layers, as illustrated in Fig. 1 [1,2]. The transition from I to II was shown to depend on external factors such as tensile stress and hydration [2–5]. Local β-helix arrangements were also proposed in fibroin [6].

* Corresponding author at: Department of Chemistry and Chemical Engineering, "Babes-Bolyai" University, 11 Arany Janos Str, Cluj-Napoca RO-400028, Romania. Tel.: +40 26 4593833; fax: +40 26 4590818.
E-mail address: carrascoza@chem.ubbcluj.ro (J.F. Carrascoza Mayen).

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The natural occurrence of the amino acids in silk II involves a ratio of 3:2:1 which can be reflected for instance into a sequence of the type $(Ala–Gly)_2–Ser–Gly$ [1].

Using NMR and empirical calculations, it was shown that alanine protons are engaged in stronger, more favorable inter-strand hydrogen bonds in silk II, as compared to glycine [7]. Computational DFT data have been employed for comparing with the experimental NMR data; notably, the geometries for such DFT computations were built directly to the desired conformations, rather than optimized [8].

With $(AG)_{15}$ peptides, mixtures of β-turns and β-sheets were demonstrated using NMR spectra, with evidence for lamellar arrangements in solution [9,10]. Introduction of serine residues into such AG polypeptides was found to provide even better similarity with fibroin; the role of the serine was proposed to involve loosening of the inter-plane interactions by means of its bulkier and more hydrophilic side-chain [10]. Molecular mechanics and dynamics calculations have been used to describe the folding of both silk I and silk II, and the instability of its aggregated states also followed by NMR data [4]. Buehler and Keten have employed molecular mechanics to show that poly-alanine structures are more likely to engage in crystalline-like β-sheet domains than the glycine rich structures, these authors also evidenced a role for each type of structure under mechanical stress in fibroin [11,12]. This was in line with experimental data showing that in $[GAGAGX]_{16}$ peptides the nature of the amino acid X (A, S, Y or V) has a measurable influence on the ability of the peptide to form β-sheets [13].

Using a small two-peptide model Asakura and co-workers in 2004 [14] applied molecular mechanics in order to show possible ways in which a tyrosine residue may be accommodated within fibroin. With such models, they further described silk I type of structures in water. The position of the tyrosine as an (AG) polypeptide was found to affect the preference for silk II [15] versus silk I conformations [16]. Zhou and co-workers have additionally shown that this tyrosine can yield a spectroscopically detectable free radical, whose signal would be diagnostic between the I and II types of silk [17].

Stewart [18] has shown that semiempirical methods may reasonably describe β-sheet structures in globular-type proteins without stacked sheets of β-strands; tensile strength parameters were then derived from a fibroin model using such semiempirical strategy, although the detailed structure of the optimized geometry has not been discussed [18]. Several studies have shown that the hydrogen bonding interactions within β-sheets are cooperative with contributions to the energy of binding from several layers [19–24].

Molecular dynamics (MD) studies has been used before to obtain information on how the molecular interactions can be understand at atomic level and draw conclusions on the final effect in the structure [25].

We have recently provided an extended analysis of computational methods for predicting another secondary structure element in proteins, the α helix [26]. Here, we report computational data on fibroin using two of the best performing methods for the task, namely PM6 and M06-2x, which were used to compute the energy of interaction between strands, after optimization. We also applied molecular dynamics methods to obtain statistically representative geometrical models and understand its interaction and stability across the time. These data allow not only the estimation of preferred geometries for fibroin-like peptides, but also of the relative energies of interactions, thus allowing formulation of conclusions on the roles of alanine and serine in fibroin. As glycine contains the smallest side-chain, we perform calculations on Gly–Gly multimers as reference, thereafter examining the effect of different side-chains with Gly–Ala and Gly–Ser peptide models.

2. Methods

Polypeptide models were built as ten — amino acid monomer units using the Spartan software package [27] and multiplied up to eighteen such units (6 × 3 monomers) as described previously. Parallel as well as antiparallel structures were considered; however, unless otherwise specified, only antiparallel structures are discussed in Section 3, in line with experimental data [4]. Within a plane each monomer was arranged so that its side-chains point on the opposite side of the layer's plane, relative to the side-chains of the two neighboring-sheets.

Regarding the construction of the layers, we study first a single decapeptide and its relationship with the solvent, then a dimer (non-covalent association of two decapeptides in antiparallel pleated beta sheet conformation) in order to explore chain to chain and dimer–solvent interactions. Subsequently, models of 4 and 6 monomers were done with the intention to observe the systematic effect of isolation of the central

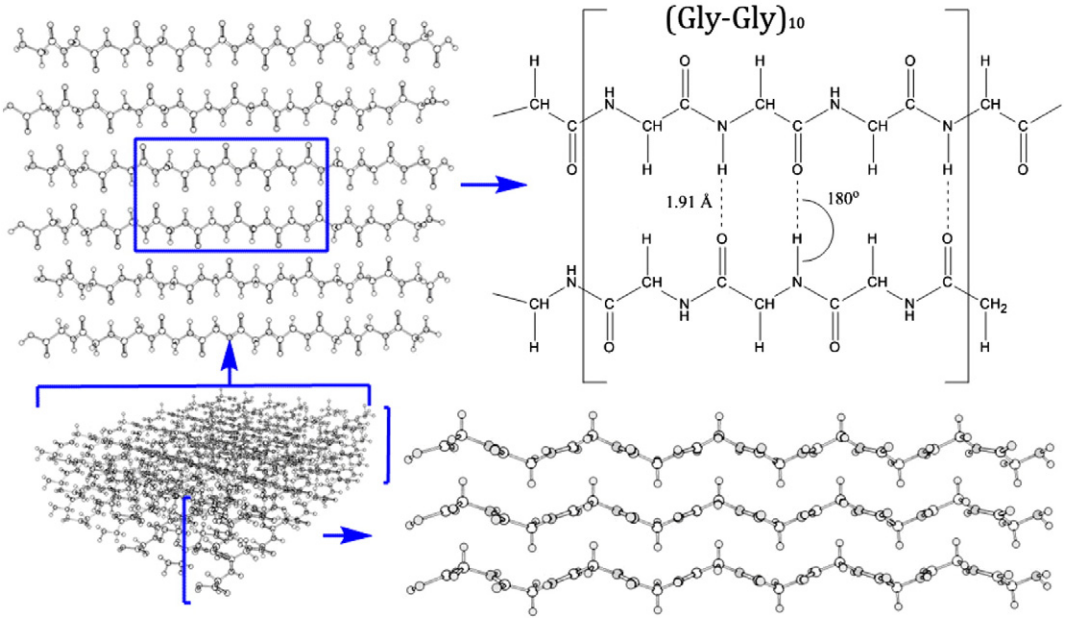

Fig. 1. Assemblies of octadecamers in β-sheets (decaglycine chosen for illustration only) organized canonically according to a putative fibroin-like silk II structure. The $[(Gly–Ala)_5]_{18}$ and $[(Gly–Ser)_5]_{18}$ assemblies are presented in the Supporting Information.