NANOSTRUCTURE CHARACTERIZATION OF BETA-SHEET CRYSTALS IN SILK UNDER VARIOUS TEMPERATURES

by

Yan ZHANG^a, Yu-Yue CHEN^{a*}, Ling LIN^b, and Pi-bo MA^{c*}

^a National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, Suzhou, China

 ^b Ningbo Key Laboratory for Advanced Textile Technology and Fashion CAD, Zhejiang Fashion Institute of Technology, Jiangbei District, Ningbo, Zhejiang, China
^c Engineering Research Center for Knitting Technology, Key Laboratory of Eco-Textiles, Ministry of Education, Jiangnan University, Wuxi, China

> Original scientific paper DOI: 10.2298/TSCI1405459Z

This paper studies the nanostructure characterizations of β -sheet in silk fiber with different reaction temperatures. A molecular dynamic model is developed and simulated by Gromacs software packages. The results reveal the change rules of the number of hydrogen bonds in β -sheet under different temperatures. The best reaction temperature for the β -sheet crystals is also found. This work provides theoretical basis for the designing of materials based on silk Key words: β -sheet crystals, hydrogen bonds, temperature, silk fiber

Introduction

Silk is a traditional protein fiber since its development in China thousands of years ago. Silk fiber has excellent mechanical properties such as high modulus, high tensile strength, and great extensibility. Because of the exceptional properties, silk has already been used not only as luxury fabrics but also as various industrial applications including medical sutures, tissue regeneration, *etc.* [1-3]. The mechanical properties of silk depend on the internal structure especially the β -sheet crystals structure.

Recently, many articles focus on the structure characters of antiparallel β -sheet crystals of silk fiber at nanoscale. A highly conserved poly-(Gly-Ala) repeats which form β -sheet crystals are found in both silk and spider silk fibers [4]. The content of β -sheet exceeds 50% for silk and spider silk fiber and at the same time determines the main properties of silk and also regenerated silk materials [5-7]. Du *et al.* [8] compare the β -sheet structure between silk and spider silk. The higher content of intermolecular β -sheet of spider silk gives rise to the strain-hardening phenomenon. Jin *et al.* [9] develop regenerated silk films with reduced β -sheet. The new silk films degrade more rapidly because of the reduced β -sheet and show excellent support for human adult stem-cell expansion. Keten *et al.* [10] reveal the size effects of β -sheet nanocrystals. The smaller β -sheet crystals confined to a few nanometers achieve higher mechanical properties.

This paper studies the changes of the number of hydrogen bonds of silk β -sheet nanocrystals under various temperatures. The results may provide theoretical support for designing of new silk based materials.

Methods

Figure 1 shows the structure of β -sheet crystal in silk fiber. The molecular model of silk is obtained from the protein data bank with the identification code 2slk [10]. The model

^{*} Corresponding author; e-mail: chenyy@suda.edu.cn, mapibo@jiangnan.edu.cn



clearly shows the highly antiparallel structure. The molecular dynamic (MD) simulations are performed with the GROMACS 4.5.1 MD software packages using Chemistry at Harvard Molecular Mechanics (CHARMM) topology and force-field parameter files [11]. The energy function form is:

$$U(\vec{R}) = \sum_{\text{bonds}} K_{b}(b-b_{0})^{2} + \sum_{\text{angles}} K_{\theta}(\theta-\theta_{0})^{2} + \sum_{\text{H} \in \mathcal{W}} K_{UB}(S-S_{0})^{2} + \sum_{\text{impropers}} K_{\varphi}(\varphi-\varphi_{0})^{2} + \sum_{\text{dihedrals}} K_{\chi} \left[1 + \cos(n\chi - \delta)\right] + \sum_{\text{nonbonded}} \frac{q_{i}q_{j}}{4\pi Dr_{ij}} + \varepsilon_{ij} \left[\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{12} - 2\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{6} \right]$$

Figure 1. Structure of the β -sheet crystal in silk drawn using the program Visual molecular dynamics (VMD)

where K_{b} , K_{UB} , K_{θ} , K_{χ} and K_{φ} are the bond, Urey-Bradley, angle, dihedral angle, and improper dihedral angle force constants, respectively, *b*, *S*, θ , χ and φ are the bond length, Urey-Bradley 1,3-distance, bond angle, dihedral angle, and improper torsion angle, respectively, with the subscript zero representing the equilibrium values for the individual terms. Coulomb and Lennard-Jones 6-12 terms contribute to the external or non-bonded interactions; ε_{ij} is the Lennard-Jones well depth, R_{\min} – the distance at the Lennard-Jones minimum, q_i – the partial atomic charge, and r_{ij} – the distance between atoms *i* and *j*.

The molecular model is then minimized and equilibrated in a transferable intermolecular potential spc/e explicit water box. Simulations are carried out in an isothermal-isobaric system and at a constant pressure of 1.01325 bar (1 atm). Five different temperatures (300 K, 340 K, 360 K, 366 K, and 372 K) are selected for simulation. The stability of the β -sheet without load applied is verified from the root mean squared deviation data obtained from the molecular dynamics trajectory.



Figure 2. Number of hydrogen bonds in β-sheet nanocrystals under different temperatures

Results and discussion

Figure 2 shows the changes of number of hydrogen bonds in the β -sheet nanocrystals under different temperatures. Generally, the number of hydrogen bonds is the function of time. The number decrease with the increase of time. During the relatively low temperatures (300 K, 340 K, 360 K), the number of hydrogen bonds decreases from about 72 to about 68. Additionally, there are not apparent different around the three temperatures. For the temperature of 366 K, the number of the hydrogen bonds decreases sharply at about 8800 ps and then maintains at about 50. When the temperature rises to the highest of 372 K, the number of hydrogen bonds decreases sharply early at about 6500 ps. After the sharp decrease, the number of hydrogen bonds increases and maintains at about 60.

In the β -sheet nanocrystals, the conserved poly-(Gly-Ala) domains are mainly linked together by hydrogen bonds. The decrease of number of hydrogen bonds means the structure of the β -sheet nanocrystal changes from tight to lose. It provides more opportunities for the β -sheet nanocrystal to contact with other materials such as polymers, inhibitor, *etc.* Therefore, the best reaction temperature for β -sheet nanocrystal is around 366 K.

Conclusions

The temperature effects of the structure of β -sheet nanocrystal are studied by MD simulations. The results show the number of hydrogen bonds decrease with the increase of temperature. When the temperature increases to 366 K, the number of hydrogen bonds reduces to the least.

Acknowledgments

The work is supported by the National Natural Science Foundation of China under grant No. 11402155, Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Jiangsu Planned Projects for Postdoctoral Research Funds (Grant No. 1302061C and 1302112B), the Open Project Program of Key Laboratory of Eco-textiles, Ministry of Education, Jiangnan University (No. KLET1311), China Postdoctoral Science Foundation (Grant No. 2014M551656), and the Natural Science Foundation of Jiangsu Province (Grant No. BK20140396 and BK20140399), Ningbo Natural Science Foundation under grant Nos. 2011A610111 and 2013A610011, and Ningbo Science and Technology Innovation Team Foundation under grant No. 2012B82014.

References

- [1] Becker, N., *et al.*, Molecular Nanosprings in Spider Capture-Silk Threads, *Nature Materials*, 2 (2003), 4, pp. 278-283
- [2] Shao, Z. Z., Vollrath, F., Materials: Surprising Strength of Silkworm Silk, *Nature*, 418 (2002), 6899, pp. 741-741
- [3] Vollrath, F., Knight, D. P., Liquid Crystalline Spinning of Spider Silk, *Nature*, 410 (2001), 6828, pp. 541-548
- [4] Hayashi, C. Y., et al., Hypotheses that Correlate the Sequence, Structure, and Mechanical Properties of Spider Silk Proteins, International Journal of Biological Macromolecules, 24 (1999), 2-3, pp. 271-275
- [5] Du, N., et al., Design of Superior Spider Silk: from Nanostructure to Mechanical Properties, Biophysical Journal, 91 (2006), 12, pp. 4528-4535
- [6] Rousseau, M. E., *et al.*, Study of Protein Conformation and Orientation in Silkworm and Spider Silk Fibers Using Raman Microspectroscopy, *Biomacromolecules*, 5 (2004), 6, pp. 2247-2257
- [7] Grubb, D. T., Jelinski, L.W., Fiber Morphology of Spider Silk: the Effects of Tensile Deformation, *Macromolecules*, 30 (1997), 10, pp. 2860-2867
- [8] Du, N., et al., Structural Origin of the Strain-Hardening of Spider Silk, Advanced Functional Materials, 21 (2011), 4, pp. 772-778
- [9] Jin, H. J., et al., Water-Stable Silk Films with Reduced Beta-Sheet Content, Advanced Functional Materials, 15 (2005), 8, pp. 1241-1247
- [10] Keten, S., et al., Nanoconfinement Controls Stiffness, Strength and Mechanical Toughness of Beta--Sheet Crystals in Silk, Nature Materials, 9 (2010), 4, pp. 359-367
- [11] MacKerell, A. D., et al., All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins, *Journal of Physical Chemistry B*, 102 (1998), 18, pp. 3586-3616

Paper submitted: January 18, 2014 Paper revised: May 10, 2014 Paper accepted: July 23, 2014