NOTE

PIXE Analysis of Silk

YONGCHENG LIU,[1] TONGYIN YU,[1] HUIYING YAO,[2] FUJIA YANG[2]

[1] Department of Macromolecular Science, Fudan University, Shanghai 200433, People's Republic of China

[2] Nuclear Science Department, Fudan University, Shanghai 200433, People's Republic of China

Received 3 February 1997; accepted 10 March 1997

ABSTRACT: The method of proton-induced X-ray emission (PIXE) was first utilized to analyze the elements in silk. Different kinds of silk from home and the wild were examined. The results show that every silk, besides C, H, O, and N, contains many types of elements such as Si, P, S, Ca, Mn, Fe, Cu, Zn, and Sr and different samples have different relative contents. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **66:** 405–408, 1997

INTRODUCTION

Silk has been one of natural macromolecules used for some centuries. *Bombyx mori* silk consists of two types of proteins: fibroin and sericin. Fibroin is the protein that forms the filaments of silkworm silk and gives silk its unique physical and chemical properties. Sericins are a group of gummy proteins that coat the fibroin filaments. Silk has good strength, elasticity, softness, luster, absorbency, and affinity for dyes and is biocompatible. Silk fibroin can be used in various forms, such as gels, powders, fibers, or membranes and can applied to biotechnology and in medicines, and textiles, etc. Recently, silk fibroin membranes were used for the separation of amino acids[1] and in water–ethanol separation.[2] We utilized silk fibroin as an immobilization matrix for enzymes and constructed several second-generation amperometric biosensors.[3–5]

To understand the relationship between its structure and properties, many methods such as FTIR,[6–9], NMR,[6] SEM,[6] ESR,[6,10] X-rays,[8,9,11] TEM,[7] Ramam,[12] and STM[13] have been used to investigate the silk fibroin structures. However, it has not been reported what elements besides carbon, hydrogen, oxygen, and nitrogen compose silk and how these elements influence its properties, the mechanism of its forming fiber, and the physiology of the *Bombyx mori* larvae. It is difficult to explore these questions with general methods.

Particle-induced X-ray emission (PIXE) has been applied in a variety of research fields including biology and medicine since the pioneering experiment of Johansson and colleagues.[14] PIXE can provide adequate and high sensitivity (0.1–1 ppm) for most elements, good precision (2–10%), simultaneous multielement analysis, and trace analysis.[14–20] In this communication, we first report the results of a PIXE analysis of silk. The detail results will be described in later research article.

EXPERIMENTAL

Materials

Regenerated silk fibroin was obtained as described elsewhere.[3–5] The gummed silk including fibroin and seri-

---

Correspondence to: Liu et al.

Contract grant sponsors: National Key Basic Topics of China; National Science Foundation of China.

Journal of Applied Polymer Science, Vol. 66, 405–408 (1997)
CCC 0021-8995/97/020405-04

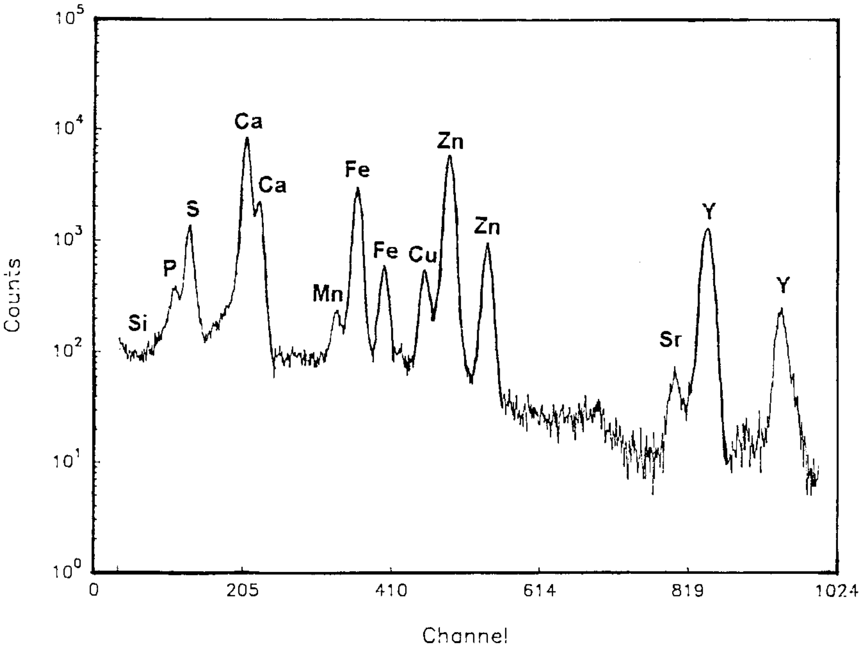

Figure 1 PIXE spectrum of regenerated silk fibroin.

cins was from a *Bombyx mori* cocoon. The wild gummed silk was from the cocoon of wild larvae. These samples were rinsed several times with ion-free water and dried in an oven under the temperature 80°C for 4–5 h, then put into the desiccator overnight and carefully weighed. The weighed samples were then placed in a quartz vial and inserted into a low-temperature oxygen plasma oven for ashing. The temperature reached was at most 120°C, and the total time for reduction to ash was 8 h. The recovery efficiency for all elements of interest was more than 90% as checked by an atomic absorption spectroscopy method. To improve the sensitivity, samples were preconcentrated using a dry-ashing procedure. The residue was then dissolved in a 4 mol/dm^3 HNO_3 solution containing an appropriate amount of yttrium which was previously doped as an internal standard. Finally, the thin target was made by pipetting 10 μL of the resulting solution onto the target backing treated previously. After being dried at room temperature in a clean vessel, the target was ready for PIXE analysis.

Measurements

The PIXE apparatus and experimental methods were described in detail in our previous articles.[21,22] A proton

Table I Element Contents of Samples

Element Symbol	Element Content (ppm) Regenerated Silk Fibroin	Gummed Home Silk	Gummed Wild Silk-1	Gummed Wild Silk-2
Si	93	320	160	203
P	419	449	456	786
S	1498	1282	968	934
Ca	3804	1990	5342	5083
Mn	6	3	177	6
Fe	107	17	23	28
Cu	17	1	5	2
Sr	14	3	11	10
Zn	228	11	30	11

beam of 2.5 MeV was produced from an NEC 9SDH-2 Pelletron tandem accelerator, which was installed in the Nuclear Science Department of Fudan University.

A target chamber made of Lucite was used for a vacuum PIXE analysis. The X-rays were detected by a 28 mm^2 × 5.2 mm ORTEC Si (Li) detector with a 165 eV resolution at 5.9 keV. The detector was placed at 90°C to the incident beam to obtain a larger solid angle. A sheet of 1.2 mg/cm^2 Kapton foil was utilized as a chamber window for outgoing X-rays. A funnel absorber made of 640 μm Mylar foil with a small hole of 0.4 mm in diameter at the center was placed in front of the detector to attenuate the intense flux of light elements X-ray.

The complex PIXE spectra were analyzed with a FORTRAN program AXIL,[23] which is based on a numerical correction method for an accurate peak-shape description and is incorporated into a nonlinear iterative least-squares fitting procedure. This program gives the net area of peak overlap for each element after subtracting the bremsstrahlung background counts. The net areas of the peak, the relative sensitivity factor of the detection system, and an internal standard are used to calculate the elemental concentrations.

RESULTS AND DISCUSSION

The PIXE spectrum of regenerated silk fibroin (Fig. 1) shows that fibroin contains many types of elements including Si, P, S, Ca, Mn, Fe, Cu, Zn, and Sr. The element contents were calculated as the peak area using the FORTRAN program AXIL.[23] The results of other samples are similar to those of regenerated silk fibroin. They also contain Si, P, S, Ca, Mn, Fe, Cu, Zn, and Sr although the relative contents of these elements are different. The results are listed in Table I.

The content of the element calcium is highest in silk containing 10 types of elements. Moreover, the content in the wild silk is about three times that in the home silk and the content in the fibroin of home silk is twice that in the gummed silk, which shows that sericin has almost no calcium element. It will be proved that the element influence depends mostly upon the mechanism of how silk is formed in the larvae and their properties such as their mechanic strength and color.

The content of the element sulfur is higher for both the H-chain and L-chain of protein and is connected by the S—S bridge. The content in the fibroin is almost as much as in the gummed silk, which proves that both the fibroin and the sericin have the S—S bridge.

The element copper has been thought to be the main factor causing the silk's yellow color although its content is not high. The content in the fibroin is much higher than that in the gummed silk. It demonstrates that sericin has a much lower content of the element copper than has the fibroin and that the yellowing of silk is mainly caused by the fibroin becoming yellow. Because the color of wild silk is much yellower than that of the home silk, the content of the element copper in the wild silk is much higher than that in the home silk.

In home silk, the contents of the elements Mn, Fe, Sr, and Zn in the fibroin are much higher than those in the gummed silk, indicating that sericin contains much less of these elements than does the fibroin. Especially, the content of the metal elements Fe and Zn in the fibroin is so much higher. However, the content of the element Si in the fibroin is much lower than in the gummed silk. In other words, the sericin contains much more silicon than does the fibroin. It has not been understood that how these elements influence the properties of the silk. Other work is in study and the other results will be reported in a later article.

This work was supported by the National Key Basic Topics of China and the National Science Foundation of China.

REFERENCES

1. Y. Tohru, K. Wataru, S. Yusuke, Y. Ryohei, and M. Keizo, *Agric. Biol. Chem.,* **52,** 2103 (1988).
2. H. Toshihiro, S. Nakajima, A. Kitamura, K. Mizuguchi, and Y. Suda, *Sen'i Gakkaishi,* **44,** 72 (1988).
3. T. Yu, H. Liu, J. Deng, and Y. Liu, *J. Appl. Polym. Sci.,* **58,** 973 (1995).
4. Y. Liu, H. Liu, J. Qian, J. Deng, and T. Yu, *Anal. Chim. Acta,* **316,** 65 (1995).
5. Y. Liu, H. Liu, J. Qian, J. Deng, and T. Yu, *Electrochim. Acta,* **41,** 77 (1996).
6. H. Yoshimzu and T. Asakura, *J. Appl. Polym. Sci.,* **40,** 1745 (1990).
7. W. S. Muller, *Langmuir,* **9,** 1857 (1993).
8. J. Ishikuro, Y. Mirai, and T. Nakajima, *Sen'i Gakkaishi,* **48,** 682 (1992).
9. D. G. Bae, *Han'guk Chamsa Hakhoechi,* **30,** 105 (1988).
10. T. Asakura, H. Kitaguchi, M. Demura, H. Sakai, M. Kaneko, A. Kurioka, and K. Komatsu, *Nippon Sanshigaku Zasshi,* **60,** 466 (1991).
11. M. Nagara, T. Ueki, Y. Hiragi, H. Tagawa, M. Kataoka, Y. Izumi, Y. Muroga, and Y. Amemiya, *J. Polym. Sci. Part B Polym. Phys.,* **25,** 2567 (1987).
12. S. Zheng, G. Li, W. Yao, and T. Yu, *Appl. Spectrosc.,* **43,** 1269 (1989).
13. T. Yu, S. Zhang, and Z. Shao, *Chem. J. Chin. Univ.,* **17,** 323 (1996).
14. T. B. Johansson, R. A. Kselsson, and S. A. E. Johansson, *Nucl. Instrum. Methods,* **84,** 141 (1970).
15. H. V. Rinsvelt, S. Bauman, J. W. Nelson, and J. W. Winchester, in *Proceedings of the 4th International*

Conference on PIXE, Nucl. Instrum. Methods, 1987, p. B22.
16. R. M. Parr, *Int. Atom. Engery Agency Bull.,* **25,** 7 (1983).
17. T. A. Cahill, *Annu. Rev. Nucl. Part. Sci.,* **30,** 211 (1980).
18. F. Yang, *Atomic Physics,* Shanghai Science and Technology Publishing House, Shanghai, China, 1985.
19. J. Chen, in *Ion Beam Analysis,* F. Yang and G. Zhao, Eds., Fudan University Press, Shanghai, China, 1985.
20. C. Ren, H. Cheng, G. Tang, J. Chen, and H. Yao, *PIXE and SPM,* Atomic Energy Publishing Corp., Beijing, China, 1981.
21. J. Chen, H. Li, C. Ren, G. Tang, X. Wang, F. Yang, and H. Yao, *Nucl. Instrum. Methods,* **168,** 437 (1980).
22. J. Chen, Y. Guo, H. Li, C. Ren, G. Tang, X. Wang, F. Yang, and H. Yao, *Nucl. Instrum. Methods,* **181,** 269 (1981).
23. P. Van Espen, H. Nullens, and Maenhaut, in *Microbeam Analysis,* D. E. Newbury, Ed., San Francisco Press, San Francisco, CA, 1979, p. 265.