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SPHERULITES OF TUSSAH SILK FIBROIN
Structure, thermal properties and growth rates

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Abstract

The crystal structure, thermal properties and growth rates of spherulites of the Tussah silk fibroin, produced upon drying of the silk taken directly from the lumen which is essentially a poly(*L*-alanine) polypeptide, are investigated. Depending on casting conditions, spherulites with either α helical chain conformation or β parallel sheet structure are produced. The growth rates display a strong positive temperature coefficient, with an apparent transition, which however cannot be related with the formation of two different crystal structures at this stage.

Keywords: growth rate, silk, spherulite, structure, thermal property, Tussah silk fibroin

Introduction

The synthesis, storage and spinning of silks continue to raise major challenges when considering the physicochemical processes involved. A major issue is the storage under physiological conditions of a highly concentrated gel of a fibrous protein made of polar amino acid residues. Indeed, the corresponding homopolypeptides or statistical polypeptides of glycine and *L*-alanine are only soluble in strong solvents such as trifluoroacetic acid, dichloroacetic acid, etc. A past research, therefore, concentrated on the stability of the silk lumen, and its conversion to a solid or fiber under natural and artificial conditions. Whereas shearing or stretching of the silk produces fibers, investigation of the solidification process under quiescent conditions can help shed light on the silk solution stability and structure. When the silk solution is left to dry under quiescent conditions, development of spherulitic entities is frequently observed.

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Sobue and Ishikawa [1] were the first to investigate the drying of *Bombyx mori* (the domestic silkworm) silk solutions. *B. mori* silk is made of two proteins, fibroin and sericin. The more crystalline fibroin has a chemical repeat of the type (glycine–*X*), where *X* stands for *L*-alanine and *L*-serine in a 2:1 ratio [2]. When dried, solutions of *B. mori* fibroin as well as of sericin yield spherulites. Furthermore, silk fibroin can be obtained in two different crystal structures: under quiescent conditions, it has an unstable (to mechanical stress) form named Silk I (Shimizu [3] and Kratky and Schauenstein [4]); when the solution is subjected to stress while drying, it has the more conventional β sheet structure, commonly observed in the spun silk thread.

The Tussah or wild silk, produced by *Antheraea pernyi*, has a molecular constitution which is very close to homo-poly(*L*-alanine), as judged from its fiber X-ray diffraction pattern (the inter sheet distance of 0.53 nm is characteristic of sheets lined with methyl side groups in poly(*L*-alanine)) [5]. Contrary to poly(*L*-alanine), it can be cast from the aqueous solution extracted from the mature silkworm gland.

In the present report, we investigate the formation of the spherulitic entities which develop upon drying of Tussah silk solution, determine their crystal structure, measure their growth rates as a function of casting temperature and discuss the analogies and differences with the behavior of the corresponding homopolypeptide, poly-(*L*-alanine).

Experimental

Materials

The liquid solution of Tussah fibroin was extracted from the posterior division of the silk gland of mature silk worms, *Antheraea pernyi*. Crystallization of the solutions was followed by casting the fresh solutions of silk fibroin on glass slides, at predetermined temperatures using thermostatically controlled ovens.

The spherulitic growth was followed in a polarizing microscope equipped with a hot stage. Diffraction patterns were recorded on flat films. A Rigaku Denki X-ray generator was operated at 35 kV and 20 mV, using Ni-filtered CuK_α radiation. Differential scanning calorimetry was performed with a Seiko DSC 6100 instrument. Standard heating rate was 5°C min^{-1}.

Results

Crystalline polymorphism, spherulitic structure and thermal properties of Tussah silk

The crystal structure and morphology of the dried film depend significantly on casting conditions, and more specifically on temperature. When cast at temperatures ranging from room temperature to 45°C and when left in the open air, the dried film does not display any characteristic morphology. Its X-ray diffraction pattern is characterized by a strong reflection at ~0.74 nm^{-1} (Fig. 1a), which is characteristic of the α helical form of poly(*L*-alanine) [6].

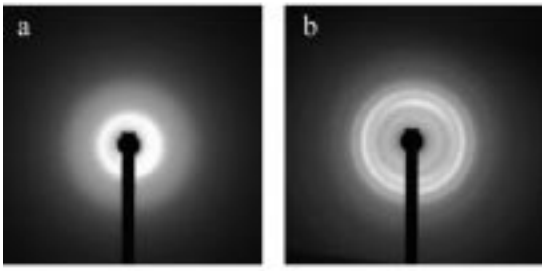

Fig. 1 X-ray diffraction pattern of Tussah silk fibroin a – at 45°C and b – at 105°C

When cast above 45 and up to about 105°C, the crystalline β modification is always produced. X-ray diffraction patterns indeed display the trademark strong reflections (Fig. 1b) at ~0.53 and ~0.45 nm^{-1} which correspond to the intersheet distance and 110 reflection of the pleated sheet structure of poly(*L*-alanine) [7, 8]. However, the crystalline morphology of the film varies significantly whether the film is exposed to air while drying, or is confined between slide and cover-slide.

When the liquid solution is left in open air, no specific crystalline morphology is observed. The drying conditions (evaporation of the solvent) are of course faster, which may explain the absence of well-developed morphology. However, previous investigations from this laboratory have indicated that the crystallization rate of silk fibroin (in this case, of *Bombyx mori*) is significantly enhanced by change of pH in samples due to the intake of carbon dioxide; It is probable that a similar feature is at play in the present case [9].

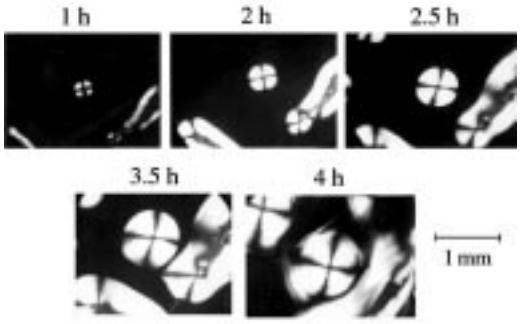

Fig. 2 Spherulites of Tussah silk fibroin as a function of casting time at 45°C

When the liquid solution is maintained between glass slide and cover-slide, large, wellformed spherulites are observed to grow (Fig. 2). These spherulites do not fill the entire field, i.e. do not end up impinging into each other, although their size is unusually large: a radius of up to 0.8 mm has been reached upon growth for 4 h at 50°C. Their optical birefringence, determined with a quartz λ plate, is found to be negative.

The above observations indicate therefore a complex pattern of drying processes. This is not however unexpected, since similar observations have been made

for the silk of *Bombyx mori,* which is also obtained in two different crystal modifications depending on casting conditions: Silk I and β form are observed below and above 45°C, respectively [10, 11]. Furthermore, the possibility to obtain Tussah silk under two crystal modifications makes it possible to investigate the thermal stability of these forms, and more precisely of the α crystal modification. Indeed, it is well established that the crystal form most stable at high temperatures is the β form.

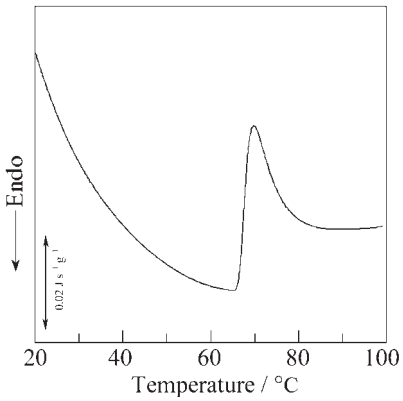

Fig. 3 DSC curve of Tussah silk fibroin

The liquid solutions of Tussah silk in the α modification heated in the DSC display the heating curve shown in Fig. 3. It displays a clear exotherm around 70°C. This peak corresponds to a crystal transformation from the α phase to the β phase, as ascertained by X-ray diffraction. Interestingly, this transition takes place at significantly lower temperatures than for most synthetic polypeptides, where it is observed between 180 and 220 °C [12, 13]. This major difference seems to be due to the small size and local environment of the α phase crystallites in Tussah silk: the crystals are still embedded in a mostly, but not completely dried gel. When heated beyond 100°C, the liquid solutions release water molecules.

Growth rates of β *phase spherulites of Tussah silk*

The development of well formed spherulites of Tussah silk fibroin makes it possible to investigate the variation of growth rates over an exceptionally large range of temperatures, which has few equivalents among biological systems. The growth rates have been determined for silk solutions between glass slide and cover slide at various temperatures. The experimental conditions do not guarantee constant conditions, in particular with respect to the variation of water content. Care has been taken however to measure the growth rates from the central parts, away from the lateral edges of the films. To emphasize these limitations (which we consider however to have relatively minor impact on the results), we prefer to plot the growth rates *vs*. casting temperature rather than crystallization temperature.

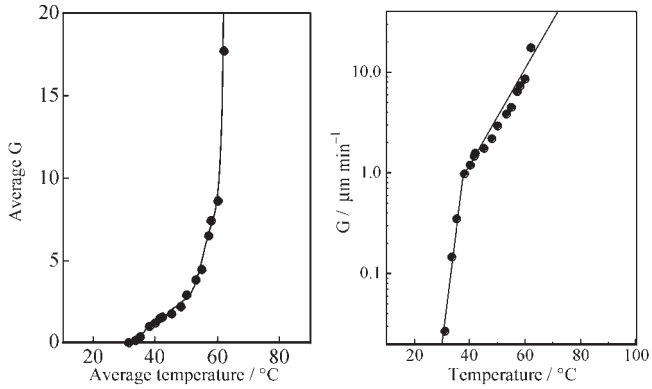

Fig. 4 Growth curve of Tussah silk fibroin spherulites

The growth rates of Tussah silk spherulites were found to be constant with time up to the larger sizes measured, which suggests that local variations of crystallization conditions (e.g. change of solution concentration) were limited. The growth rates are plotted in a linear and semi-logarithmic form as a function of casting temperature in Fig. 4. Most growth rates span one decade, but very slow rates have been measured at room temperature, thus extending the range to nearly three decades. The major feature which emerges from these data is the very strong positive coefficient of the growth rate with temperature, as is frequently observed with crystallizing gels.

Discussion

The above observations on the crystal polymorphism and properties of Tussah silk fibroin are of particular relevance in that they demonstrate significant differences with the behavior of poly(*L*-alanine). As stated in the introduction, the crystalline fraction of Tussah silk is nearly isomorphous with poly(*L*-alanine) in its β modification. Also, the chemical sequence of Tussah displays significant stretches of poly-(*L*-alanine). The differences in stability, transition temperatures, etc. therefore indicate the impact of physiological conditions (i.e. the possible presence of counterions in the silk gel) and the non-crystallizable and more water-soluble sequences of amino acids in maintaining acceptable conditions for crystallization of silk solutions. In particular, the present investigation has indicated the significant difference in α→β transition temperature between Tussah silk fibroin and poly(*L*-alanine).

Such differences in solution stability make it also possible to investigate the behavior of poly(*L*-alanine) (or at least sequences of poly(*L*-alanine)) under conditions which cannot be achieved for the homopolymer: the latter cannot be produced as a water-soluble gel and, to our knowledge, no report on growth of β poly(*L*-alanine) spherulites exists.

Large spherulites of poly(*L*-alanine) were however reported in the past [14]. They were produced by slow casting (at room temperature) of a solution of poly-

(*L*-alanine) in a 50/50 mixture of trifluoroacetic acid/trifluoroethanol – which is far from physiological conditions. The spherulites had radii in the hundred micrometers range, and displayed a regular pattern of concentric rings with ring-to-ring distances of 6 to 9 μm. These rings were (probably wrongly) attributed to 'periodic thickness variations' [14] but are more probably the result of a twisting orientation of radial lamellae, which would in particular account for the apparent variation from positive to negative birefringence within each ring (optical biaxial indicatrix with the intermediate refractive index oriented radially in the twisting lamella). The crystal structure of these spherulites is however neither α nor β: the diffraction pattern is characterized by several diffraction rings with a strong one at ~0.7 nm^{-1}, which does not correspond to any known crystal structure of poly(*L*-alanine). It is probable that this is a solvated phase, possibly stabilized by water bridges in the hydrogen bonds.

The spherulites of Tussah silk examined here, correspond therefore to a different crystalline morphology of poly(*L*-alanine), obtained under conditions which are not accessible to the homopolymer. Work is in progress to further exploit these differences, and to learn more about both systems by comparing their behavior under unorthodox physicochemical conditions.

Reference

1 H. Sobue and H. Ishikawa, Kogyo Kagaku Zasshi, 64 (1961) 1320.
2 D. J. Strydom, J. Haylett and R. H. Stead, Biochem. Biophys. Res. Commum., 79 (1977) 932.
3 M. Shimizu, J. Sericult. Rep., 10 (1941) 477.
4 O. Kratky and E. Schauenstein, Diss. Faraday Soc., 11 (1951) 363.
5 C. H. Bamford, A. Elliott and W. E. Hanby, Synthetic polypeptides, Academic Press, 1956, p. 287.
6 Y. Kondo, K. Hirabayashi, E. Iizuka and Y. Go, Sen-i Gakkaishi, 23 (1967) 311.
7 C. H. Bamford, L. Brown, A. Elliot, W. E. Handy and I. F. Trotter, Nature, 171 (1953) 1149.
8 C. H. Bamford, L. Brown, A. Elliot, W. E. Handy and I. F. Trotter, Nature, 173 (1954) 27.
9 J. Magoshi, Y. Magoshi, M. A. Becker and S. Nakamura, Polymeric materials encyclopedia, 1 (1996) 667.
10 J. Magoshi, N. Kasai and M. Kakudo, Koubunshi Kagaku, 30 (1973) 649.
11 J. Magoshi, Y. Magoshi, M. A. Becker and S. Nakamura, Proc. of the 9th International Wool Textile Research Conference: Protein Chemistry, 1996, p. 323.
12 D. B. Green, F. Happey and B. M. Watson, European Polymer Journal, 6 (1970) 7.
13 J. Brandrup, E. H. Immergut and E. A. Grulke, Ed., Polymer Handbook, 4th Ed., John Wiley & Sons, New York 1999.
14 L. C. Anderson, H. Brumberger and R. H. Marchessault, Nature, 216 (1967) 52.