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DETERMINATION OF THE CRITICAL CONDITIONS FOR FIBER FORMATION BY THE FIBROIN OF NATURAL SILK BY POLARIZATION-OPTICAL METHODS

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The processes of fiber formation by the fibroin of natural silk have been studied by the methods of birefringence in a longitudinal hydrodynamic field and optical rotatory dispersion. The experiments were performed with the direct drawing of a fiber from the secretion of the silk glands of the silkworm Bombyx mori. *The position of appearance of a longitudinal hydrodynamic field within the gland has been detected and experimental results have been obtained which permit an evaluation of the critical condition for the $\alpha-\beta$ structural transition of the fibroin chains.*

The molecules of natural silk fibroin are characterized by the presence of sections with an α-helical conformation in the secretion of the silk gland of the silkworm and a β-structure in the fiber [1]. Frenkel' [2] has given a scheme of the formation of the fiber as the result of an $\alpha-\beta$ transition during the longitudinal flow of a fibroin solution and has reported that in the realization of this process the chains are oriented along the streamlines, and the α-helical sections break down and slip so as to achieve the necessary number of stabilizing intermolecular hydrogen bonds in the β-structure. However, because of the complexity of the performance of experimental investigations of the processing taking place in the secretion, the position of appearance of the longitudinal field was not found and its definitive function in fiber formation was not established, and, consequently, it was impossible to answer the question of the critical conditions for the $\alpha-\beta$ structural transition of fibroin.

The secretion of the silkworm is produced in the silk gland, which consists of two long, thin, flexible, transparent tubular sheaths each having three divisions and joining together in a discharge papilla. Fibroin, synthesized in the fibroin division in the form of a viscous aqueous solution, passes into a reservoir where it is mixed with a bioadhesive — sericin — and, in paired excretory ducts, with a fatty wax [3]. The silk gland is extracted during the dissection of the body of the silkworm. In this process, fiber formation can be artificially prolonged by "drawing" the secretion. This makes it possible to perform optical polarization investigations by, in particular, the method of studying the orientation-deformation behavior of macromolecules with different rigidities, based on the measurement of the birefringence effect appearing during the longitudinal flow of polymers [4]. We have used this method previously for studying structural features of the unfolding of the chains of a fibroin fiber in salt-containing solutions [5].

Supplementing this, to reveal an α-helical or β-structural conformation of the chains it is desirable to use measurements of the optical activity of the protein [6]. The task of the present work was to determine the critical conditions for the formation of natural silk fiber on the basis of the $\alpha-\beta$ transition of the fibroin chains.

Details of the concentration and molecular characteristics of the components of the secretion obtained from various divisions of the silk gland are given in Table 1 in comparison with the analogous information for the cocoon fiber. The secretion obtained from all the divisions of the gland is a molecularly disperse system sensitive to variations in the temperature and in the concentrations of the components. The values of the weight fraction of the components of the secretion that we have determined agree well with literature figures [1]. The slight fall in the molecular masses of the fibroin in the secretion–fiber transition is possibly connected with a partial degradation of the chains at the stage of preliminary purification.

A study of the optical polarization properties of the secretion over the whole length of the gland showed a marked increase in the birefringence effect at the junction of the reservoir and the paired excretory ducts and a monotonic rise in it

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TABLE 1. Concentration and Molecular-Mass Characteristics of the Secretion of the Silkworm Silk Gland and of Silk Fibers

	Weight proportion of the components of the secretion				[η], cm³/g		$\overline{M}_n$
	fibroin	sericin	fatty wax	water	fibroin	sericin	fibroin
Fibroin division	0.351			0.649	145		374000
Reservoir	0.286	0.132		0.574	142	46	368000
Paired excretory ducts	0.512	0.130	0.008	0.350	140	44	
Fiber	0.743	0.245	0.012		131	40	345000

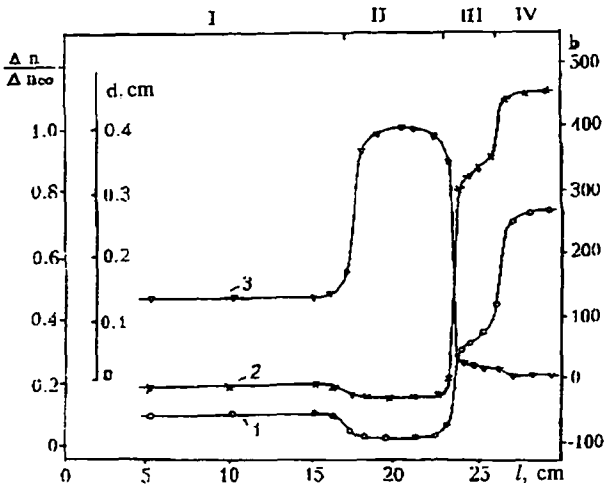

Fig. 1. Change in the values of the parameters $\Delta n/\Delta n\infty$ (*1*), b_0 (*2*), and d (*3*) in various sections of the silk gland during the secretion–fiber transition.

within this division up to the formation of the fiber. At the junction of the reservoir and the paired excretory ducts the diameter of the reservoir decreases conewise from 0.5 to 0.02 cm and forms a profile of the rates of flow that are characteristic for a conoidal aperture [10]. The birefringence effect arising on this has the form of a thin anisotropic "cord" with a diameter not exceeding 2/3 of the diameter of the paired excretory duct. According to previous work [5], the appearance of such a cord confirms the existence of a longitudinal hydrodynamic field.

The fibroin chains have right-handed α-helices and, according to the literature [9], the values of the constant b_0 that we have determined show by their sign and magnitude a predominance of α-helical sections in the secretion of the fibroin division and the reservoir, and of β-structure in the secretion of the paired excretory duct division and in the fiber.

Figure 1 shows the dependences of $\Delta n/\Delta n\infty$ (*1*), b_0 (*2*), and d (*3*) on l during the drawing of the fiber at a rate V = 1 cm/sec, close to the average rate of spinning a cocoon. It can be seen that the reduced birefringence had the minimum value in the reservoir ($\Delta n/\Delta n\infty$ = 0.01) and the maximum value in the fiber ($\Delta n/\Delta n\infty$ = 0.7). If we start from the fact that fibroin has an α-structural conformation in the secretion of the reservoir and a β-form in the fiber, the realized condition leading to a sharp increase in $\Delta n/\Delta n\infty$ at the junction of the reservoir and the paired excretory duct must be the beginning of the $\alpha-\beta$ structural transition of the chains. Whether this condition is critical for fiber formation by fibroin was analyzed in the following way.

The secretion of the reservoir is an aqueous solution of fibroin mixed with sericin. Preliminary experiments conducted as described in [11] have shown a thermodynamic incompatibility of these proteins in common solvents, but under certain conditions it is possible to obtain kinetically stable mixtures. In fact, in all the solution of the mixtures that have been studied a separation of protein phases takes place with changes in the temperature, in the pH of the medium, and in the concentrations of the components, and under the influence of external mechanical forces. It likely that in the reservoir secretion the condition characteristic for a kinetic mixture is realized, since the fiber formed as a result of mechanical drawing is a two-phase system consisting of fibroin — the base of the fiber — and sericin — its sheath.

An investigation of the orientation-deformation behavior of solutions of fibroin and sericin, and also mixture of them, in a longitudinal hydrodynamic field showed the absence of a contribution of sericin to the optically anisotropic phase represented in the form of a thin cord [5]. It is known that a similar cord is formed by a system of oriented unfolded macromolecules along the longitudinal flow of solutions. Sericin, unlike fibroin, does not suffer an appreciable deformation action from a longitudinal field because of its low molecular mass. Consequently, the appearing optical anisotropy of the phase characterizes the conformational states of the fibroin chains.

In the anisotropic phase of the secretion of the paired excretory ducts, the concentration of fibroin increases and the anisotropic cord is converted into a physical gel. This is confirmed by the facts that, in the first place, a quantitative analysis of the components of the secretion showed a high concentration of fibroin in the cord; in the second place, optical rotatory dispersion measurements showed the presence of β-structural sections in the chains in the cord (Fig. 1, curve 2), i.e., the formation of seeds of the semicrystalline structure of the fiber; and, in the third place, the anisotropic cord retains the structure it has attained in the excretory ducts after the cessation of the drawing of the fiber. The facts mentioned permit the assumption that in the initial section of the paired excretory ducts the α-helical sections oriented along the flow of the chains break down and an energetically favorable (apparently both thermodynamically and kinetically) situation arises for the spontaneous formation of the structure of a physical gel. Since, now, the process of drawing continues, the gel-like cord is slightly deformed. This is shown by the gradual decrease in the diameter of the cord as the secretion passes through this division (Fig. 1, curve *3*).

With the formation of the physical gel structure, difficulties could arise in the movement of the secretion in the paired excretory ducts; however, this does not take place since fats playing the part of lubricants are elaborated in the walls of this section.

Thus, the results of the investigations performed show that a longitudinal hydrodynamic field arises within the silk gland at the junction of the reservoir and paired excretory duct divisions, and also that at the critical velocity gradient G_{cr} = 1000 sec^{-1} a transition of the α-helix into the β-form and a slipping of the macromolecules relative to one another in the gel-like medium of the secretion take place.

EXPERIMENTAL

We used the silk gland of a silkworm at the cocoon-spinning age. The proportions by weight of the components of the secretion and of the fiber were determined as described in [7].

The intrinsic viscosities $[\eta]$ of the fibroin samples obtained were determined in a 2.5 M lithium chloride–dimethylformamide (LiCl–DMFA) solution at 25°C in an Ubbelohde viscometer. The molecular mass of the fibroin was calculated from the equation $[\eta] = 1.23 \times 10^{-3} M^{0.91}$ ml/g [8].

To study the structural states of the fibroin during the secretion–fibroin transition, we used a combination of optical methods of measuring polarization effects of polymer solutions — birefringence in a longitudinal hydrodynamic field [5, 9] and optical rotatory dispersion [10]. The silk gland was placed in a Frank-Keller [9] apparatus at the position of the hydrodynamic cell and the fiber-forming process was continued, the secretion being drawn onto a special rotating drum. In this way it was possible to determine the optical polarization properties of the fibroin over the whole length (l) of the gland and the fiber.

The degree of unfolding of the chains was determined from the reduced birefringence $\Delta n/\Delta n\infty$, where Δn is the measured and $\Delta n\infty$ the maximum possible birefringence. The procedures for measuring and calculating these parameters are described in [5].

The specific optical rotations $[\alpha]_\lambda^T$ were measured at different wavelengths (450 and 600 nm) and graphs were plotted of the relation

$$3\,M_a[\alpha]_\lambda^T / 100(n^2-2) / (\lambda_0^2 / \lambda^2-\lambda_0^2)) = b_0\lambda_0^2 / (\lambda^2-\lambda_0^2) + a_0.$$

where $M_a = 125$ is the mean molecular mass of an amino acid residue in proteins; $\lambda_0 = 212$ nm is the constant of rotatory dispersion (both taken from [6]); n is the refractive index of the solvent; and a_0 and b_0 are constants characterizing the structural-conformational state of the chains.

Following [9], we judged the existence of α-helical, β-structural, and coil-like states of fibroin from the sign and magnitude of the constant b_0.

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