SOFT MATTER

STRUCTURAL CHANGES IN A FIBROIN FIBER INDUCED BY AN ALKALINE SOLUTION

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A comparative study of acoustic properties of fibroin fibers treated and untreated in an alkaline solution has been carried out. The dependence of the sound velocity on the static tensile stress in a fiber is determined and is used to calculate the elastic moduli of the second and third orders. A conclusion is made that the treatment of a fibroin fiber in the alkaline solution modifies its structure; in particular, in the course of the treatment, there emerge defects (voids) in unordered areas, and the chains become oriented along the fiber axis in those areas.

1. Introduction

It is known [1, 2] that fibroin fibers–silk – are produced by silkworms – e.g., *Bombyx mori*. The secretion of their glands contains the aqueous solution of fibroin protein mixed with another protein, sericine, which plays the role of lubricator and glue. A silkworm, when having pasted a droplet of secretion to any subject around, emits the content of its gland in the form of a liquid jet, which, solidifying in air, transforms into a thread. Threads are spun by the silkworm into a cocoon. The integrity of cocoons is preserved by sericine, which glues the threads (fibers) together. In the industry, fibers are cleaned from sericine by treating cocoons in the aqueous solution of NaOH. This work aimed at detecting the structural modifications in a fibroin fiber induced by this solution.

It is well known that the phrase "The system is structured" is used, when the system can be imagined as a combination of individual parts, which are called "structural elements". Generally speaking, the system can be divided into components on the basis of the sizes of chosen structural elements. In this case, the different struc-

tural levels of systems are implied. According to the modern viewpoint [3] on the structure of fibroin fibers, one may say about the existence of four structural levels in them:

1) macroscopic (the structural element size is comparable with the fiber diameter),
2) cellular (the structural element is a cell),
3) fibrillar (the structural element is a fibril),
4) atomic.

In this work, the structure is analyzed at the fibrillar level. The corresponding structural model is depicted in Fig. 1.

In this case, the structural element is a fibril. The latter is a cylindrical cluster of chains. The axes of fibers are parallel to that of the cylinder. The cylinder diameter is 7.5–8 nm. Fibrils are separated from one another by unordered interfibrillar areas. Ordered and unordered intrafibrillar sections alternate along a
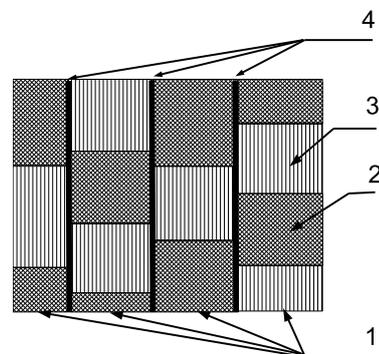
Fig. 1. Fibril structure at the fibrillar level: fibrils (*1*), intrafibrillar unordered areas (*2*), intrafibrillar ordered areas (*3*), interfibrillar unordered areas (*4*)
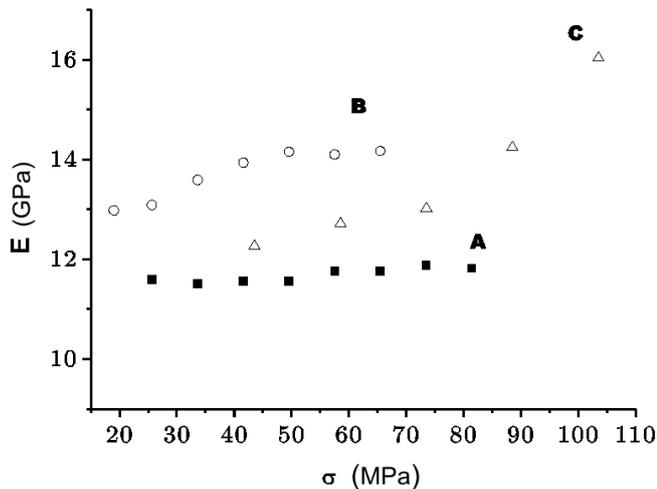
Fig. 2. Dependences of the elastic modulus on the loading, $E(\varepsilon)$, for various specimens: fibroin fiber obtained immediately from silkworm's glands (A), fibroin fiber obtained from silkworm's glands and treated in an alkaline solution (B), and fibroin fiber obtained from a silkworm cocoon (C)

fibril. The ordered area has a crystalline lattice formed by fibroin chains, the axes of which are parallel to the fibril axis. The configuration of those chains is a plane zigzag.

Now, when the model of fiber structure, which will be dealt with in this work, is described, the aim of the work can be formulated more specifically. Thus, we intend to determine, at the fibrillar level, the structural modifications in a fiber that occur owing to the action of an alkaline solution.

2. Experimental Technique

The structure of a fibroin fiber is studied on an acoustic interferometer. We measured the sound velocity c in fibroin fibers subjected to the static tensile stress σ. This method is traditionally used while researching the acoustic properties of fibers. Its description can be found, e.g., in work [4] and elsewhere. Frankly speaking, the aim of our experiment consisted in the determination of the dependence of the fiber elastic modulus E on the stress σ. It was the character of this dependence that served as the basis for our conclusions concerning the structure of a fibroin fiber.

The elastic modulus is known [5] to be calculated by the formula

$$E = \rho c^2, \tag{1}$$

where ρ is the density. Therefore, the experimentally measured sound velocities c's can be easily recalculated

into the values of elastic modulus E. It is also known [5] that, at temperatures within the interval of about 300–400 K, relaxation processes in polymers become "defrosted". They induce some changes in the structure, which combine with the structural modifications emerging owing to the action of an alkaline solution. In this work, out task consisted in determining only those structural modifications that arose owing to the fiber treatment in an alkaline solution. Therefore, the influence of the relaxation process on the structure of a fibroin fiber had to be excluded from consideration. With this purpose in view, the measurements were carried out at the temperature $T = 153$ K.

3. Specimen Fabrication

It was obvious from the very beginning that, for the formulated problem to be solved, the experimental data obtained for treated fibers and fibers untreated in an alkaline solution should be compared. However, how can we obtain raw fiber specimens? A silkworm produces a cocoon, in which fibers are glued together by sericine. To obtain a fiber from a cocoon for the study, we have to treat the cocoon – and, hence, the fiber – with an alkaline solution.

Thus, the cocoon turned out a stumbling-block for our researches. Therefore, it became clear that raw fibers could be obtained only by changing the "work" conditions for the silkworm, not allowing it to spin a cocoon. We have tackled this problem by pulling out a secretion jet immediately from silkworm's glands. The fibers obtained in such a way will be referred to as raw ones. This group of specimens will be denoted below by the letter A.

Two more groups of specimens, B and C, were also studied. Specimens B were obtained from specimens A by treating them in an alkaline solution. Specimens C comprise silk threads produced following the traditional technology, namely, by treating cocoons in an alkaline solution.

4. Experimental Results and Their Discussion

For the majority of biological materials, the relation between the stress σ and the strain ε is known to be non-linear. As a result, the elastic modulus E turns out dependent on the stress σ. This fact is confirmed by our experimental data. In Fig. 2, the dependences of the elastic modulus on the static stress calculated by formula (1) are plotted, with the corresponding density value being taken from the literature [6]. The figure

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testifies that the plotted dependence can be considered linear and satisfying the formula

$$E = E_0 + B\sigma. \tag{2}$$

Since $\sigma = E_0\varepsilon$ [5], where ε is the tensile strain, Eq. (2) can be rewritten in the form

$$E = E_0 + E_1\varepsilon, \tag{3}$$

where the notation

$$E_1 = BE_0 \tag{4}$$

is used. Expressions (2) and (3) demonstrate that, from the viewpoint of rheology, fibroin is classed to nonlinear elastic media (for the attributes of this class, see, e.g., work [7]). The linear deformation properties of such media are conventionally characterized by the elastic modulus of the second order, E_0, and the nonlinear ones by the modulus of the third order, E_1. In this work, we tried to make conclusions concerning the structure of specimens at the fibril level on the basis of values obtained for the elastic moduli E_0 and E_1. It is clear that this task can be solved only provided that the contribution given by that or another structural area to the modulus is known.

Let E_F denote the elastic modulus of the fibril as a whole; E_C, E_P, and E_S stand for the elastic moduli in the ordered, interfibrillar (unordered), and intrafibrillar, respectively, areas; and n_C, n_P, and n_S mean the corresponding volume fractions of the areas. We may assert *a priori* that the elastic modulus for the interfibrillar unordered area, where – by definition – the degree of chain orientation is insignificant and is substantially smaller than that for a fibril, which contains ordered areas with chains aligned along the fibril axis, i.e.

$$E_p \ll E_F. \tag{5}$$

It is generally agreed [8] that the unordered areas between fibrils arise as transient layers. From such a definition of the transient layer, it follows that the layer thickness should be substantially narrower that the size of the adjacent area. In our case, this means that the inequality

$$n_p \ll n_C + n_S \tag{6}$$

should be obeyed. The smallness of the parameters $\alpha = \frac{E_P}{E_F}$ and $\beta = \frac{n_P}{n_C+n_S}$ allows the elastic modulus E to be expanded in a series

$$E = E^0 + E^1 + \ldots, \tag{7}$$

where E^0, E^1, and so forth are the terms of the zeroth, first, and higher orders of smallness in the parameters α and β. In what follows, we confine the consideration to the zero-order approximation, by writing down the expression

$$E \approx E^0. \tag{8}$$

The zero-order approximation in the parameters α and β means that the followings equalities are considered to be valid:

$$n_P \approx 0, \tag{9}$$

$$E_P \approx 0. \tag{10}$$

Hence, in this approximation, the presence of interfibrillar unordered areas in the structure is ignored. In other words, in the zeroth approximation of the structural model, the interaction between fibrils is absent so that fibrils deform independently of one another under the action of an external force. Provided such assumptions, the whole volume of the fiber turns out to be occupied by fibrils and, respectively, the following approximate equality proves to be valid for the fibril elastic modulus E:

$$E \approx E_F. \tag{11}$$

Every fibril is a series of sequentially connected ordered and unordered areas. The series connection means [5] that the fibril compliance $\frac{1}{E_F}$ is a sum of compliances for separate areas, i.e.

$$\frac{1}{E_F} = \frac{n_C}{E_C} + \frac{n_S}{E_S}. \tag{12}$$

According to Eq. (9),

$$n_C + n_S = 1. \tag{13}$$

Therefore, for the fiber elastic modulus, we have the formula

$$E = \frac{E_S E_C}{(1 - n_C)E_C + n_C E_S}. \tag{14}$$

Writing down the expressions

$$E_C = E_0 + E_{0C}\varepsilon, \tag{15}$$

$$E_S = E_0 + E_{0S}\varepsilon, \tag{16}$$

let us introduce the elastic moduli of the second and third orders for ordered, E_{0C} and E_{1C}, and unordered, E_{0S} and E_{1S}, areas. The dense packing of links in the ordered areas creates considerable obstacles for the development of nonlinear deformations in them, in contrast to unordered areas, where the presence of a free volume and, hence, a lower intensity of the interaction between chains promote the emergence of nonlinear deformations. These circumstances allow us to assert that the nonlinearity of deformations, which was observed in experiment, is introduced exclusively by unordered areas, i.e.

$$E_{1C} = 0. \tag{17}$$

Substituting equalities (3) and (15)–(17) into formula (14), expanding the right- and left-hand sides of the obtained equation into a series in ε, and confining it to the first-order terms, we obtain

$$E_0 = \frac{E_{0S}E_{0C}}{(1 - n_C)E_{0C} + n_C E_{0S}}, \tag{18}$$

$$E_1 = E_{1S} \frac{E_{0C}}{(1 - n_C)E_{0C} + n_C E_{0S}} \times$$

$$\times \left(1 - \frac{n_C E_{0S}}{(1 - n_C)E_{0C} + n_C E_{0S}} \right). \tag{19}$$

Formulas (18) and (19) enable us, on the basis of experimental values obtained for the fiber moduli E_0 and E_1, to determine the moduli E_{0S} and E_{1S} for unordered areas, provided that the quantities n_C and E_C are known. We took the value $E_C = 23$ GPa from work [9], where it was determined in the framework of the radiographic method by analyzing the reflex displacements under the action of an external force.

The concentration n_C can be calculated on the basis of the following reasoning. It is known [1] that the primary structure of fibroin chains can contain 18 amino acid residues. It is adopted [10] that only four of them–Gli, Ala, Ser, and Tir–enter into the content of chains that fill the ordered areas. Proceeding from this statement, we consider the volume fraction n_C as the relative volume occupied by four mentioned amino acid residues. Using the data of work [10] for the relative volume of amino acid residues, we obtain $n_C = 0.53$. The corresponding values of moduli E_{0S} and E_{1S} calculated according to formulas (18) and (19) are listed in Table.

Let us analyze the tabulated data. First, we consider the quantity E_{0S}, the elastic modulus of the second order

for unordered areas. One can see that the corresponding value for specimens C is substantially smaller than its counterpart for specimens A. A reduction of the elastic modulus testifies, as a rule, to the growth of structure imperfection. Therefore, we may assert that the treatment of fibroin fibers in an alkaline solution increases the number of defects in unordered areas.

The following speculations can be made concerning the origin of those defects. When a fibroin fiber is treated in the alkaline solution, NaOH molecules together with water molecules penetrate into unordered areas and fill the space between fibroin chains. After those molecules having been removed, the fiber structure contains voids. In our opinion, these voids are defects that stimulate the decrease of the modulus E_{0S} after the fiber having been treated in an alkaline solution.

This conclusion seems to contradict the circumstance that the modulus E_{0S} for specimens of type B is larger than the corresponding value for specimens A. Specimens B were fabricated by treating specimens A in an alkaline solution. However, the difference between the E_{0S}-values for specimens A and B practically falls within the error limits resulting from both the experimental measurements and the approximations made while calculating E_{0S}. Therefore, we may assume that the tendency is preserved in this case; namely, after the specimens having been treated in an alkaline solution, the structure in their unordered areas becomes also defect. However, the corresponding structural variations remain in the error limits.

Concerning the elastic moduli of the third order, E_{1S} and E_{1C}, the main experimental fact, in our opinion, consists in that they are positive for all three specimens. The third-order elastic moduli for fibers were studied in work [4], and the positive sign of the third-order modulus was shown to be a result of noncentral forces. In turn, the prevalence of the forces of this type is a consequence of the chain orientation along the fiber axis. Therefore, on the basis of our result, we may assert that the fibroin chains in unordered areas are mainly oriented along the fiber axis in all three specimens.

The unordered areas contain chain sections that cannot form a lattice. In the ordered areas, the fibroin

T a b l e. Deformation parameters of unordered areas in fibroin fibers

Fiber type	Second-order modulus, GPa	Third-order modulus, GPa
A	7.22	0.06
B	8.29	0.33
C	4.52	1.31

chains are known to have the shape of a plane zigzag [11]. The fact of the chain orientation in the unordered areas revealed by us testifies that the chains in those areas have a geometry close to the plane zigzag. The configuration of protein chains can be regarded [12] as a sequence of virtual bonds that connect carbon atoms not entering the peptide groups. Accordingly, the deviation of a peptide chain configuration from the plane zigzag can be described in terms of the rotation angles of virtual bonds with respect to one another (in the plane zigzag, all virtual bonds lie in-plane). Therefore, an impossibility for certain amino acid residues to arrange themselves in the zigzag plane – as such residues, glycine, alanine, serine, tyrosine, asparagine, arginine, histadine, glutamine, lysine, valine, leucine, phenylalanine, proline, threonine, methionine, cysteine, tryptophan, and isoleucine are recognized – results in a rotation of the corresponding virtual bond or, equivalently, in a deviation of this bond from the zigzag plane.

The elastic modulus of the third order, provided that it has a positive value, can be considered as a measure for the contribution made by noncentral forces to the total stress in a specimen. Therefore, proceeding from the tabulated data, we may assert that the treatment of fibroin fibers in an alkaline solution increases the contribution given to the stress by noncentral forces. The noncentral character of forces, in turn, is a consequence of the chain orientation along a fiber.

The revealed fact that the third-order elastic modulus increases, if the fiber has been treated in the alkaline solution, testifies that this treatment enhances the orientation of chains along the fiber axis in the unordered areas. On the basis of this reasoning, it is possible, in our opinion, to assert that the deformation of unordered areas under the action of an alkaline solution is governed by two factors. On the one hand, the treatment in an alkaline solution increases the imperfection in the unordered areas, which leads to a reduction of the second-order modulus. However, simultaneously with an increase in the number of defects, the chains become aligned so that the rotation angles for virtual bonds approach the values that correspond to the arrangement of those bonds in the zigzag plane. All the speculations given above are illustrated in Fig. 3. In the figure, the chains are exhibited in the form of broken lines, with every piece corresponding to a definite virtual bond. Figure 3,a illustrates the structure of an untreated fiber. Here, the slopes of those pieces with respect to the fiber axis are larger than the slopes of analogous
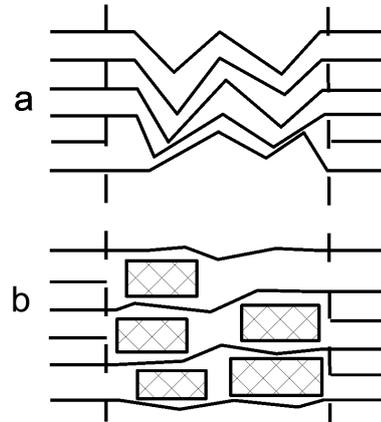
Fig. 3. Models of unordered area structure before (a) and after (b) the fiber treatment in an alkaline solution

pieces shown in Fig. 3b that illustrates a treated fiber. In addition, Fig. 3b, shows voids denoted as hatched areas.

5. Conclusions

To summarize, the treatment of fibroin fibers in an alkaline solution leads to the following structural changes:

1) in the unordered areas, there emerge defects, namely, voids between fibroin chains;

2) in the unordered areas, chains, whose virtual bonds substantially deviate from the fiber axis in the untreated state, become oriented along it, which manifests itself in that the slope of virtual bonds with respect to the fiber axis decreases.

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СТРУКТУРНІ ЗМІНИ У ФІБРОЇНОВОМУ ВОЛОКНІ ПІД ДІЄЮ ЛУЖНОГО РОЗЧИНУ

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Р е з ю м е

Проведено порівняльне дослідження акустичних властивостей фіброїнових волокон, оброблених і необроблених лужним розчином. Отримано залежності швидкості звуку від статичного напруження, розтягуючого волокно. За цією залежністю розраховано значення модулів пружності другого та третього порядків. На основі одержаних значень зроблено висновки про зміни структури, що відбуваються у фіброїновому волокні під час обробки його лужним розчином, а саме: встановлено, що обробка приводить до появи дефектів типу порожнин у невпорядкованих областях та орієнтації ланцюгів в цих областях уздовж осі волокна.