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PROPERTIES OF CONCENTRATED SOLUTIONS OF FIBROIN AND ITS DERIVATIVES

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Rheological properties of concentrated solutions of fibroin from natural silk and its chemically and physically modified derivatives were studied. The structure and properties of these solutions, which have characteristically anomalous viscous flow, are determined by the degree and type of modification.

Key words: fibroin, structure, concentrated solutions, effective viscosity.

Macromolecules of fibrillar (fibrous) proteins like fibroin have porous-ball conformations in aqueous solution. These are formed by complicated constructs that combine in concentrated solutions into complex supramolecular associates [1-4]. Destruction of the regular structure of the macromolecular fibroin chain during chemical and physical modification, e.g., hydrolysis and mechanical destruction of polymer chains, decreases the molecular weight, changes and decreases the strength of intermolecular interactions, and changes the structure of concentrated solutions. This is reflected primarily in the viscosity. Therefore, the study of the rheological properties of concentrated solutions of fibroin and its derivatives under various flow conditions provides important information about the macromolecular structure.

Silk fibroin is a protein of natural origin with β-structure. The structural layers in silk thread are bonded to each other only through Van-der-Waals interactions [2]. The polypeptide chains in silk fibroin are situated antiparallel. Silk fibroin undergoes slight swelling in an aqueous solution of sodium thiocyanate at 8-19 mass % concentrations. Fibroin swells significantly at NaSCN concentrations >30 mass % and partially dissolves at room temperature. Silk completely dissolves at 46.5 mass % NaSCN due to peculiarities in the interaction of water and NaSCN with fibroin. In the initial stages, fibroin absorbs water and NaSCN mainly in amorphous regions of the polymer. The dissolution of silk fibroin in the studied medium (46.50% NaSCN, 24.975% acetic acid, and 28.525% water) is accompanied not only by peptization of the macromolecules due to cleavage of H-bonds between β-chains but also by destruction of fibroin at peptide bonds and conformational changes of the polymer chains because the mobility of the macromolecules in solution increases sharply. Transitions such as $\beta \rightarrow \alpha$, $\alpha \rightarrow \beta$, $\alpha \rightarrow$ball, and ball→spiral can be observed. It has been found experimentally that the viscosity of the studied fibroin solutions in this solvent remains constant with time. The molecular weight is $M_w = 139{,}000$ [5]. We believe that fibroin macromolecules in the studied aqueous salt solutions undergo a series of conformational changes that produce the porous-ball conformation in which the majority of hydrophilic polar groups of amino acids are externalized (Table 1).

Figure 1 shows flow curves of solutions of fibroin and its derivatives in the complex solvent (46.50% NaSCN, 24.975% acetic acid, and 28.525% water) plotted as functions of temperature. The curves characterize non-Newtonian flow of polymers of various nature [3].

The structure of concentrated solutions of fibroin in these aqueous NaSCN and acetic acid systems is apparently modeled by a labile structural network formed from fluxional associates. The strength of the intermolecular interactions in these are determined mainly by specific interactions between functional groups of tyrosine and serine that are located on the outside of the fibroin porous-ball. The structure is also due to specific intramolecular H-bonds and other interactions in the high-molecular-weight matrix. Protein molecules are partially cleaved upon partial acid hydrolysis of fibroin silk in HCl (4 N) at 95°C for 1 h. The hydrolysis mechanism probably includes substitution of the protonated peptide N atom by carboxyl anion [2].

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TABLE 1. Amino-Acid Composition of Intact and Hydrolyzed Fibroin

Amino acid	Amino-acid content, g/100 g protein	
	intact fibroin	hydrolyzed fibroin
Lys	0.51	0.51
His	0.30	0.11
Arg	1.00	0.99
Asp	2.43	2.38
Thr	1.25	1.245
Ser	13.18	13.01
Glu	2.09	2.04
Pro	0.53	0.53
Gly	33.34	29.92
Ala	26.61	24.08
Val	2.62	2.62
Ile	0.87	0.75
Leu	0.67	0.59
Tyr	11.42	11.35
Phe	1.2	1.16

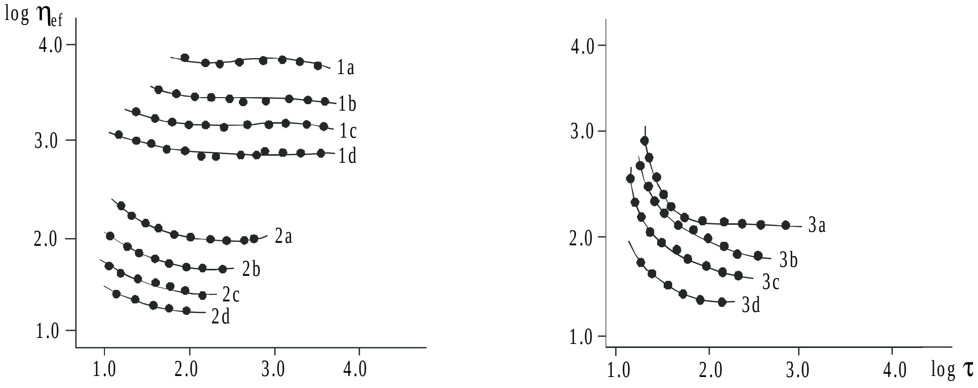

Fig. 1. Flow curves of concentrated (15%) solutions of starting fibroin (1) and mechanically hydrolyzed fibroin of natural silk (2) and hydrolyzed fibroin (3) at various temperatures (°C): 25 (a), 40 (b), 55 (c), 70 (d).

The amino acids are known to form the sequence X-Gly-Ala-Gly-Ala-Gly-, where X is serine or any other amino acid, in the largest peptide fragment of silk fibroin (*Bombyx mori*) after partial cleavage of protein. Experimental results indicate that cleavage of the polypeptide at peptide bonds under these conditions occurs specifically and regularly to yield 36% of fragments up to M_w 64,700. The most stable peptide bond under the acidic conditions occurred for His and then Ile, Leu, Gly, etc. (Table 1).

The principal reason for the viscosity anomaly of concentrated solutions of natural and synthetic polymers is the progressive destruction of the structural network formed by fluxional associates as the shear stress increases [1]. In addition to this, the deformation and orientation of polymer chains and their associates along the flow by the applied shear stress can affect the viscosity anomaly of concentrated solutions of polymers. The role of these factors is explained by the nature and concentration of the polymer, which determine the ability to form interchain contacts in solution. The packing of fibroin macromolecules into associates and the contacts of associates with each other are determined by the primary chemical structure, the structural configuration of the macromolecules acquired in the solvent used, the balance of interactions of functional groups with each other, the solvent, etc. Modification of fibroin has a substantial effect on the destruction process during flow of concentrated solutions [4, 6, 7].

Solutions of starting fibroin have the maximum effective viscosity (up to 7000 mPa·s). According to the rheograms obtained, the start of extensive disintegration of the solution structure is characterized by high shear stresses and is shifted to values exceeding $1\cdot10^3$ Pa. Effective viscosities in the studied range of τ change insignificantly. This is characteristic of systems with elevated chain aggregation due to strong intermolecular interactions and high molecular weight. This is explained by the formation of intermolecular associates of fibroin macromolecules.

The decreased values of τ_{cr} (<10 Pa) at which extensive destruction of solutions begins for hydrolyzed fibroin is apparently due to a sharp drop in the molecular weight, the cleavage of peptide bonds, and changes in the primary structural configuration, in general, and the three-dimensional molecular conformation. This disrupts and weakens the evenness of the network of intermolecular contacts. As a result, the effective viscosities and stresses of extensive structural destruction significantly decrease. The fact that the lowest Newtonian viscosity is achieved is consistent with this [1]. This effect to a large extent is characteristic of solutions of polymeric systems with low molecular weights and weak intermolecular contacts.

Further mechanical treatment of hydrolyzed fibroin at shear stresses of 600 Pa leads to some physical destruction of molecular chains that is accompanied by a noticeable decrease of effective viscosities of the solutions (by almost 100 times) and τ_{cr} (Fig. 1).

The viscosity of the studied solutions as a function of temperature in the range 25-70°C is described by the Arrhenius—Frenkel equation [8] $\eta = A\cdot e^{\Delta E/RT}$, where ΔE is the activation energy of viscous flow. The apparent activation energy of viscous flow is found from the slope of a curve in coordinates of log η and 1/T. This follows from the equation $\log\eta = \log A + \Delta E/2.303RT$. The apparent activation energies of viscous flow ΔE, which are a measure of the extent of intermolecular interactions of the macromolecules in solution, are 40.21 kJ/mol for starting fibroin; 34.47, for hydrolyzed fibroin; and 30.64, for hydrolyzed and mechanically treated (at τ = 10 Pa) fibroin. This phenomenon can be explained by hypothesizing the existence in fibroin solutions of stronger types of structural constructs consisting of a fluxional network and determined by the primary configuration of the protein macromolecules. The formation of stronger structural units in solution has a significant effect on the increase of activation energy. This is explained by the rather high ΔE for fibroin solution in the complex solvent. As already noted, hydrolysis changes the primary structure of the macromolecules (destruction of the macromolecules at functional groups, along the chain length, at radicals). As a result, the strength of the intermolecular interaction in solutions of hydrolyzed fibroin and ΔE decrease. The primary structure of fibroin undergoes even greater destruction with further mechanical treatment. The formation of even greater quantities of weaker structural fragments in solutions of mechanically treated and hydrolyzed fibroin significantly decreases ΔE.

Thus, depending on the nature of the polymer, fibroin from natural silk, and the conditions under which fibroin is modified, the intermolecular interactions that play the main role in forming a network of intermolecular contacts, associates and aggregates, and determine the principal structural parameters of concentrated solutions can vary.

EXPERIMENTAL

Starting fibroin from natural silk was isolated by the boiling soap-soda method [9]. **Hydrolyzed fibroin** was obtained by hydrolysis of starting fibroin from natural silk in aqueous HCl (4 N) at 95°C for 1 h. **Mechanically treated fibroin** was obtained by grinding hydrolyzed fibroin on an AGO-2U device with shear stress 600 Pa for 10 min and 1:10 modulus.

The amino-acid composition of hydrolysate was determined on an amino-acid analyzer (Hungary).

Solutions (15%) in solvent containing 46.50% NaSCN, 24.975% acetic acid, and 28.525% water were prepared from the obtained fibroin samples and its modified derivatives.

Rheological properties of concentrated solutions of polymers were investigated in a Rheotest-2 rotational viscometer. The effective viscosity was measured at shear stresses up to $\tau = 3\cdot10^3$ N/m^2 at 25-70°C. The principal method for studying rheological properties of flowing systems is determining the dependence of the deformation rate on shear stress [1], $\log D = f(\log\tau)$, and the numerical values of its parameters where τ is the shear stress, i.e., the force acting tangentially to the laminar flowing layers per unit area, D is the rate (deformation rate) gradient that characterizes the rate change per unit distance measured at a right angle to the rate direction or $\log\eta = f(\log\tau)$, where η is the effective viscosity of the system and $\eta = \tau/D$. This function is displayed graphically by the flow curve.

REFERENCES

1. G. V. Vinogradov and A. Ya. Malkin, *Rheology of Polymers* [in Russian], Khimiya, Moscow (1977), p. 440.
2. Yu. A. Ovchinnikov, *Bioorganic Chemistry* [in Russian], Prosveshchenie, Moscow (1987), p. 815.
3. B. Jirgensons, *Natural Organic Macromolecules*, Pergamon Press, Oxford and New York (1962).
4. C. Zheng and Y. Wang, *Sichuan Daxue Xuebao, Ziran Kexueban*, **30**, No. 3, 365 (1993); *Chem. Abstr.*, **121**, 257738c (1994).
5. S. D. Kamilova, Author's Abstract of a Candidate Dissertation in Chemical Sciences, Tashkent (1988).
6. Z. H. Ayub, M. Arai, and K. Hirabayashi, *Biosci., Biotechnol., Biochem.*, **57**, No. 11, 1910 (1993).
7. S. Fu, J. Xu, J. Li, and Z. Tan, *Sichuan Daxue Xuebao, Ziran Kexueban*, **36**, No. 1, 99 (1999).
8. A. A. Tager and O. G. Botvinnik, *Vysokomol. Soedin., Ser. A*, **16**, No. 6, 1284 (1974).
9. F. I. Sadov, I. M. Sokolova, and E. O. Vil′dt, *Laboratory Practicum for a Course in Chemical Technology of Fibrous Materials* [in Russian], Moscow (1963), p. 84.