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MOLECULAR BIOPHYSICS

The Molecular Dynamics of the Self-Assembly and a Rheological Model of the Superhelical Structure of a Spiderweb Protofibril

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Abstract—In this study, we performed a dynamics simulation of the formation of a protofibril of the nanofilament of a spiderweb. A bundle of parallel poly-*L*-alanine β-strands of sufficient length was shown to be self-arranged into a stable right-handed superhelix. The rheological properties were investigated and nonlinear generalization of the Zener model was given for the description of the rheological behavior of a superhelix using numerical analysis.

Keywords: spiderweb, structure of a protofibril, molecular dynamics
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Recently, the interest in protein nanofibers has been significantly stimulated. In particular, spiderweb, which has both unique strength properties and practically complete biocompatibility, is of special interest [1, 2]. The creation of artificial web fibers using recombinant spidroin proteins is very promising for a number of areas of technology and biomedicine [3]. However, little is still known about the mechanism of the formation of the spider fiber and the influence of the amino-acid composition of spidroins on the unique mechanical properties of the fiber [4, 5]. Rather long polyalanine regions are involved in the spidroin amino-acid sequences. These regions alternate with glycine-rich segments. The latter are considered to form coil structures that are responsible for the elasticity of the fiber. The role of the long polyalanine regions has still not been elucidated in detail. Earlier, Winkler et al. [1] demonstrated that the web fiber protein predominantly has the β-sheet conformation and its separate molecules form long spider filaments. Below, we investigate the process of self-assembly in a bundle of polyalanine peptides that simulate the corresponding spidroin regions. Spiderweb is a new object for materials science and the dynamics of stretching of the formed structures and comparison of the rheological characteristics of the model of the web protofibril with those of well-known materials (for example, rubber) are of interest. On the other hand, the molecular dynamics of the self-assembly of macromolar structures is of special interest [6]. Recently, some geometric relationships between the length of the chemical bond in the monomers and their Van der Waals size have been found. These relationships result in the possibility of the formation of helical foldings of a definite type that is similar to those in biopolymer structures [7]. From this point of view, the arrangement of superhelical elements from fragments of helixes could be a very natural means of the self-assembly of polypeptide structures for the formation of tough fibers.

MATERIALS AND METHODS

The Gromacs 3.3 software [8] and the OPLS-AA field of force [9] were used for molecular-dynamics calculations. Scholastic dynamics served [12] as a thermostat for the uniform distribution of energy through the degrees of freedom [10, 11]. An external force from 1 to 100 kcal/(mol A) was applied to the peptides in the experiments of the stretching of the peptide complex. The trajectory length was 10 ns in all the experiments. The cutoff radius for the Van der Waals and Coulomb interactions was 20 Å.

RESULTS

The molecular dynamics of the peptide complexes. The complete atomic dynamic modeling demonstrated that the bundles that consisted of at least five parallel polyalanine β-strands provided the formation of a sufficient number of hydrogen bonds between the molecules, resulting in the stabilization of the structure. The supramolecular complexes formed a stable right superhelix (Fig. 1), which was clearly observed for peptides that involved more than eight amino-acid residues.

Condensation of the parallel β-strands stabilizes the structure and the strands form a right superhelix. The formation of only a right superhelix is explained by the fact that the proteins consist of *L*-amino acids.

A left superhelix forms if *D*-amino acids are used for the modeling.

The pronouncement of the superhelical structure depends on the peptide length. The poly(A)-elements in the protein molecule of the spiderweb are probably the basis that forms a protofibril similarly to the fibers of a cable with the rigid helical structure.

Stretching of the peptide complex. Studies of the rigidity of the secondary structure using the applied external force allows the characterization of the system behavior near a local minimum on the surface of the potential energy. The application of large force values provides an evaluation of the system behavior on the slopes of the hypersurface of the potential energy, which lead to the local minimum (the same approach was used in the [7]).

The structural elasticity was determined using superhelical complexes that were relaxed for 10 ns. The *N*-termini of the peptides were fixed and force was applied to their *C*-termini along the peptide axis, i.e., digital experiments involving the stretching of the superhelix under the action of various forces were performed (Fig. 2) and the values of the relative elongation were evaluated (Fig. 3).

Investigation of the dependence of the relative elongation on the applied force revealed three linear regions of superhelix stretching: within the force intervals from 0 to 2 kcal/(mol A), from 3 to 5 kcal/(mol A), and higher than 50 kcal/(mol A).

The presence of several linear regions with considerably different slopes corresponds to different stages of the stretching of the supramolecular complex.

The studies of the structures of the complexes that are formed under the action of forces of different values demonstrate that the right superhelix of the supramolecular complex begins to gradually untwist under the action of forces from 0 to 2 kcal/(mol A) (the first linear region). The structure of the complex at a force of 3 kcal/(mol A) already corresponds to a β-sheet; its elasticity is not associated with the deformation of the superhelix. Thus, the elasticity of the supramolecular complex at the initial stage of the stretching depends on the elasticity of the superhelical structure.

The peptide bundle preserves the β-sheet conformation under the action of forces from 3 to 5 kcal/(mol A). The structural rigidity in this force interval is supported by hydrogen bonds between the individual molecules.

The action of higher forces deforms the secondary structure of the complex and its geometric parameters no longer correspond to a β-sheet. Deformation of the valent angles and stretching of valent bonds affect the structural elasticity.

The value order of the obtained coefficient of rigidity (110 N/m) is close to that of the valent angles.

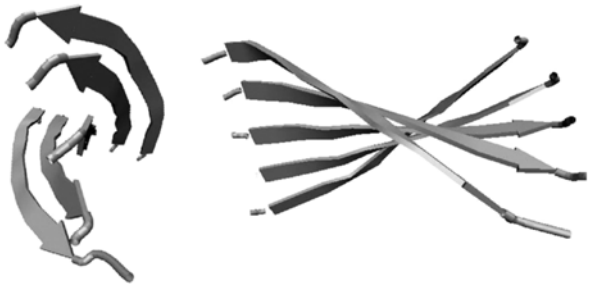

Fig. 1. The helical structure that was formed by five polyalanine β-strands, each of which consisted of 12 amino-acid residues.

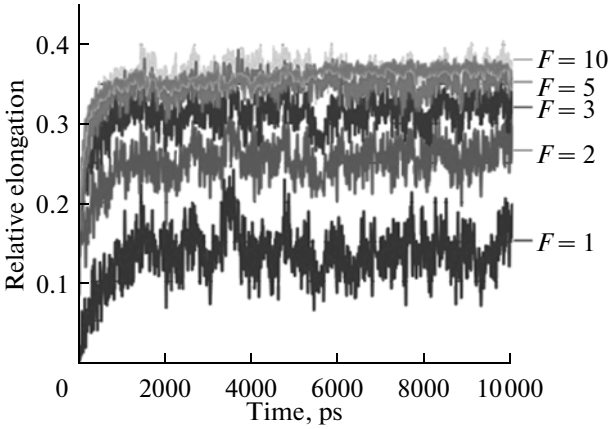

Fig. 2. The dynamics of the relative elongation of the superhelix under the action of the applied external forces (in kcal/(mol A)

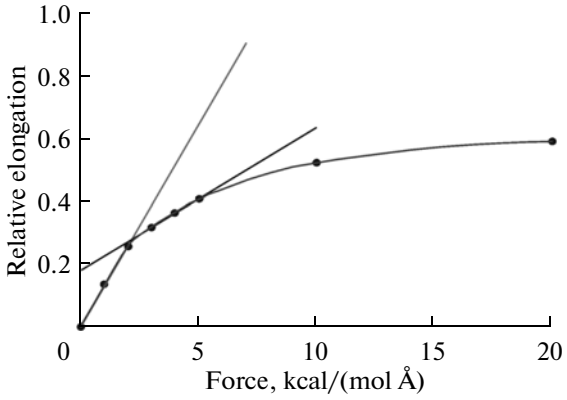

Fig. 3. The dependence of the relative elongation on the applied force. The linear approximation of the first and the second regions is also shown.

The rigidity coefficients can be converted to the effective Young modulus. It should be kept in mind that the stretching dynamics of the examined fragment of the web protofibril (Fig. 2) only roughly corre-

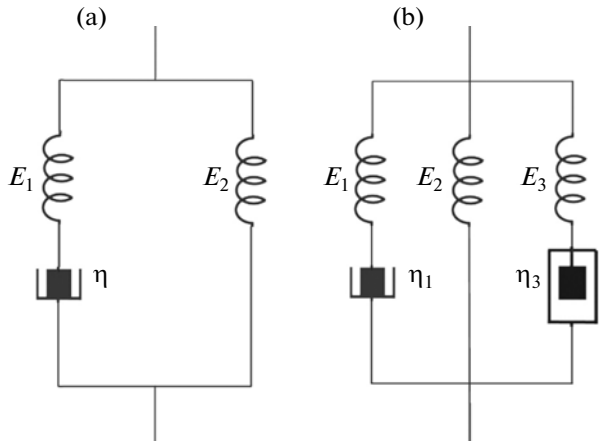

Fig. 4. Rheological models for the viscoelastic materials: (a), the Zener model; (b) the refined rheological model of the protofibril with an additional elastic element and a nonlinear viscoelastic element that limits the "piston" stroke.

sponds to the standard model of a linear body (the Zener model [13], Fig. 4a):

$$\varepsilon = \varepsilon_0 + (\varepsilon_\infty - \varepsilon_0)(1 - e^{-t/\tau}),$$

where

$$\varepsilon_0 = \frac{\sigma_0}{E1 + E2}, \quad \varepsilon_0 - \varepsilon_\infty = \frac{\sigma_0 E1}{(E1 + E2)E2},$$

$$\tau = \left(\frac{\eta(E1 + E2)}{E1E2}\right).$$

The protofibril fragment is characterized by a strong nonlinear elasticity and nonlinear internal viscosity (table). The nonlinearity is especially dramatically exhibited at low and very high loads. For example, the elastic element that is linked to the viscous element (see the model in Fig 4a) appears to be practically nonstretching ($E1 \gg E2$) at a force of approximately 1 kcal/(mol A) (which corresponds to the tension of about 25 atm). The effective viscosity also proves to be significantly higher than that at larger loads. The degrees of freedom in the viscoelastic Maxwell element are unbroken at the high loads (Fig. 4a). The effective Young modulus varies within the range from ~18 MPa to approximately double the values with a fivefold increase of the load. Approximately the same tendency is observed for the elasticity that is characterized by the $E2$ modulus (Fig 4a). The effective internal viscosity remains practically constant in this load range (approximately 5 centipoises). This value is almost five times higher than the water viscosity. A dramatic increase of the effective Young modulus and the effective internal viscosity of a protofibril are observed at the large load increase (by 100 times). This jump is explained by the fact that the deformation resource due to the relatively mild conformational degrees of freedom appears to be depleted at deformations of 50% and higher and we proceed to the regime of the deformation of exclusively valent angles and valent bonds. The refined rheological model of a protofibril is given in Fig. 4b. The added viscous-elastic element is distinguished by its significantly higher elasticity and considerably lower viscosity in comparison with the above-discussed Maxwell element. However, the "piston" stroke in this viscous element is limited by a definite range of deformations. The viscosity sharply increases as the limit is approached and further relaxation becomes impossible.

Taking the picture of the structural changes during the stretching of a fragment of the protofibril of the spiderweb into account, we could probably classify the basic viscoelastic elements in Fig. 4b. The $E2$ elastic element corresponds to the superhelix. The $E1$ elastic element is the equivalent of the hydrogen bonds of the secondary structure. The internal viscosity is caused by overcoming barriers during the transition between conformational substates during the deformation of the secondary structure (β-sheet). The $E3$ elastic element can be compared with the deformation of valent angles and bonds. The conjugated $E3$ nonlinear viscous element can probably be considered as the relaxation of the valent angles and bonds (the effective viscosity for low-amplitude variations less than 0.1 Å is rather low [14]). The nonlinearity is associated with

The coefficients of the Zener model for the polyalanine superhelix from five @[beta]-strands at different values of the external force

Force, kcal, (mol A)	ε_0	ε_∞	$E1$, GPa	$E2$, GPa	τ, ps	η, Pa s
1	0	0.14	∞	0.018	474.21	0.009
2	0.14	0.26	0.017	0.19	527.51	0.005
3	0.14	0.31	0.030	0.024	305.89	0.004
4	0.19	0.37	0.026	0.027	334.55	0.004
5	0.19	0.41	0.036	0.031	287.97	0.005
10	0.31	0.52	0.033	0.049	243.57	0.005
100	0.54	0.75	0.131	0.337	121.19	0.011

the limitation of the "piston" stroke and corresponds to the depletion of possibilities of the protofibril deformation due to the collective adjustment of the torsion and valent angles of the β-filaments.

Note that the above-mentioned values of the elasticity of the protofilament fragment are somewhat lower than that of the body filament of a spiderweb (2 GPa), which is published on the basis of indirect data. The difference is possibly associated with macroscopic effects that are absent at the more elementary level. It should be stressed that the value of the Young modulus that was obtained in the digital experiments for the protofibril fragment is higher than the typical values for rubber (~0.01 GPa) and, at small deformations, is one order of magnitude lower than those for globular proteins (~1 GPa) that have three-dimensional "sewing" with hydrogen bonds.

CONCLUSIONS

Polyalanine complexes generate bundles of parallel β-filaments and tend to form a right superhelix. The superhelix is stabilized in molecular-dynamics experiments when the number of the filaments is no less than five and every β-filament consists of no less than eight residues.

The superhelix stretches in three stages. At the first stage, the superhelical structure plays a determining role. Further, deformation of the hydrogen bonds of the secondary structure occurs, and, finally, valent angles and bonds are deformed.

The viscoelastic properties of the superhelix do not fit in the framework of the model of a linear viscoelastic body (the Zener model). The necessary elements of the complication of the rheological model are the introduction of the dependence of the elasticity and the viscosity of the Maxwell element on the tension and the elongation, as well as the introduction of a new element with high rigidity and a threshold increase in the internal viscosity that varies from very low to very high values during the elongation of the fibril higher than the critical value.

The values of the Young modulus of the superhelical fragment of the protofibril at small loads and low elongations are somewhat higher than those of rubber. The Young modulus rapidly increases with the enhancement of the load and the elongation and approaches the values that are characteristic of elastic bodies during elongations on the order of 75%.

In this study, we demonstrated a tendency towards the formation of superhelixes for the fragments of the peptide sequence of spidroin. This tendency could be a rather common stage in the course of the self-assembly of fibers of a peptide nature.

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