BIOPHYSICS AND BIOCHEMISTRY

Elastic Modulus of the Thin Filament under Normal Conditions and during Heart Failure

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 5, pp. 498-503, May, 2004
Original article submitted June 21, 2003

We developed an original approach to prepare samples for electron microscopy in electric field allowing calculation of the Young's modulus of the thin filament in a direction perpendicular to the axis of the filament (longitudinal to the protomer) under normal conditions and during heart failure induced by 10-day toxic and allergic myocarditis. Electric field stretches thin filaments in the transverse direction and the increase in its diameter linearly depends on the applied voltage. The elastic modulus was in inverse proportion to the applied voltage. We found that during heart failure thin filament had an extreme conformation and to a great extent lost its mobility.

Key Words: *structure; thin myocardial filaments; directed electric field; structure of thin filament*

Thin filaments (TF) of myofibrils are characterized by high conformational mobility, which manifested in variability of the major indexes and diameter of the helix [4,6,11]. Dynamic properties of TF depend on elasticity, which is characterized by the Young's modulus. We proposed a method for preparing samples for electron microscopy. This technique allowed evaluation of the effect of electric field of varying strength on TF helix and calculation of the Young's modulus for normal myocardium and during heart failure (HF) caused by myocardial inflammation.

MATERIALS AND METHODS

Experiments were performed on natural TF of myofibrils from rabbit myocardium with a specific electrical conductivity of 0.716 Ω/m [6]. TF were prepared as described previously [8]. A copper disk (diameter 15 cm, height 10 cm) cooled to 277°K was covered with a Teflon film (0.1 μ). The space between the film and the disk was filled with 40% ethyl alcohol to improve heat conduction. The TF preparation (0.25 ml, ρ_f=0.1 mg/ml) was mounted on the cooled film. The carbohydrate film (5-6 nm) was maintained on the surface of TF, placed on a drop of 2% uranyl acetate, and then on copper meshes.

For evaluation of the effect of electric field on TF the preparation procedure was performed between condenser plates at 500 and 800 V potential difference (U) across the condenser and electric field strength (E) 5×10^4 (E_1) and 8×10^4 V/m (E_2), respectively.

For evaluation of dielectric permeability of the solution with TF preparation procedure was performed on a copper disk as described above, but two parallel platinum electrodes (length, g_2=10 mm; diameter, h_2= 0.1 mm) were placed into the solution (distance between electrodes u_2=8 mm, U=10 V). Some preparations were not exposed to electric field. They were coated with platinum on a JEE-4C device (JEOL) at a pressure of 10^{-5} Torr and φ=9° shadowing angle (tgφ=1/6). Thin carbohydrate films were prepared by carbon coating of fresh mica chips. Treatment was performed on the same device under similar conditions.

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0007-4888/04/1375-0435

The diameter of TF was measured on microphotographs (×300,000). We calculated the mean of 3 measurements [10]. A direct image of TF was studied in contrasted preparations. The shadow width (t) was measured in shadowed preparations. The diameter (d) was calculated as follows: $d=t\times\mathrm{tg}\varphi=t/6$. The arithmetic mean

$$(\overline{d}=\frac{\Sigma k_i d_i}{\Sigma k_i})$$

and weighted average diameter were evaluated:

$$\overline{d}_{wa}=\frac{\Sigma k_i d^2{}_i}{\Sigma k_i d_i}.$$

where d_i is the diameter of TF in the i-th measurement; and k_i is the number of equal values of the diameter in the i-th measurement.

The results were analyzed by standard statistical methods. The significance of differences was determined by Student's t test.

RESULTS

The diameter of TF in normal sarcomeres at the potential differences between the condenser plates of 0 and 800 V was 3.5 and 4.25 nm, respectively (~40% of the mean value). The diameter of normal TF at a potential difference of 500 V was 3.0 nm (Table 1). During HF the diameter of TF varied from 4.2 (0 V) to 4.0 nm (800 V), which corresponded to ~30% of the mean value. Electric field increased the maximum mean-square deviation (s) of the diameter of myocardial TF under normal conditions and during HF by 28 and 5.7%, respectively. Therefore, the rate of electric field-produced conformational changes in TF of normal myocardium (mobility of the structure) was much higher than during HF. This disorder was characterized by high stability of TF. Our results are consistent with published data [6].

It is important to estimate the statistical center of gravity for the diameter of TF characterizing changes during functioning of actin. Table 1 shows that these indexes ($\overline{d}_{wa}$) did not differ from the average value ($\overline{d}$) within the limits of variation. The tendency to increase the diameter illustrates asymmetry of the distribution curve (Fig. 1).

Indexes for the helix of TF (*e.g.*, diameter) were characterized by discreteness. It was confirmed by asymmetry of the distribution curve and appearance of the saddle point (mean diameter of TF). The appearance of this saddle point indicates the presence of at least 2 independent distribution families for the diameter distributions and reflects structural nonequivalence of actin protomers in TF (Fig. 2). The saddle point was absent under the influence of electric field

TABLE 1. Diameter of TF under Normal Conditions and during HF (nm)

Group	d_{min}	d_{max}	Estimated			Calculated after 300 measurements			$\overline{d}_в$
			d	s	m_x	d	s	m_x	
Normal (n=329), 0 V	7.5	11.0	9.01	0.609	0.03	9.01	0.608	0.03	9.05
Normal (n=145), 500 V	8.0	11.0	9.51	0.597	0.05	9.51	0.592	0.03	9.55
Normal (n=1262), 800 V	8.0	12.25	9.91	0.764	0.02	9.90	0.751	0.04	9.97
HF (n=543), 0 V	11.5	15.75	13.39	0.699	0.03	13.40	0.690	0.04	13.43
HF (n=199), 800 V	12.0	16.0	14.51	0.738	0.05	14.51	0.730	0.04	14.54
Normal (n=76), shaded	10.0$^+$	14.7$^+$	11.11$^+$	1.01$^+$	0.10$^+$	—	—	—	11.2$^+$
Normal (n=112), 10 V	8.0$^+$	11.0$^+$	9.33$^+$	0.731$^+$	0.07$^+$	—	—	—	9.39$^+$

Note. d-Δd'=d^*_1, or $\overline{d}$+Δd'=d^*_2, where d^*_1 and d^*_2 are centers for symmetrical distribution of filament diameters; and Dd' is the step of discreteness characterizing the structure or minimum changes in the diameter resulting in new energy conformation of TF. The average value of d should be less probable for structures capable of undergoing active conformational changes. Here and in Table 2: $^+$experimental data; other numerals, study of curve.

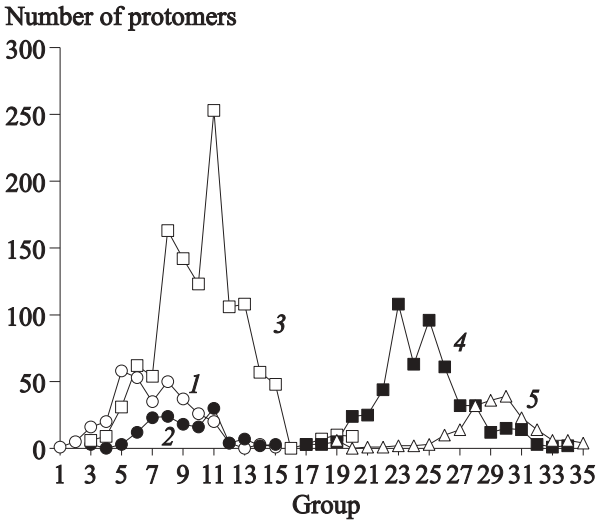

Fig. 1. Diagram for the distribution of diameters of thin filaments depending on the series of measurements (0.25-nm increments). Here and in Fig. 2: S1 (*1*), S2 (*2*), and S3 (*3*) are thin filaments of normal myocardium at 0, 500, and 800 V, respectively. S4 (*4*) and S5 (*5*): 0 and 800 V, respectively.

on TF during HF, which attested to conformational inertness of the protomer not capable of undergoing mobile changes. These characteristics are typical of the protomer with a strongly developed structure, which reflects extreme conformational changes in TN of myofibrils during HF. These results can be extended to other indexes of the helix. It can be hypothesized that the probability of the mean indexes is minimum in the helix of normal muscle. The charge distribution gradient (*grad P*) was perpendicular to the axis of TF along the field lines. The direction of field lines depended on electric properties of the filament. These properties are determined by the state of functional units in TF (7 protomers combined with tropomyosin) [3]. When tropomyosin is located near the axis of TF, the outer surface of the protomer is not exposed to the effect of tropomyosin charges. *Grad P* is directed along the field lines and has maximum value. The diameter of TF increases to maximum. When tropomyosin is located near the outer surface of the protomer in actin (Fig. 3), external charges of the protomer are partially shielded. *Grad P* decreases and, therefore, diameter of TF increases to a lesser extent. The difference between the maximum and minimum increase in the diameter of TF is ~0.25 nm. It should be emphasized that electric field (800 V) has no effect on the structure of TF during HF. Thus, TF gains a strongly developed conformation in the initial state. Tropomyosin cannot open functional units, and the system has only one conformation. These specific features are manifested in the absence of the saddle point.

Electric field increased the diameter of TF (Fig. 3). The higher was *E*, the more pronounced was the increase in the diameter of TF. The diameter of TF increased by 1.4 times during HF, but electric field further increased this parameter. It reflects greater elasticity and reserve conformational mobility of TF. While the strength of isometric contraction of myofibrils decreases by 13 times compared to normal, it nevertheless occurs [2]. Probably, the increase in the diameter (Δd) linearly depends on the electric field strength or applied voltage at condenser plates.

The study of TF in electric field of different strength allowed us to derive an equation that describes mechanical properties of TN (elastic modulus or Young's modulus):

$$J \approx 1.3 \frac{(g \times r_0)}{(aU)^2} \times 10^{13} = 3.7 \frac{(d \times d_0)^3}{U^2} \times 10^{13}. \quad \textbf{(1)}$$

TABLE 2. Elastic Modulus of TF (Transverse Direction) and Changes in Internal Energy under the Influence of External Electric Field ($M \pm m$)

Group	U, kV		d^*, nm	d_0, nm	Δd, nm	J ($\times 10^8$), N/m^2	ΔW_{IN}/ ($\times 10^{-12}$), J/m
	actual	arbitrary					
Normal	0.3		9.3	9.01	0.3	4.52±0.3	2.22±0.55
	0.5$^+$		9.51$^+$	9.01$^+$	0.50$^+$	1.74±0.12$^+$	6.33±0.48$^+$
	0.8$^+$		9.91$^+$	9.01$^+$	0.90$^+$	0.77±0.03$^+$	19.01±0.81$^+$
		1.2	10.4	9.01	1.4	0.39±0.04	46.54±3.2
		2.0	11.3	9.01	2.3	0.18±0.02	138±43
		3.0	12.5	9.01	3.5	0.11±0.02	350±31
HF	0$^+$	3.8$^+$	13.39$^+$	9.01$^+$	4.38$^+$	0.08±0.01$^+$	593±53$^+$
	0.5	4.3	14.1	9.01	5.1	0.08±0.01	823±74
	0.8$^+$	4.6$^+$	14.51$^+$	9.01$^+$	5.50$^+$	0.07±0.01$^+$	977±88$^+$

Note. Arbitrary value was derived after studying the dependence of changes in the diameter of TF on applied voltage.

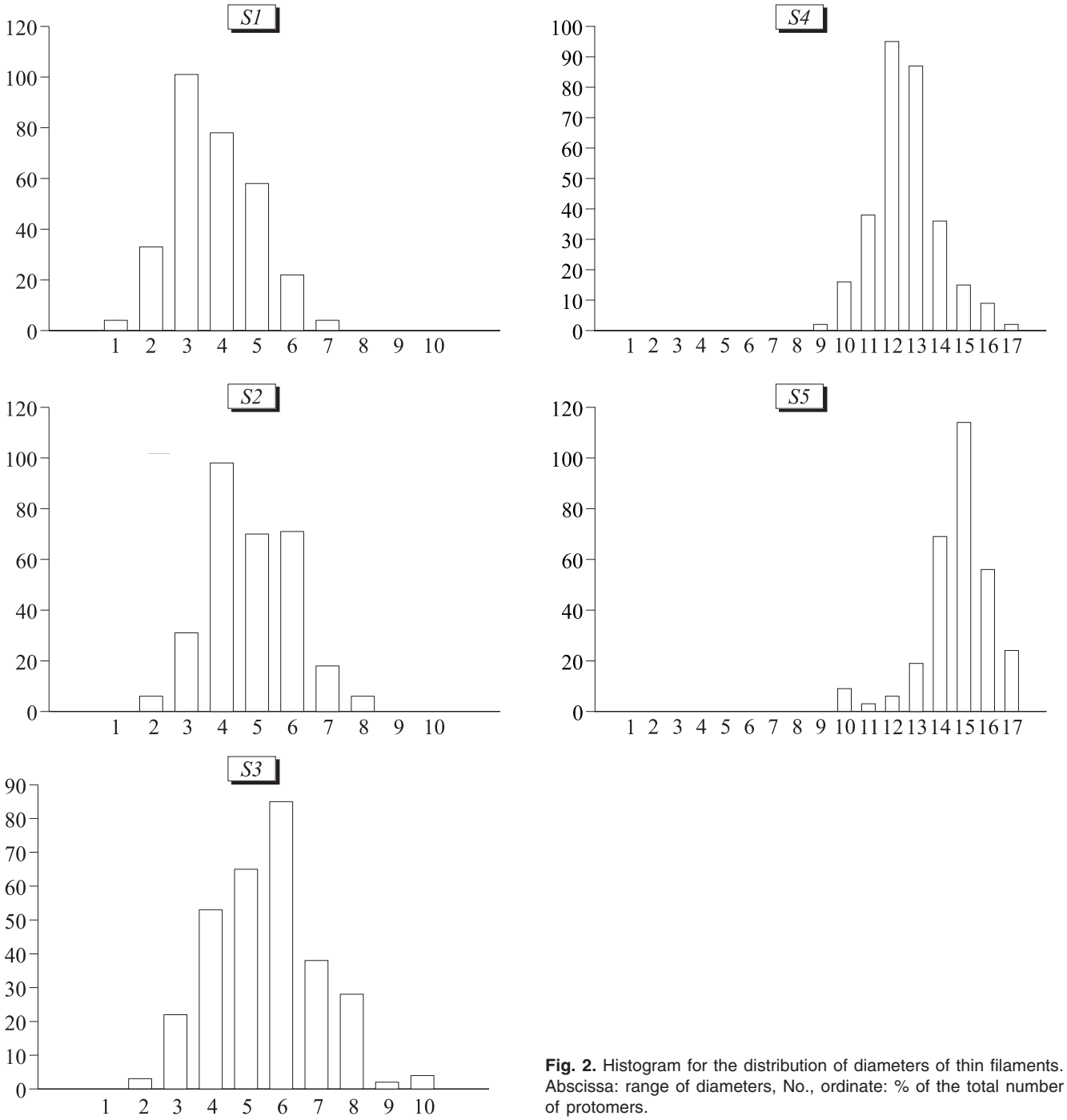

Fig. 2. Histogram for the distribution of diameters of thin filaments. Abscissa: range of diameters, No., ordinate: % of the total number of protomers.

The elastic modulus (Table 2) was calculated by substituting values of the diameter of TF and electric field strength into Eq. **(1)**. Eq. **(1)** shows that the elastic modulus is in inverse proportion to applied voltage at plates of the preparative condenser. The elastic modulus was unstable and decreased with increasing the load on TF. The increase in the diameter was accompanied by stabilization of the elastic modulus. The elastic modulus remained practically unchanged under the influence of electric field during HF. Therefore, TF undergoes less pronounced conformational changes during HF. The state of TF can be characterized by not only the elastic modules, but also ΔW_{IN} of the system. This value is determined by activity of the macrosystem: $\Delta W_{IN}=F_F\Delta d$, where F_F is the volume force applied to TF. Simple mathematical treatment and substitution of constant indexes for TF produced the following equation:

$$\Delta W_{IN}=2.4\times10^{-11}\times Ul\ \frac{d^*\Delta d}{d_0}, \qquad \textbf{(2)}$$

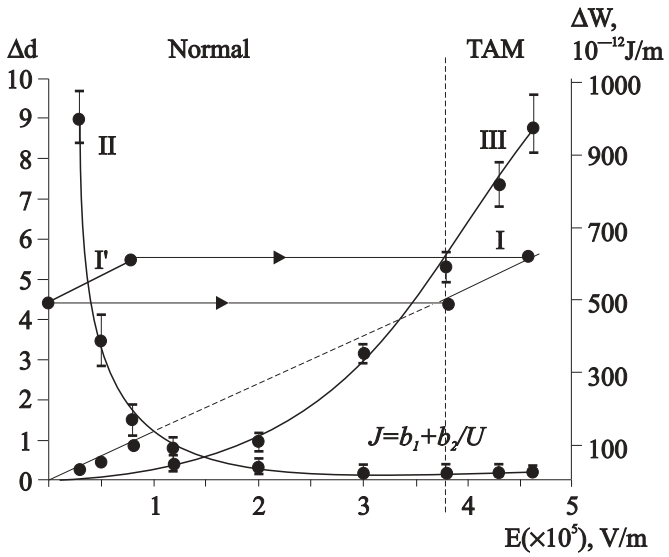

Fig. 3. Histogram for the distribution of diameters of thin filaments (TF) and dependence of changes in the diameter of TF (I), elastic modulus (II), and internal energy on the strength of external electric field (III).

where l is the average length of TF, Δd is an increase in the diameter of TF under the influence of electric field, d^* is the diameter of TF inside the field, d_0 is the diameter of TF outside the field, and U is applied voltage at condenser plates. The length of F-actin *in vitro* differs under normal conditions and during HF (1.2 and ~0.4 μ, respectively) [9]. It is necessary to normalize ΔW_{IN} by the filament length to compare internal energies (Table 2). ΔW_{IN} is minimum under the influence of electric field with $E_1=5\times10^4$ V/m (1.92×10^{-12} J/m). ΔW_{IN} is 5.7×10^{-12} J/m at $E_2=8\times10^4$ V/m. The diameter of filaments (Δd) and internal energy increase by 1.78 and 2.97 times, respectively, with a 1.6-fold increase in E. Therefore, the increase in the diameter of TF during extension is accompanied by a decrease in its resistance to external forces. The near-extreme state of the filament can result in mechanical rupture (Fig. 3). Electric field with $E_2=8\times10^4$ V/m produced other changes during HF. Variations in internal energy corresponded to 6.91×10^{-12} J/m (relative to the absence of electric field). ΔW_{IN} increased by 1.21 times compared to that for normal TF exposed to electric field of the same strength. The diameter of TF underwent similar changes (1.23-fold increase). Although the diameter of TF during HF surpasses the normal by 1.61 times, they retain conformational characteristics of the actin protomer resistant to transverse deformations.

These findings attest to complete conformational reconstruction of the actin protomer in HF, rather than to its simple extension in the transverse direction (longitudinal to the protomer). TF exposed to electric field are characterized by low conformational mobility. It should be emphasized that TF are conformationally mobile before exposure to electric field. Reversibility of conformational reconstructions illustrates their functional importance. In the working muscle these conformational changes are necessary for the contraction and relaxation of myofibrils.

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