BIOPHYSICS AND BIOCHEMISTRY

Effect of Polymerization on Thermodynamic Parameters of Melting and Stabilization of F-Actin Structure in Normal and Dystrophic Canine Myocardium

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Differential scanning microcalorimetry was used to determine changes in enthalpy, entropy, and free energy of melting of purified myocardial fibrillar (F) actin from normal dogs and dogs with 2-3-month L-thyroxin-induced and athyreotic cardiomyopathy. Polymerization of globular (G) actin stabilizes protomer structure in both pathologies. However, the conformational changes in actin monomer caused by L-thyroxin-induced and, especially, by athyreotic cardiomyopathy decrease the free energy of the bonds between protomers in the synthesized F-actin. Binding energy between actin protomers modified in athyreotic cardiomyopathy (-12 kJ/mol) is 4 times below the control value (-48.7 kJ/mol), while in L-thyroxin-induced cardiomyopathy it little differs from the normal value (-40.8 kJ/mol).

Key Words: *myocardium; L-thyroxin-induced cardiomyopathy; athyreotic cardiomyopathy; actin polymerization; thermodynamics*

Previously we described considerable changes in thermodynamic parameters of melting of purified G-actin from the myocardium modified by 2-3-month L-thyroxin-induced (LTC) and athyreotic (ATC) cardiomyopathy (myocardial dystrophy according to G. F. Lang).

Pronounced conformational changes in monomers were revealed in these pathologies. The structure of myocardial G- and F-actin in dogs with acute and chronic heart failure (in particular, caused by ATC) was studied by the method of circular dichroism [1,4].

In this paper we describe the relative changes in thermodynamic parameters of melting of F-actin in LTC and ATC.

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MATERIALS AND METHODS

Experiments were carried out with actin isolated and purified [6] from the myocardium of healthy dogs (*n=5*), and dogs with LTC (*n=6*) and ATC (n=5). The monomeric form of this actin was studied in the previous work, the methods and procedures were also described in detail [2]. Polymerization of monomeric actin was initiated by addition of a polymerizing solution to purified G-actin [6] (final concentrations in mM): 125 KCl, 1 $MgCl_2$, 0.2 Na-ATP, 0.2 $CaCl_2$, 0.5 dithiothreitol, 1 NaN_3, and 2 Tris HCl (pH 8.0 at 25°C).

The standard free energy of protomer binding (ΔG°_B) was calculated according to the equation:

$$\Delta G^\circ_B = \Delta G^\circ_{FA} - \Delta G^\circ_{GA} - \Delta G^\circ_{ATP},$$

where ΔG°_{FA} and ΔG°_{GA} are changes in free energy of stabilization of F- and G-actin structure, respectively,

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and DG^{o}_{ATP} is change in standard free energy of ATP hydrolysis.

The melting cooperativity index was calculated as the ratio of van't Hoff enthalpy and calorimetric enthalpy.

RESULTS

Visual evaluation of experimental melting thermogram of F-actin in ATC (Fig. 1) attests to pronounced deviation of its form and value from normal. The peak approximates the ideal form characteristic of extremely cooperative melting, which goes on in a narrow temperature range by almost "all-or-nothing" mechanism. The thermogram has no low-temperature peak observed during melting of G-actin in ATC [2].

Despite the drastic decrease in melting cooperativity index for G-actin from 0.8 to 0.5 compared in ATC, this parameter in the case of F-actin only little differs from the normal value (Table 1) [2]. Correspondingly, the experimental calorimetric enthalpy little differs from van't Hoff enthalpy calculated from maximum heat capacity, although in normal actin this difference is evident (Table 1). Judging from these parameters, polymerization stabilizes the protomer structure in F-actin, in particular in the small domain region [2]. In LTC, the melting cooperativity index increased due to an increase in calorimetric enthalpy, while van't Hoff enthalpy remained unchanged, which indicates protomer stabilization in the filament due to a decrease in basic heat capacity after denaturation (Table 1).

Comparison of the ratios of melting and stabilization parameters of F-actin in LTC and ATC to these parameters in normal actin with the corresponding

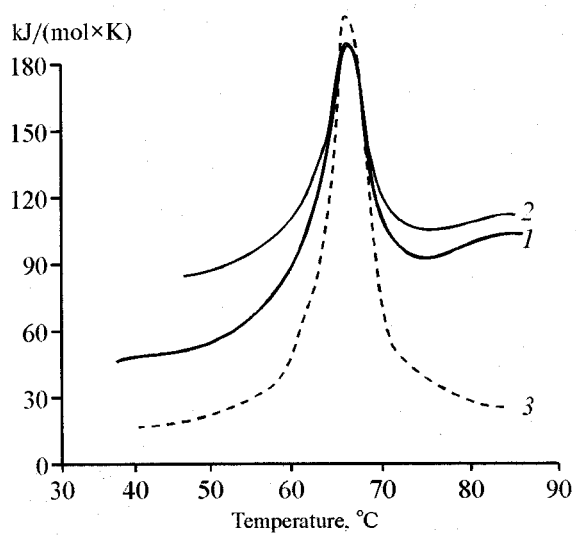

Fig. 1. Melting thermograms of purified F-actin from dog myocardium under normal conditions (*1*) and in L-thyroxin (*2*) or athyreotic (*3*) cardiomyopathy. Scanning rate 2 K/min, protein concentration 1 mg/ml.

TABLE 1. Changes in Thermodynamic Parameters of Melting and Stabilization of F-Actin from Canine Myocardium under Normal Conditions and in L-Thyroxin (LTC) or Athyreotic (ATC) Cardiomyopathy (*M±m*)

Group	Temperature of melting peak, °C	Cooperativity index	Changes (Δ)					Changes (Δ) of standard parameters		
			calorimetric enthalpy, kJ/mol	van't Hoff enthalpy, kJ/mol	heat capacity in melting peak, kJ/(mol×K)	denaturation entropy, kJ/(mol×K)	postdenaturation basic heat capacity, kJ/(mol×K)	enthalpy, kJ/mol	entropy, kJ/(mol×K)	free energy, kJ/mol
Normal	69.0±0.7	660±6	711±26	1.10±0.01	131±10	1.9±0.1	13.2±0.5	78±5	0.11±0.01	45.3±2.0
LTC	69.9±0.7	425±25***	657±42	1.55±0.05***	112±15	1.2±0.1***	8.0±0.7***	64±10	0.11±0.02**	31.0±3.0
ATC	70.1±0.1	855±61**	862±71*	1.04±0.01*	194±34***	2.5±0.2**	10.7±2.3***	380±45***	1.03±0.16***	73.3±8.3**

Note. Here and in Table 3: *$p<0.05$, **$p<0.02$, 0.01, and ***$p<0.001$ compared to the control, *$p<0.05$ and **$p<0.001$ compared to LTC.

TABLE 2. Ratio of the Changes in Thermodynamic Parameters of Melting and Stabilization of G- and F-Actin in LTC and ATC to Changes (Δ) of These Parameters in Normal Actin

Group	Calorimetric enthalpy		van't Hoff enthalpy		Cooperativity index		Heat capacity in melting peak		Postdenaturation basic heat capacity		Denaturation entropy		Enthalpy		Entropy		Free energy	
	G	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F
LTC	0.8	0.66	0.09	0.09	0.66	1.0	0.8	0.8	0.8	0.66	0.8	0.6	0.8	0.8	0.8	1.0	0.8	0.6
ATC	2.0	1.4	0.12	0.12	0.2	1.4	1.4	1.4	0.66	0.8	2.0	1.2	5	5	5	10	2.5	1.6

TABLE 3. Changes in Enthalpy, Entropy, and Free Energy of Stabilization of F- and G-Actin under Normal Conditions and in LTC and ATC (*M±m*)

Parameter	Normal	LTC	ATC
Enthalpy, kJ/mol	134.4± 0.5	116.7±4.3	470.5±37.2
Entropy, kJ/(molxK)	0.46±0.01	0.37±0.02	1.66±0.07
Free energy, kJ/mol	-0.7±0.001	7.2±1.0	36.0±3.7

ratios for G-actin allowed us to evaluate the changes of F-actin parameters caused by conformational changes of G-actin and related to protomer-protomer interaction (Table 2).

Since the relative changes of van't Hoff enthalpy, melting peak heat capacity, and standard enthalpy for normal and pathological F- and G-actin coincide, it can be proposed that the formation of F-actin structure in LTC and ATC is primarily affected by conformational changes in G-actins.

The fact that changes in calorimetric enthalpy, denaturation entropy, and free energy in normal and modified F-actin tended to 1 in contrast to the corresponding parameters of G-actin in ATC probably attests to a stabilizing effect of polymerization on protomer structure in this pathology (Table 1).

On the other hand, marked increase in the cooperativity index and standard entropy in normal and ATC-modified actin attests to considerable local intramolecular conformation changes that are increased after polymerization. Therefore, energy stabilization is achieved at the expence of conformational destabilization (Table 2, Fig. 2). This inference is not applied to LTC, because in this case the changes in considered ratios are close to normal.

Analysis of differences in the changes of enthalpy, entropy, and free energy of G- and F-actin stabilization (Table 3) makes it possible to evaluate the

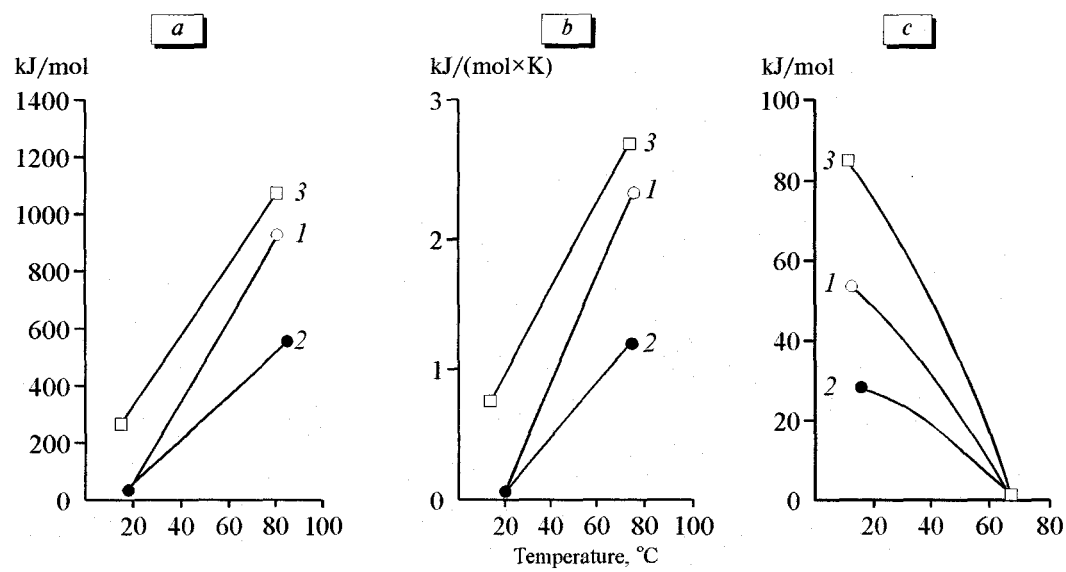

Fig. 2. Denaturation increment of changes in enthalpy (*a*), entropy (*b*), and free energy (*c*) of melting of F-actin from dog myocardium under normal conditions (*1*) and in L-thyroxin (*2*) or athyreotic (*3*) cardiomyopathy. Protein concentration 1.0-1.2 mg/ml.

thermodynamic parameters characterizing protomer-protomer bonds.

Since melting releases energy of ATP hydrolysis stored in F-actin, the resulting free energy should decrease by 48 kJ/mol [5]. Therefore, the free energy of protomer binding in normal actin is –48.7 kJ/mol, while in ATC and LTC it is –12 kJ/mol and –40 kJ/mol, respectively.

Thus, despite the opposite conformational changes in G-actin induced by ATC or LTC, both pathologies weaken interaction between the protomers, which presumably leads to similar disturbance in the functional properties of thin filaments in the myocardium.

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