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Conformational Changes in Myocardial Actin in Heart Failure Caused by Toxic Allergic Myocarditis

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The fluorescence resonance energy transfer method was used to study myocardial actin in rabbits with severe heart failure caused by toxic allergic myocarditis. It caused changes in orientation and microenvironment and increased excitation lifetime of fluorophores at the labels to Cys374 and Cys10 (subdomain 1), as well as at Lys61 and Tyr69 (subdomain 2). In addition, it increased the distance between Cys374 and Lys61, Cys10, and Tyr69, as well as between Tyr69 and Cys10. This attests to enlargement of the outer actin domain and more exposed and open arrangement of the tertiary structures of the N- and C-terminal regions and subdomain 2, accompanied by reduced conformational mobility. The relationships between actin conformation changes and decrease in contractile force and rate, as well as efficiency of actomyosin contraction are revealed, which agrees with the hypothesis on the leading role of actin in disturbances of contractile activity and energy transduction in the system of contractile proteins in heart failure.

Key Words: *myocardium; actin; heart failure; conformation; fluorescence*

Heart failure (HF) caused by 10-day toxic allergic myocarditis is characterized by reduced contractile activity and efficiency of myocardial myofibril contraction Ca,Mg-ATPase activity and contractile capacity of hybrid actomyosin macromolecules containing normal myosin and modified actin from rabbits with toxic allergic myocarditis are impaired [4,5]. The tertiary actin structure in the region of aromatic amino acid residues is modified in patients and animals with HF, although its secondary structure remains unchanged [3]. Fibrillar actin from HF myocardium forms a more rigid filament structure with considerably (1.4-fold) larger diameter and reduced conformational flexibility [8].

Our aim was to study conformation and mobility of amino acid residues located near or immediately in the physiologically important centers of myocardial actin: Cys374 (participates in force generation and in polymerization), Cys10 (center of interaction with myosin), Tyr69, and Lys61. The latter amino acids form a cleft between the two actin domains and participate in polymerization (blockade of Lys61 inhibits, and blockade of Tyr69 decelerates polymerization); they also take part in binding of bivalent cations and myosin (proteolytic availability of the 61-69 residues upon interaction with myosin head sharply decreases) [13,14,16]. According to the atomic model, the N- and C-terminal regions (residues 1-79 and 261-375, respectively) are located in the outer domain. Specifically, Cys374 and Cys10 are located in actin subdomain 1, while Lys61 and Tyr69 in actin subdomain 2 (residues 33-69) [13,14].

MATERIALS AND METHODS

The study was carried out on myocardial actin from normal rabbits (n=47) and rabbits with severe HF caused

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by toxic allergic myocarditis (n=48) [1]. The rabbits were sacrificed under hexenal narcosis.

Actin was isolated as described previously [19]. Fluorescent measurements were performed on MPF-4 (Hitachi) and SLM 4800 spectrofluorimeters (SLM-Instruments). Fluorescence (FL) quenching time was determined by phase-modulation method at modulation frequencies of 18 and 30 MHz [2,6].

The distances between amino acid residues in actin molecules were calculated by fluorescent resonance energy transfer (FRET) in the dipole pair interaction; polarization and anisotropy were calculated as described elsewhere [2,6].

Cys374 was labeled with fluorophore 5-2-(iodoacetyl)amino]ethyl]amino-naphthalene-1-sulfonic acid (1,5-IAEDANS), Lys61 was labeled with fluorescein 5-isothiocyanate (FITC) [16,17], and Tyr69 was labeled with fluorescence donor 5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl chloride, Dns) [9,10]. Cys10 was selectively labeled with N-[4-(dimethylamino)-3,5-dinitrophenyl]-maleimide (DDPM) [10,11,17] in a medium containing (in mM): 0.2 ATP, 0.1 $CaCl_2$, and 2 Tris-HCl (pH 7.4). Actin polymerization was performed at increased medium ionic strength by adding KCl and $MgCl_2$ to final concentrations of 80 and 2 mM, respectively (pCa 4.5).

The data were statistically analyzed as small samples of dependent and independent groups [7].

RESULTS

Heart failure was characterized by a shift in 1,5-IAEDANS-monomeric actin FL maximum from 464±3 to 472±2 nm (p<0.05, Fig. 1). The shift was accompanied by a 2.2-fold decrease in FL intensity of 1,5-IAEDSANS donor. Anisotropy of 1,5-IAEDANS-Cys374 FL in normal monomer indicates fluorophore rotation predominantly in the surface perpendicular to the molecular axis with a small reorienting of environment. By contrast, under HF this fluorophore to Cys374 rotates out of monomer axis with higher disorder in environment. Moreover, orientation of the fluorophore in actin from HF myocardium is opposite to that in normal actin, the microenvironment of actin from animals with HF being considerably disturbed. In HF myocardium the lifetime of excited 1,5-IAEDANS attached to Cys374 increases from 19.7±0.9 nsec to 29.1±2.1 nsec, which indicates that actin from failing heart is characterized by a more rigid attachment of 1,5-IAEDANS and more stable conformation of the Cys374 region.

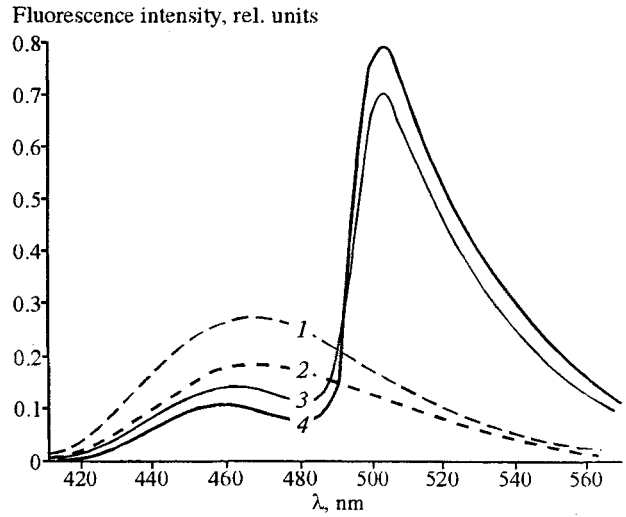

Fig. 1. Corrected fluorescence spectra of 1,5-IAEDANS-actin (*1,2*) and fluorescence resonance energy transfer in 1,5-IAEDANS-FITC-actin (*3,4*) under normal conditions (*1,3*) and in heart failure (*2,4*).

TABLE 1. FRET Parameters in Actin Monomer from Normal and Failing Heart ($M\pm m$)

Donor/Amino acid residue	Acceptor/Amino acid residue	R_0	Energy transfer efficiency, %		Distance, nm		References
			control	HF	control	HF	
1,5-IAEDANS-Cys374	FITC-Lys61 (n=7)	5.16	66±11	45±7	4.64±0.14	5.43±0.08***	
		5.10	*65*		*4.56*		*[16]*
	Dns-Tyr69 (n=5)	2.31	21±4	16±4	2.62±0.06	3.17±0.09*	
		2.23			*3.86*		*[9]*
	DDPM-Cys10 (n=7)	3.0	60±8	42±4	2.52±0.07	3.09±0.06**	
		2.9*			*3.2*		*[10,12]*
Dns-Tyr69	DDPM-Cys374 (n=7)	2.27	32.5±6.0	20±4	2.62±0.06	3.17±0.09**	
		2.19	*42.5*		*2.27±0.04; 3.6*		*[10,17]; [14]*
	DDPM-Cys10 (n=5)	3.19	58±5	43±4	2.90±0.10	3.50±0.05**	
		3.1	*68*		*2.72±0.04*		[10,17]

Note. R_0 is the characteristic distance at which efficiency of energy transfer is 50%. Italic marks the published data for normal skeletal muscle. *p<0.1, **p<0.01, ***p<0.001 compared with the control.

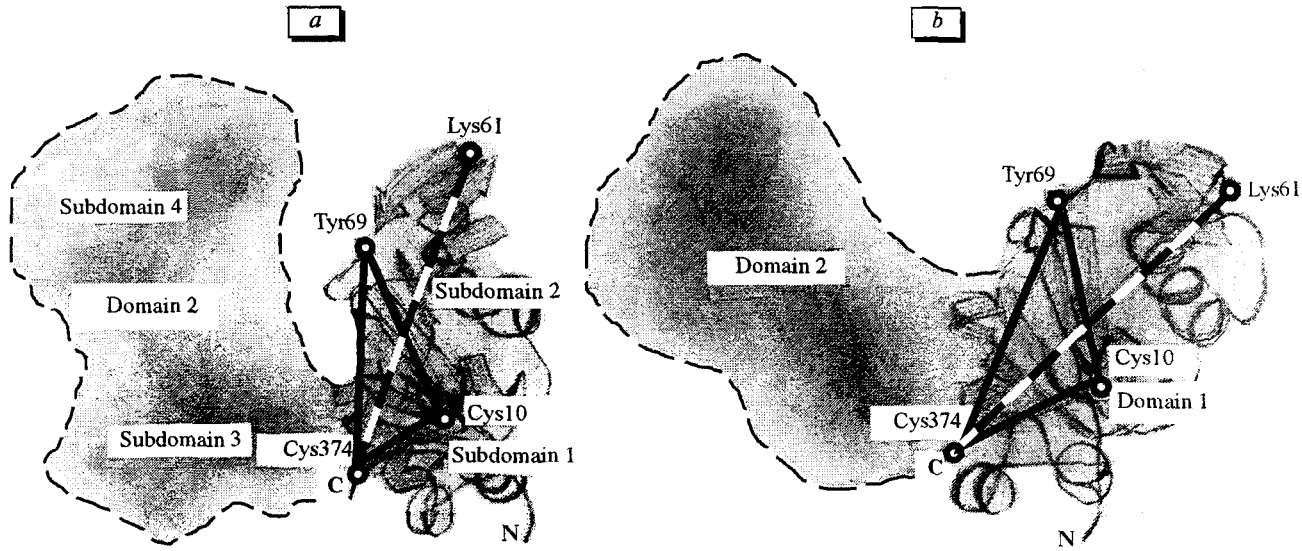

Fig. 2. Hypothetical model of actin monomer under normal conditions (*a*) and in heart failure (*b*). The model is based on actin atomic structure [13,14] and 3D-reconstruction of thin filament in heart failure [8] attesting to an increase in the diameter of F-actin filament.

Attachment of FITC to Lys61 reverses polarity of 1,5-IAEDANS FL in both normal and modified actin, so it changes orientation and microenvironment of Cys374. Maximum of FL emission of FITC-actin in HF did not differ from the control value and even demonstrated a tendency to increase despite a more than 2-fold decrease in the excitation energy (Fig. 1). This indicates a more surface-exposed (less shielded) arrangement of Lys61 in actin from failing heart probably due to opening of the interdomain cleft.

The intensity of FRET between 1,5-IAEDANS-Cys374 and FITC-Lys61 at λ=470 nm in the normal actin was 66±2% of 1,5-IAEDANS-actin FL (FITC did not contribute to FL, so the decrease in the intensity was due to change in FRET). This intensity determines the distance between Cys374 and Lys61 in the monomeric actin: 4.64±0.14 nm (Table 1, Fig. 2). In HF, the intensity of FRET between these centers in actin monomer decreased to 45±1.5%, which indicates an increase in the distance between these amino acid residue by 8 Å or by 20% (p<0.001, Table 1).

Under normal conditions, FRET efficiency in the 1,5-IAEDANS-Dns pair was 21% of FL intensity of 1,5-IAEDANS-actin. The corresponding value in actin from failing heart was 16%, which attests to a 17% increase in the distance between these sites (p<0.001, Table 1). In case of 1,5-IAEDANS-Cys374-DDPM-Cys10, FRET intensity decreased by 30%, reflecting a 17% increase in the distance between centers, without significant changes in donor and acceptor anisotropy.

The distance between two Cys-residues in the N- and C-terminal regions of subdomain 1 of the outer actin domain also increased in HF (Table 1).

Heart failure had no effect on the FL emission maximum of the monomeric Dns-actin (Fig. 3), although FL intensity increased 1.6-fold at the same actin/Dns ratio=1.0 and at the same actin concentration. Additional attachment of DDPM to Cys374 in actin from failing heart decreased FL intensity of Dns-actin by 59% (in normal actin the corresponding value was 46%). After trypsin (0.4 mg/ml) cleavage FL intensity increased to 81 and 75% relatively to FL intensity of cleaved Dns-actin at λ=510±4 nm under normal and HF conditions, respectively. Thus, the corrected transfer efficiencies were 37.5 and 21%, which indicates a 20% increase in the distance between these amino acid residues (Table 1).

Therefore, changes in the orientation and microenvironment of the Lys61 and Tyr69 regions in subdomain 2 of the outer actin domain result from exposition of these centers at the protein surface. It profoundly increases FL emission of the fluorophores at both residues not only in normal, but also in actin from failing heart, which also attests to widening of the cleft between two actin domains.

Polymerization of 1,5-IAEDANS-actin decreased FL intensity by 25%, shifted λ_{max} of FL emission spectrum by 10 nm, and changed considerably the polarization of FL. When equal amounts of actin from failing heart labeled with FITC and 1,5-IAEDANS were polymerized by phalloidin, the efficiency of FRET between Cys374 and Lys61 of adjacent protomers (FRET over one protomer is forbidden) in the resultant F-actin corresponded to a distance of 4.0±0.02 nm between these amino acid residues. For actin from the normal myocardium or skeletal muscle, the corresponding values were 3.43±0.03 nm (p<0.01) and 3.34 nm [16].

Polymerization of monomeric double-labeled actin (Cys374 and Lys61) was accompanied by a multiacceptor energy transfer from the common donor Cys374

to Lys61 in the own and adjacent protomers. In this case, the distances between Lys61 and Cys374 in normal protomer and monomer were 3.92±0.07 and 4.64 nm, respectively, while in actin from animals with HF the corresponding values were 5.36 and 5.43 nm (Table 1). Therefore, incorporation of actin monomer into F-actin filament decreased the distance between Cys374 and Lys61 in actin from normal rabbit myocardium by 18%, and remained unchanged in actin from failing heart. In contrast to normal actin, incorporation of actin monomer from the myocardium of animals with HF into actin filament was not accompanied by structural rearrangement. This inference completely agrees with the circular dichroism data [13, 15]. The distances obtained in our experiments in the presence of regulatory proteins (tropomyosin-troponin complex) characterize the protomer at pCa>8 both under normal and HF conditions. When pCa was decreased to 5 in the presence of 2 mM $MgCl_2$, the distance between Lys61 and Cys374 in the normal protomer increased from 3.9 to 4.45±0.12 nm, while in actin protomer from failing heart this distance remained unchanged at both pCa>8 (4.0±0.07) and pCa=5 (4.1± 0.10 nm).

Moreover, polymerization of normal monomeric actin completely reversed FL anisotropy, while anisotropy of labeled Cys374 in actin monomer and polymer from failing heart remained unchanged. Binding of the second fluorophore (FITC) to Lys61 in normal actin increased FL anisotropy, while in actin from failing heart it resulted exclusively in the extraplanar rotation of the pair.

The distance between 1,5-IAEDANS-Cys374 and Dns-Tyr69 in adjacent protomers was 2.0±0.1 nm in normal actin and 3.56±0.08 in HF. For multiacceptor energy transfer the distance between Cys374 and Tyr69 in the normal protomer decreased to 1.56±0.11 nm (by 40%), while in HF decreased to 2.1±0.1 nm (by 25%).

According to 3D-reconstruction, the major distinction of thin filament in HF is its widening by 40% and changed reflex intensities and their rations [8]. This evidence agrees with our data obtained by FRET method and in light of the modern concept on actin structure [13,14,18] it can be hypothesized that the structure-conformational (recombinant) changes in the outer domain of the protomer increase its length in actin from failing heart (Fig. 2).

Assuming the myosin heads (50 kD of the domain) to interact with actin subdomain 2 during filament sliding, the flexibility and rotation of actin subdomain 2 probably contribute into force generation, while translocation of Lys61 and Tyr69 to the surface can affect force generation and polymerization of actin in HF (Fig. 2). These results and the data on 3D-reconstruction of thin filament from failing heart [8] suggest that mobility of a bulk region of subdomain 2 and the

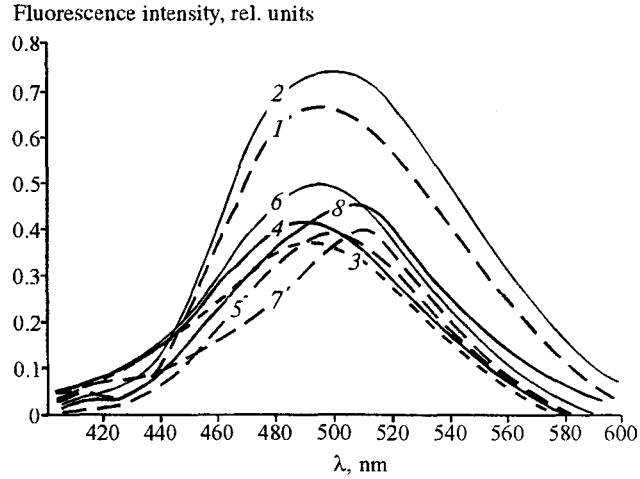

Fig. 3. Corrected fluorescence spectra of Dns-actin (*1,2*), Dns-DDPM-actin (*3,4*), Dns-actin (*5,6*), and Dns-DDPM-actin (*7,8*) cleaved with trypsin under normal conditions and in heart failure.

interdomain link is changed. Since cross-linking between Cys10 and Cys374 prevents actin polymerization but not binding of the subfragment 1 of the myosin head [12,16], it might be conjectured that the decrease in the rigor tension developed by the system of contractile proteins in HF can be related to changes in the N-terminal fragment (1-12 amino acid residues) of actin molecule, which directly participates in the formation of the strong bond with 20-50 kD domains in the myosin head. These changes disturb energy stability of the actomyosin complex.

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