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Structural and Functional Changes in Myocardial Thin Filaments in Experimental Hypothyrosis

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Fluorescence resonance energy transfer study revealed decreased intermonomer mobility of Ca-actin and Mg-actin filaments of myocardial myofibrils in myocardial dystrophy caused by diffuse endocrine disorders, *e. g.* hypothyrosis. Cis374 axial distance in Ca-actin filament protomer increased in hypothyrosis. Intracellular pH has no effect on intermonomer mobility of Ca-actin filament.

Key Words: *hypothyrosis; actin; myocardium; structural and conformation changes*

The development and progress of thyroid hormone deficiency lead to an increase in systemic vascular resistance, impairment of systolic and diastolic functions of the heart, prolongation of diastolic relaxation [3,4], and heart rhythm deceleration [6]. This can be due to changed isoenzyme composition of myosin (the main protein of the thick filament of myofibrils), impaired excitation/contraction coupling, and Ca^{2+} homeostasis disorders [5]. Impairment of contractile activity of myocardial myofibrils in hypothyrosis can be explained by low effectivity of the contraction process, disorders in Ca^{2+} regulation of the contraction process by the thin filament, and changed tertiary structure of actin [1].

We studied conformation mobility of actin, the main thin filament protein, in myocardial contractile dysfunction caused by hypothyrosis.

MATERIALS AND METHODS

The study was carried out on 27 albino rats of both sexes kept under standard vivarium conditions on the same ration. The animals were divided into groups at random: control (n=9) and experimental (n=18). For hypothyrosis modeling, 9 experimental rats received 0.06% propylthiouracyl with drinking water for 3 months.

After euthanasia, the heart was removed and actin was isolated from the left and right ventricles [9]. Ca-F-actin was polymerized from monomeric actin by increasing Ca concentration (pCa) to 4.5 M and KCl concentration to 0.1 M (in a medium containing 0.1 mM $CaCl_2$, 0.2 mM ATP, 10 mM imidazole, pH 7.4 or 6.5) for 12 h at 4°C. For studies of Mg-actin, monomeric actin was obtained as described previously [11] and dissolved in a medium containing 0.2 mM EGTA and 0.1 mM $MgCl_2$ at ambient temperature. Polymerization of Mg-actin was performed by increasing the concentration of KCl to 0.1 M and of $MgCl_2$ to 2 mM at ambient temperature for 2 h. Cis374 in subdomain I of myocardial actin was labeled with 1,5-IAEDANS (N-iodoacetylaminoethyl-5-naphthylamine-1-sulfonate, donor) and 5-IAF (5-iodoacetamidofluorescein, acceptor), the fluorescence spectra of 1,5-IAEDANS actin and 5-IAF actin were recorded, and fluorescence resonance energy transfer (FRET) study was performed [2,11]. The molar ratio of 1,5-IAEDANS to 5-IAF monomeric actin in studies of inter-monomeric mobility during polymerization was 1:10.

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The fluorescence spectra were recorded on an SLM-Instruments 4800 spectrofluorometer in the modulation and phase modes. The temperature in the cuvette was monitored with a miniature thermoresistor; it was 36°C in all cases. The distance between amino acid residues in actin was evaluated by radiation-free electron energy transfer during resonance interactions of the dipole couple of fluorophores. The distance (R) for the donor-acceptor couple was calculated by the formula: $R=R_o[(1-E/E)]^1/_6$, where R_o is a characteristic distance at which the efficiency of fluorescence energy transfer is 50% and estimated by the formula: $R_o^6=8.79\times10^{-25}\times J\times n^{-4}\times k^2\times Q_d$, where J is overlap integral (M^{-1} cm^3), reflecting the degree of overlap between donor emission and acceptor absorption, n is refraction index equal to 1.39 for organic molecules, k^2 is a factor of mutual orientation, Q_d is quantum yield of the donor in the absence of acceptor; E is measured energy transfer efficiency equal to $1-F_{dd}/F_d$, *i.e.* ratio of donor emission to donor fluorescence in the presence of acceptor, respectively. In order to rule out the effect of acceptor fluorescence emission during RTFE, the emission of 1,5-IAEDANS was evaluated for the 400-460 nm interval. Corrected spectra $F_{corr}=F_{abs}$ antilog ($I_{absorption}+I_{emission}$) were used for calculation of J. The mean fluorescence life-time was evaluated [7]. The significance of differences between the means was evaluated using Student's t test.

RESULTS

The fluorescence intensity of 1,5-IAEDANS attached to Cis374 in the monomeric actin subdomain 1 decreased by 2.5 times in hypothyrosis, the maximum fluorescence emission was shifted from 472±2 to 485±2 nm ($p<0.05$). Polymerization of 1,5-IAEDANS actin at pCa=4.5 in hypothyrosis, in contrast to normal myocardial actin, was not associated with the decrease in quantum yield of fluorescence (by 17%), shift of maximum fluorescence emission to the short-wave region (by 6 nm), and drastic changes in the fluorescence anisotropy (by 56%), in other words, the conformation status of Cis374 location area did not change. In parallel, the correlation life-time of excited state of 1,5-IAEDANS attached to myocardial actin Cis374 increased in hypothyrosis by 15.8% at pH 6.5 and by 19.2% at pH 7.4 (Table 1), hence, Cis374 area became more rigid. Despite the fact that correlation life-time of excited Ca-actin in normal myocardium does not depend on pH, the development of acidosis in hypothyrosis transfers the actin filament (at least in areas of Cis374 location) into a more rigid conformation state.

Similarly as with Ca-actin, polymerization of myocardial 1,5-IAEDANS actin in the presence of Mg^{2+} in hypothyrosis was associated with a decrease in fluorescence quantum yield by just 8% (but not with 23% reduction) and fluorescence anisotropy by just 16.5% (but not by 56%, as with normal myocardial Mg-actin). This is paralleled by prolongation of correlation life-time of excited status of 1,5-IAEDANS attached to myocardial Mg-actin Cis374 in hypothyrosis by 15.8% at pH 6.5 and by 19.2% at pH 7.4, *i.e.* the Cis374 area becomes more rigid.

The axial distance Cis374 in the Ca-actin filament protomer in hypothyrosis reaches 2.31±0.09 nm (*vs.* 1.88±0.05 nm in normal; $p<0.01$), in Mg-actin filament 2.12±0.05 (normal 1.94±0.04; $p<0.01$).

In hypothyrosis, the distance between 1,5-IAEDANS (Cis374) and 5-IAF (Cis374) in the neighboring protomers in myocardial Ca-actin filament increased from 4.62±0.04 (normal) to 4.78±0.08 nm.

The Cis374 radial distance in the myocardial myofibrillar actin filament increases significantly (by 0.6 nm) in hypothyrosis at pCa=4.5 (contraction conditions) in comparison with the normal (1.96± 0.06 nm): subdomain 1 is shifted from the filament axis.

TABLE 1. Correlation Life-Time of F-Actin Excited Status of Myocardial in Health and Hypothyrosis ($M\pm m$)

pH group	Ca-actin		Mg-actin	
	1,5-IAEDANS	5-IAF	1,5-IAEDANS	5-IAF
Control				
6.5	18.7±0.6	4.6±0.3	17.9±0.5	4.4±0.3
7.4	18.3±0.5	4.4±0.2	16.6±0.6$^+$	4.6±0.3
Experiment				
6.5	26.9±0.7**	5.4±0.4*	22.3±0.6**$^{++}$	5.8±0.3**
7.4	25.4±0.6**	5.1±0.3*	21.2±0.8**$^{++}$	5.6±0.3**

Note. *$p<0.05$, **$p<0.01$ compared to normal; $^+p<0.05$, $^{++}p<0.01$ compared to Ca-actin.

As Cis374 is located near the center of actin-myosin interaction [8,10], this ncreases the probability of more loose, weaker tangential attachment of the myosin head to actin filament, attenuation of strong force-generating bond in the actomyosin ensemble of myocardial myofibrils in cardiac insufficiency.

These results are in complete agreement with increased diameter of natural actin filament in cardiac insufficiency, detected by optic diffraction and 3D reconstruction method and by direct measurement of the filament diameter on electron microphotographs of F-actin filaments [1].

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