Structural and Conformational Changes in Myocardial and Erythrocyte Actin during Cardiac Ischemia

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Structural and conformational changes in myocardial and erythrocyte actin during cardiac ischemia were studied by the method of fluorescence resonance energy transfer with highly selective fluorescent probes. In contrast to 15-min coronary artery occlusion, 120-min ischemia was accompanied by irreversible structural and conformational changes in the small domain of erythrocyte actin. Posttranslational changes during myocardial ischemia concerned the N- and C-terminal regions of actin and went beyond the allowed conformational fluctuations in the actin molecule without breaking the energy barrier. Our results suggest that under conditions of ischemia, actin of the myocardium and erythrocyte cytoskeleton loses its ability to acquire conformation required for force generation by cardiomyocyte myofibrils and maintenance of elasticity and integrity of the erythrocyte membrane.

Key Words: *structural and conformational state of actin; myocardium; ischemia; NAD; fluorescence resonance energy transfer*

Profound structural and conformational changes in actin (major protein of fine threads in myocardial myofibrils) accompanying 15-min ischemia are followed by a sharp adaptive decrease in the rate of contractions and force generated by contractile proteins of the myocardium [2-4]. Prolonged ischemia is accompanied by an increase in energy deficiency and decrease in the concentrations of ATP and NAD in the myocardium and erythrocytes. These changes decrease contractile activity of myofibrils in the ischemic zone and impair erythrocyte elasticity [1]. Erythrocyte passage through the capillary bed and oxygen supply to peripheral tissues are impaired under these conditions [1,7,13]. Actin is the major protein of the erythrocyte cytoskeleton in humans. Short actin threads are cross-linked with long spectrin molecules and tropomodulin to form a quasi-hexagonal grid [5,7].

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Here we studied changes in the concentrations of adenyl and pyridine nucleotides and conformational state of actin in cardiomyocytes and erythrocytes under conditions of ischemic myocardial injury.

MATERIALS AND METHODS

Experiments were performed on hearts and blood erythrocytes from conventionally healthy dogs (n=12) and animals with coronary artery occlusion (CAO). CAO was produced by ligation of the upper third in the left descending anterior coronary artery for 15 (n=9) or 120 min (n=12). By the 120th minute the *ST* segment was elevated to 7.5±1.7 mm. The maximum rate of the rise and drop in left ventricular pressure was reduced by 62 and 56%, respectively. Minute volume decreased by 34%. Signs of tissue hypoxia and acidosis were found in the ischemic zone. CAO for 15 and 120 min was followed by a decrease in Po_2 by 26.3 and 28%, respectively. Under these conditions pH decreased from 7.20±

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0.03 to 6.90±0.07. The animals were anesthetized with hexenal and euthanized 60 and 120 min after artery ligation.

Erythrocytes were isolated by centrifugation of sodium citrate-stabilized blood at 4300*g* for 10 min. Erythrocyte actin was extracted as described elsewhere [12]. Actin was isolated from the zone of myocardial ischemia [11]. ATP concentration was measured by the luciferin-luciferase method. The concentrations of NAD and NADH were estimated as described previously [5].

Actin was dissolved in medium I containing 0.1 mM $CaCl_2$, 0.2 mM ATP, and 2 mM Tris-HCl (pH 7.4, pCa 4.7). Labeling of Cys374 in subdomain 1 of myocardial actin and recording of 1,5-*IAEDANS* fluorescence spectra were performed at room temperature for 1 h (label/protein ratio 10:1) [10]. Labeled F-actin was depolymerized by intensive dialysis against a solution containing 10 mM KCl, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 20 mM Na-phosphate buffer (pH 6.8) and repeatedly repolymerized. Labeled F-actin was pelleted by centrifugation at 100,000*g* for 1 h. The pellet was dissolved in medium I (G-medium with monomeric actin) and intensively dialyzed against the same medium. The coefficient of absorption for 1,5-*IAEDANS* bound to actin at 336 nm was 6100 $M^{-1}\times cm^{-1}$ [6]. Under normal conditions and CAO for 15 or 120 min, the labeling ratio was 0.82±0.02 and 0.81±0.02; and 0.85±0.03 and 0.82±0.02 mol 1,5-*IAEDANS*/mol monomeric myocardial actin, respectively. In experiments with erythrocytes these values corresponded to 0.80±0.02 and 0.78±0.04; and 0.79±0.02 and 0.83±0.04 mol 1,5-*IAEDANS*/mol monomeric erythrocyte actin, respectively. Labeling of actin by Lys61 was performed using fluorescein isothiocyanate (FITC) at a label/protein ratio of 20:1 for 3 h [10]. FITC concentration was measured spectrophotometrically. The coefficient of absorption at 493 nm was 74,500 $M^{-1}\times cm^{-1}$. Under normal conditions and CAO for 1-2, 15, or 120 min, the labeling ratio was 0.82±0.02, 0.81±0.02, 0.85±0.03, and 0.82±0.02 mol 1,5-*IAEDANS*/mol monomeric myocardial actin, respectively. In experiments with erythrocytes these values corresponded to 0.80±0.02, 0.78±0.04, 0.79±0.02, and 0.83±0.04 mol 1,5-*IAEDANS*/mol monomeric erythrocyte actin, respectively. The overlap integral for 1,5-*IAEDANS-FITC* was $19.6\times10^{-4}\times M^{-1}\times cm^{-1}\times nm^4$. The fluorescence quantum yield was 0.61. Fluorescence spectra were recorded on a MPF-4 spectrofluorometer (Hitachi). Several recordings were performed on a SLM-Instruments 4800 spectrofluorometer. The distance between amino acid residues in actin was estimated by the value of radiationless electron energy transfer during resonance dipole-dipole coupling of fluorophore molecules [8]. The distance for a donor-acceptor pair was calculated as follows:

$$R=Ro\ [(1-E/E)]^{1/6},$$

where E is the estimated efficiency of energy transfer ($1-F_{da}/F_d$, ratio between donor emission and donor fluorescence in the presence of an acceptor, respectively). Ro is a characteristic distance at which the efficiency of energy transfer reaches 50%. This parameter is calculated by the formula:

$$Ro^6=8.79\times10^{-25}\times J\times n^{-4}\times k^2\times Q_d,$$

where Q_d is the donor quantum yield without acceptor; k^2 is the coefficient of mutual orientation; n is the index of refractoriness (1.39 for organic molecules); and J is the overlap integral ($M^{-1}cm^3$) reflecting the degree of overlap between donor emission and acceptor absorption [8]. The results were analyzed by parametric Student's *t* test.

RESULTS

The concentrations of ATP and ADP in the myocardium remained unchanged, while the amount of AMP decreased by 42% after 15-min CAO. Increasing the duration of ischemia to 120 min was accompanied by a decrease in the concentrations of ATP, ADP, and AMP by 65, 32, and 44%, respectively. Similar changes in the concentrations of adenyl nucleotides occurred in erythrocytes. The concentrations of adenyl nucleotides little changed after 15-min CAO. The concentrations of ATP and ADP decreased by 32 and 21%, respectively, after 120-min ischemia. Under these conditions the concentration of AMP remained unchanged. Two hours after CAO the decrease in the energy charge reflecting the reduction of energy supply to the ATP—ADP—AMP system and dysregulation of accumulation and utilization of high-energy bonds in intracellular metabolic processes was more pronounced in the myocardium (compared to erythrocytes). Despite the absence of significant changes in the system of adenyl nucleotides, NAD concentration in the zone of myocardial ischemia decreased by 15% (Table 1). The increase in the duration of ischemia to 120 min was accompanied by a 27% decrease in NAD concentration. Blood NAD level increased after 15-min CAO, but decreased by the 120th minute of ischemia (by 54%). The concentration of oxidized NADH remained practically unchanged under conditions of 15-min CAO, but

increased by 35% after 120-min ischemia. Our results indicate that significant biochemical changes in the early period of myocardial ischemia concern NAD concentration and NAD/NADH redox potential. A negative correlation was found between NAD concentrations in the myocardium and blood during short-term ischemia. These data indicate that NAD is washed out from the myocardium, while the synthesis of pyridine nucleotides does not significantly change. Correlations are not observed under conditions of 15-min CAO, but return to normal after 120-min ischemia.

Energy deficiency in the myocardium and erythrocytes was accompanied by structural-and-conformation changes in actin (major protein of fine threads in myofibrils and erythrocyte cytoskeleton). Fluorescence of 1,5-IAEDANS linked to Cys374 in subdomain 1 of monomeric actin in the zone of ischemia (in the presence of ATP, pCa 4.7) decreased by 1.8 times after 15-min CAO. Fluorescence of the label in erythrocytes did not differ from that typical of monomeric actin in normal erythrocytes. Conformational mobility of Cys374 in actin decreased in the zone of myocardial ischemia. The lifetime of excited 1,5-IAEDANS (Cys374) increased from 18.06±0.7 (normal) to 24.2±1.3 nsec ($p<0.001$). These changes reflect a stronger binding of 1,5-IAEDANS to Cys374 of actin in the ischemic zone. The lifetime of erythrocyte actin little changed after 15-min ischemia (15.9±0.9 and 16.8±1.3 nsec). The fluorescence emission maximum of erythrocyte actin was shifted from 467±2 to 478±2 nm ($p<0.05$).

CAO for 15 min was accompanied by changes in the location of FITC linked to Lys61 in monomeric actin. Lys61 is located on the surface of subdomain 2 near the cleft. These changes in the ischemic zone were more pronounced than in erythrocytes. Similarly to monomeric actin in skeletal muscle myofibrils, the monomer of myocardial and nonmuscle actin represent a kidney-like two-domain structure (6.7×4.5×4 nm) with a deep cleft between domains (amino acid residues 62-74 and 83-90) [5,9]. The presence of β-structures in monomeric actin (18% of all structures) provides high cooperativeness of conformational changes in the actin molecule [9,10]. The lifetime of excited-state actin in the zone of myocardial ischemia and erythrocytes was 4.25±0.08 nsec (*vs.* 3.98±0.04 nsec under normal conditions, $p<0.05$) and 4.30±0.03 nsec (*vs.* 4.08±0.04 nsec under normal conditions, $p<0.05$), respectively. Fluorescence of FITC-actin increased by 18 and 12%, respectively. Thus, conformational and structural changes in Lys61 are accompanied by a shift toward lower chemical shielding (despite a slight decrease in conformational mobility).

TABLE 1. Concentration of Pyridine Nucleotides in the Myocardium and Erythrocytes during Myocardial Ischemia (*M*±*m*, μmol/mg protein)

Group	Control		CAO			
			15 min		120 min	
	myocardium	erythrocytes	myocardium	erythrocytes	myocardium	erythrocytes
NAD	5.45±0.21	18.2±0.4	4.55±0.32**	26.7±.3.0***	3.9±0.2	8.50±0.95***
NADH	3.9±0.5	19.9±0.4	4.5±0.3	27.9±1.8**	5.3±0.8*	31.5±2.3***
NAD+NADH	9.4±0.4	38.2±2.4	9.1±0.3	44.5±0.5	9.2±0.6***$^{+}$	40.0±1.0***$^{++}$
NAD/NADH	1.2±0.4	0.95±0.10	0.96±0.15	0.6±0.2*	0.74±0.11	0.27±0.04***

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to normal; $^{+}p<0.05$ and $^{++}p<0.001$ compared to 15-min CAO.

TABLE 2. Distance between Cys374 and Lys61 in Monomeric Actin of the Ischemic Zone in the Left Ventricle and Erythrocyte under Conditions of CAO (*M*±*m*, nm)

Amino acid residues, distance, nm	Normal		CAO			
			15 min		120 min	
	myocardium	erythrocytes	myocardium	erythrocytes	myocardium	erythrocytes
Cys374 and Lys61	4.68±0.18	4.72±0.08	5.0±0.1*	4.87±0.09	5.32±0.08**	5.27±0.10**

Note. *$p<0.05$, **$p<0.001$ compared to normal.

During excitation of 1,5-IAEDANS (Cys3740) a change in fluorescence of FITC (Lys61) due to fluorescence resonance energy transfer in the active zone of ischemia did not differ from normal or became more pronounced (despite a 2-fold decrease in the degree of excitation). The observed changes in erythrocyte actin even exceeded the normal. These variations occur in the early period of heart failure and are associated with changes in profile characteristics (geometry) of the cleft between domains 1 and 2 of actin (particularly in myocardial actin). After 15-min CAO the distance between Cys374 and Lys61 in monomeric actin of the ischemic zone and erythrocytes increased by 0.31 (15%, $p<0.01$, Table 2) and 0.15 nm (8.5%, $p<0.05$), respectively. The maximum shift in amino acid residues under physiological (energetically allowed) fluctuations of free energy does not exceed 0.25 nm [14]. Therefore, the change in the distance results from a significant decrease in internal energy and structural damage to actin. It should be emphasized that these reconstructions were not observed in erythrocyte actin. It probably reflects a reversible adaptive shift in the conformation of actin.

After long-term ischemia (120 min) structural and conformational changes in the site of Lys61 and Cys374 far surpassed conformational changes allowed by low-energy functional transitions. It was found not only in actin of the ischemic zone, but also in actin of the erythrocyte cytoskeleton. The hardly reversible structural and conformational changes in erythrocyte actin are accompanied by breaking the energy barrier and occur at the posttranslational level. They probably result from energy deficiency (ischemia) and underlie a sharp decrease in elasticity of erythrocytes and impairment of oxygen transport function during myocardial ischemia.

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