Conformational State of Thin Myofilament Proteins in Normal and Chronically Failing Heart

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Abstract.Circular dichroism (CD) spectra of myocardial G-actin significantly differ from those of F-actin, and the spectra of G- and F-actins differ from those of myocardial tropomyosin, native tropomyosin and α -actinin. In heart failure in man and experimental animals, characterized by a significantly decreased ability of the contractile protein system to generate force, considerable changes in the tertiary structure of Straub G-actin are observed. During polymerization a monomer of this actin is included in F-actin as a protomer without corresponding conformational changes of a part of G-actin globule; G-actin from the failing myocardium loses its conformational mobility. According to CD data the secondary protein structure is not altered. CD spectra analysis with regard to the regions of aromatic amino acid residue localization in active sites of actin suggests that the sites of actin-myosin and actin-actin interactions do not assume the conformation necessary for normal functioning of thin filaments.

Key words: Human and animal myocardium — Heart failure — Contractile protein conformation

Introduction

Earlier (Karsanov et al. 1986; 1988) it has been shown that the Straub actin from the myocardium of human and animal failing heart is polymerized significantly less than the normal one; at the same time, tropomyosin, α - and β -actinin interaction sites on actin and actin interaction sites on these proteins remain unchanged. The decline of actin polymerization activity is due neither to the influence of α - and β -actinins nor to the proteins of tropomyosin—troponin complex present in Straub actin.

The data shown in the present communication suggest significant changes to occur in heart failure in Straub G- and F-actin conformation, in the regions of aromatic amino acid residue localizations which lose polymerizability, and consequent changes in the nature of actin-myosin interaction.

Materials and Methods

Autopsy and experimental material was used. The autopsy material was obtained from two groups of patients who had died of: acute heart failure due to myocardial infarction (6 cases) and congestive heart failure due to cor pulmonale (6 cases). Effectively healthy persons who had died of grave physical traumatisms were included in the control group (6 cases).

The experimental part of the work was performed on dogs and rabbits with various heart lesions. Four groups were formed: i) 5 dogs with 2-month coronary occlusion (CAO); ii) 9 dogs with cor pulmonale (CP) of 2 -3 months duration: iii) 6 dogs with athyroid eardiomyopathy (ACM) of 2-3 months duration; iv) 8 rabbits with toxo-allergic myocarditis (TAM) of 10-days duration.

Control data were obtained for Straub G- and F-actin from 5 normal dogs and 6 rabbits.

CAO was produced by ligation of the anterior descending branch of the coronary artery in its upper third; CP was induced by daily insufflations of air into the pleural cavity (400-700 ml) during two months; in this way, the intrapleural pressure at expiration increased to 20-40 mm H₂O (Govorov 1952). ACM animals underwent thyroidectomy (Kabak 1968) and TAM was induced according to Andreev and Sokolov (1968).

The autopsy material was withdrawn within 10-20 hours of death. The experimental animals were sacrificed under hexenal anesthesia simultaneously with the controls.

Actin was obtained from myocardium by the method of Spudich and Watt (1971) and Straub (1943) (the extraction was carried out with bidistilled water for 2 h at 0° C), tropomyosin was prepared according to Bailey (1948), native tropomyosin and α -actinin by the method of Ebashi et al. (1966), and thin myofilaments by the modified technique based on the method of Driska and Hartshorne (1975) and Obinata et al. (1974).

Circular dichroism (CD) spectra were recorded by means of a Cary-60 spectropolarimeter fitted with a CD attachment (Model 6001). It was calibrated according to the ellipticity value of 0.1% solution of d-10-camphorsulfonic acid at 290 nm. The CD spectra were recorded in the far-(200-250 nm) and near- (250 -300) UV regions. When studying the spectra in the far-UV region a cuvette with an optical pathlength of 0.5 mm was used, whereas a cuvette with 1 cm pathlength was used for the near-UV region. Protein concentration was 1-2 mg/ml.

The specific ellipticity was calculated by the equation: $\left[\Theta\right] = \frac{M \Theta^{\circ}}{100 \, lC}$ (Balasubramanian

and Kumar (1976), where Θ° is ellipticity in degrees, *l* is the cell optical pathlength in cm, *C* is the protein concentration in g/ml, *M* si the molecular mass, which in the of far-UV region was taken equal to 115 (the average molecular mass of amino acid residue in protein) and in the near-UV region it corresponded to the molecular mass of the protein studied. The secondary structure was calculated according to Chen et al. (1974).

UV absorption spectra were recorded with a double beam spectrophotometer Cary—118 C. The protein concentration was determined spectrophotometrically using absorption coefficient of $A_{290}^{0.1\%} = 0.63$ for purified G-actin (Houk and Ue 1974); Straub actin and other proteins were determined by the biuret method (Bailey 1962).

SDS polyacrylamide gel electrophoresis of proteins was performed according to Laemmli (1970).

The contractility of the cardiomyocyte contractile protein system was estimated from contractile properties of myocardial glycerinated fiber bundles (MGFG) prepared according to Szent-Gyorgyi's (1949) method, in auxotonic regime of contraction, tensometrically (Karsanov et al. 1971).

MGFB data and CD curves were processed statistically, using the formulas for few observations and inequal groups. The significance (P) of differences in mean values was estimated according to Student's *t*-test (Bailey 1959).



Fig. 1. Near-UV CD spectra of myocardial actin: Straub actin of man (full line), dog (triangles), rabbit (crosses); Spudich and Watt actin of dog (filled circles), thin filament of dog myocardium (empty circles); m.psoas of rabbit (dotted line). A - G-actin, B - F-actin.

Results

CD spectra of thin myofilament proteins from normal myocardium. Far-UV CD spectra of myocardial G-actin and also that of Straub and those of thin myofilaments from dog myocardium appeared practically identical. They showed a negative maximum at 208 nm and a shoulder at 220 nm. The spectra of these actins and of the thin myofilament from dog myocardium coincided with those of Straub actin from the human and rabbit myocardium. Calculations revealed similar contents of α -helix, β -structure and random-coil conformation in Straub actin thin myofilament from the canine myocardium and in actin purified according to Spudich and Watt (the differences up to 3 % were insignificant).

Near-UV CD spectra of Straub G-actins from the human and animal myocardium (dogs, rabbits) as well as those of Spudich and Watt actins from the canine myocardium and rabbit m.psoas were also alike. With ignoring the differences observed at 255 nm (due to high noise in this region and to difficulties to find the peak locations of the spectra bands) between Spudich and Watt actin and Straub actin from dog myocardium no significant differences were found (Fig. 1*A*).



Fig. 4. CD spectra of Straub actin dog myocardium: normal (full line), coronary artery occlusion (dashed-dotted line); pellet (crosses) and supernatant (dashed-double dotted line) obtained by centrifugation $(10^{5}g x 3h)$ of myocardial F-actin in coronary artery occlusion.

and by that developed MGFB of the left ventricle was 1.54 ± 0.17 and $1.46 \pm 0.13 \text{ mN/mm}^2$, respectively. The control values were 2.75 ± 0.22 and $2.44 \pm 0.13 \text{ mN/mm}^2$ for the right and left ventricle respectively. These data are consistent with those obtained previously (Karsanov 1979).

The considerable decrease in contractility of the conctractile protein system from acutely and chronically failing human myocardium is accompanied by a sharp change of CD spectra of Straub G-actin at 250—300 nm: the intensity of the positive ellipticity of bands at 268—272 nm rises sharply, while the negative ellipticities at 286 and 292 nm become positive (Fig. 3.4).

During polymerization of Straub actin of the failing heart myocardium the bands at 255—278 nm did not become negative, as it is the case in normal heart, but remained positive, with intensities not below those of bands of normal Straub G-actin. The blue shift of these bands did not occur either. However at 287 and 292 nm, as with normal heart, the intensity of these negative bands was significantly increased and the bands shifted by 1.5 nm to the left. The changes in CD spectra of F-actin were similar in acute and congestive heart failure (Fig.



Fig. 5. CD spectra of Straub actin of dog myocardium: normal (full line), cor pulmonale (dotted line), athyreosis (dashed line). A = G-actin, B = F-actin.

cantly increased and the bands shifted by 1.5 nm to the left. These changes in CD spectra of F-actin were similar in acute and congestive heart failure (Fig. 3B). In acute heart failure the content of α -helix in G-actin was 29 ± 3 %, that of β -structure was 22 ± 3 %, and random-coil conformation was 49 ± 5 %; the respective values for G-actin in congestive heart failure were 31 ± 3 , 25 ± 5 and 44 ± 4 %. These values showed no marked changes after polymerization.

Contractility of MGFB, and CD spectra of the thin filament proteins in experimental heart damages. Similarly as in human myocardium, MGFB of experimental animals with various myocardial damages developed considerably weaker tension than those of the control group animals: the decrease in tension developed by MGFB in CAO, CP and ACM, as compared to the control group, was 33 %, 26 % and 41 %, respectively, for the right ventricle and 26 %, 21 % and 42 %, respectively, for the left one. The tension developed by MGFB of the TAM rabbit for left heart ventricle was by 43 % below normal. These data are also in agreement with the data obtained earlier (Karsanov 1979).

The 200—250 nm range spectra of Straub G- and F-actins of all experimental animals were similar to those of control animals, like with human Straub actin.



Fig. 6. CD spectra of Straub actin of rabbit myocardium: normal (full line), toxoallergic myocarditis (dashed line). A - G-actin, B - F-actin.

At 250—300 nm, similarly as for human failing heart, CD spectra of myocardial Straub G-actin in CAO (Fig.4), CP (Fig.5) and ACM (Fig.5) animals were significantly different from the control ones: the differences concerned the region of 255—278 nm in CAO (Fig.4), 255—292 nm in TAM (Fig.6) and 285 —292 nm in CP and ACM animals (Fig.5).

For F-actin the peaks at 255—276 nm remained positive in CAO, ACM, CP and TAM animals (Figs.4,5,6), similarly as it was the case with human heart failure; they do not turn negative value, as observed with normal tissue. Peak positivity was observed at 265—285 nm. In ACM animals (Fig.5) the changes concerned also the band at 292 nm. The secondary structure of actin remained unchanged regardless of the pathologic state studied.

CD spectra of tropomyosin and its complex with troponin (native tropomyosin) extracted from ACM canine myocardium (n = 6) and CAO animals (n = 3) were identical and almost overlapped with those of tropomyosin and native tropomyosin of normal animals.

The presence of a normally conformed actin fraction in Straub actin from the failing heart. In CAO animals a normally polymerized actin fraction was pre-



Fig. 7. SDS-gel electrophoresis of myocardial Straub actin of dog with CAO: A — initial; B — pellet; C — supernatant obtained after centrifugation at $(10^5 g \text{ x h})$.

cipitated from canine myocardial Straub F-actin using centrifugation at 100,000 g for 1 hour. Poorly polymerized or nonpolymerized actin remained in the supernatant. SDS polyacrylamide gel electrophoresis of the precipitated actin revealed one intensive band corresponding to that of actin (containing 94% of protein material) and very weak bands corresponding to trace amounts of troponin-tropomyosin complex proteins. In the supernatant the amount of actin was reduced to 45% of protein material and that of tropomyosin increased to 18%; troponins increased to 25%, α -actinin to 3% and non-identified proteins made up 9% (Fig.7).

The precipitated actin showed CD spectra typical of Spudich and Watt actin, but the supernatant proteins showed a pronounced spectrum pattern of failing heart actin (Fig.4). All the ellipticities, as with human failing heart actin, were located in the positive area of the spectral curve despite the presence of significant amounts of tropomyosin-troponin complex proteins (43 %), with negative ellipticity (Fig.2).

Large amounts of material were needed for this experiment and Straub actin was extracted from pooled acetone dried myocardial powder of five CAO dogs. Thus, the obtained results represent mean values of 5 experimental animals.

Discussion

The results of this study suggest that myocardial actin (monomeric and polymeric), α -actinin, tropomyosin and also ist complex with troponin (native tropomyosin) have specific CD spectra in the range of 200—300 nm, and that the secondary structure and conformation of these thin filament proteins in the regions of aromatic amino acid residue localization correspond to those of Gand F-actins (Hegyi and Venyaminov 1980; Murphy 1971; Nagy and Strzelecka-Golaszewska 1972; Oriol et al. 1977; Ptitsyn et al. 1975), tropomyosin (Nagy 1977; Staprans and Watanabe 1970) and native tropomyosin (Staprans and Watanabe 1970) from skeletal muscles.

A comparative analysis of CD spectra of Spudich and Watt G- and F-actins from the myocardium and m.psoas of rabbit as well as Straub actins of human, dog and rabbit myocardium, supports the belief that both the secondary structure and conformation of the aromatic amino acid residues microenviroment of the normal actins lacks specific and tissue features: slight differences in the primary structure of myocardial and skeletal mucsle actins (Elzinga et al. 1973; Vandekerckhove and Weber 1979), concerning differences in 4 amino acid residues (Vandekerckhove and Weber 1979) do not affect significantly the secondary structure and conformation of the regions of aromatic amino acid residue localization.

Sharp changes in the optical activites of phenylalanyl (255-265 nm (Horwitz et al. 1969; Murphy 1971)), tyrosyl (272-287 nm (Murphy 1971; Strickland et al. 1972; Su and Jirgensons 1977)) and tryptophanyl (287-292 nm (Murphy 1971; Strickland et al. 1971)) amino acid residues observed during G-actin polymerization without any changes in protein optical activity in the range of 200-250 nm suggest that incorporation of G-actin into F-actin significantly changes the tertiary structure conformation, at least in the regions where a certain part of aromatic amino acid residues is localized. The change in the conformation of these regions is also suggested by the blue shift of spectral bands which means that aromatic amino acid residues, due to a change in their microenvironment conformation, plunged into the areas characterized by a greater hydrophobicity, i.e. become less accessible to water (Murphy 1971; Vedenkina et al. 1972); hydrophobic bonds are formed, probably to stabilize (Cantor and Schimmel 1980) the actin structure. The same picture is observed with skeletal mucsle actin polymerization (Murphy 1971; Oriol et al. 1977). In this case Cys-374, for example, becomes less mobile (Brauer and Sykes 1986).

Thus, the obtained data suggest that polymerization of normal actin is associated with the formation of a new and more stable conformational state of actin protomers. This is necessary for filament self-assembling^{*} and for the formation of sites capable of normal functioning, particularly, the sites of a-actinin (Estes and Gershman 1978), myosin and tropomyosin interaction."

The high specific optical activity of actin and a significantly smaller one of proteins of tropomyosin-troponin complex and α -actinin made CD a very sensitive by itself method of investigation of local conformational changes in proteins (Cantor and Schimmel 1980), an effective technique of choice for studies of the failing heart actin structure in crude preparations (containing 71 – 77% of the main protein (Karsanov et al. 1986)) contaminated both with the tropomyosin-troponin complex proteins (up to 15%) and with small quantities of α -actinin. Our experiments have shown that "contamination" of the purified actin even with significantly larger quantities of tropomyosin-troponin complex proteins (Karsanov et al. 1986) does not significantly change CD spectra within both 200–250 nm and 250–300 nm.

With Straub actin from failing heart with ellipticity of bands in the range of 255—272 nm which remain markedly positive after polymerization, the presence of tropomyosin-troponin complex proteins and α -actinin as contaminants would only lead to a decrease of the positive band intensity, to a modulation of a markedly pathologic picture rather than to its intensification. This is also confirmed by the fact that in CAO and ACM animals the optical activity of tropomyosin-troponin complex is not changed and remains negative over the whole spectral range.

The above results allow the interpretation of the data obtained on crude actin preparations as being due to changes in actin conformation. The CD method can be employed for the investigation of failing heart when actin is purified by polymerization—sedimentation—depolymerization cycles leading to a loss of the pathologically changed part of actin (Karsanov et al. 1986 and this paper).

In acute and chronic heart failure in man as well as with different experimental heart damage (CAO, CP, ACM and TAM), associated with a reduced ability of the myocardial myofibril contractile protein system to generate force and perform work (Karsanov 1979), the optical activity of phenylalanyl and

^{*}Conformational changes in actin globule of skeletal muscles under the influence of salts have been shown to occur before actin polymerization. (Frieden 1983; Frieden et al. 1980; Gershman et al. 1983; Harwell et al. 1980; Ohara 1981; Rich and Estes 1976; Rouayrenc and Travers 1981; Tellam 1985).

[&]quot;Neither tropomyosin, nor troponin-tropomyosin complex can form complexes with G-actin (Chantler and Gratzer 1976; Lal and Korn 1986) even when the monomer is in F-conformation (Estes and Gershman 1978; Ohara et al. 1981; Rouayrenc et al. 1985) and if myosin subfragment S_1 does form G-acto- S_1 complex, there is no activation of its Mg-ATPase activity, unlike with F-actin (Chantler and Gratzer 1976); α -actinin does not form complexes with G-actin either (Holmes et al. 1971).

tyrosyl amino acid residues in the range of 255—272 nm does not turn negative during polymerization (as it is the case with normal tissue) but remains markedly positive; the amino acid residues do not plunge into more hydrophobic ("oily") regions of the molecule and there probably is no formation of hydrophobic clusters. This means that G-actin of the failing heart is incorporated in F-actin as a protomer without a proper change in conformation observed in normal tissue and necessary for both protomer stabilization and active site formation. In this case the absence of changes in the secondary structure shows that the protein as such remains native.

The identity of CD spectra for both Straub G- and F-actins in the human and animal myocardium (both normal and failing) provides good reasons to conclude that after death the actin structure does not undergo detectable changes at least during the first 20 hours, and that the differences observed whith failing heart tissue are not due to postmortem alterations or those of agonal period. Rather, they develop during the lifetime. This conclusion is consistent with the data that fibrillar actin is not exposed or only slightly exposed to proteolysis (Rich and Estes 1976). Besides, the postmortem intact heart F-actin remains in complex with tropomyosin, which stabilizes the filament and prevents its fragmentation (Wegner 1979; 1982).

This conclusion as well as the identity of CD spectra of Spudich and Watt actin and the myocardial thin myofilament justifies extrapolation of data obtained in vitro for Straub actin to the myocardial thin myofilament in vivo, its main protein actin, and to assume that the process occurring (or rather not occurring) during actin polymerization in heart damage plays an important role in the reduction of the ability of the myocardial protein system to generate force and perform work (Karsanov et al. 1985).

His-40 (Hegyi et al. 1974), the region of 52—62 amino acid residues (Bender et al. 1976; Burtnick 1984), Tyr-69 (Chantler and Gratzer 1975) and 3-met-His--73 (Johnson and Perry 1970) are responsible for actin polymerization. The chemical modification of Tyr-53 (Bender et al. 1976) and Lys-61 (Burtnick 1984) completely blocks polymerization while that of His-40 and Tyr-69 slows it down. Hence, two aromatic amino acid residues in the N-terminal part of actin molecule are among the residues responsible for (or involved in) actin polymerization should be fully reflected also by alteration of tryptophanyl peak ellipticity, since Trp-74 is bound to 3-met-His-73 which, when modified, also blocks polymerization completely (Johnson and Perry 1970).

Modification of Cys-374 (Brauer and Sykes 1986; Porter and Weber 1979) and Lys-373 (Detmers et al. 1981) does not prevent polymerization but "responds" to it, and since Phen-375 is bound to Cys-374, this aromatic amino acid residue should also "respond" to actin polymerization. It can be presumed that

Cys-217 (amino acid residue binding ATP (Faust et al. 1974; Miki and Mihashi 1978)) "responds" to polymerization as well, and so does Tyr-218 associated with it. During polymerization ATP is hydrolyzed and this is accompanied by conformational changes of the above mentioned region of actin (Carlier et al. 1984; West 1970).

The myosin heavy chain is also attached to the N-terminal part of actin in the region of the first eleven amino acid residues (Duke et al. 1976; Sutoh 1982). Consequently, the conformational changes in this region can apparently reflect the change of Phen-21 optical activity. The myosin alkaline light chain LC₁ binds to actin in its C-terminal part (Sutoh 1982), to the 361-, 363- and 364-amino acid residues (Duke et al. 1976). Tyr-362 is located between 360- and 363-amino acid residues, Trp-366 is next to Glu-364 and Phen-375 is at some distance. Hence, alteration of tyrosin, tryptophanyl and phenylalanyl peak ellipticities can reflect conformational changes in the binding sites of the heavy and light myosin chains.

Tropomyosin binds to actin with its one part attached to Arg-95 (Johnson and Blazyk 1978), the second part to Lys-238 (El-Saleh et al. 1984) and the third part to the C-terminal in the region common with myosin (Tao and Cho 1979). This means that Tyr-71, Phen-90, Tyr-212 and -362 and Phen-375 can respond to conformational changes of tropomyosin binding sites.

It can be suggested from these data that changes in optical activity of tyrosyl, tryptophanyl and phenylalanyl in actin polymerization reflect the state and conformational rearrangements in functionally active sites of G- and F- actins which are responsible for actin-actin, actin-myosin and actin-tropomyosin interactions.

The fact that the C-terminal part of actin polypeptide chain "responds" to the process of polymerization is apparently due to polymerization concurrent with conformational rearrangements of this part of molecule which is necessary, as it has been mentioned above, for normal functioning of actin globule as a thin filament protomer. According to our unpublished data, this occurs as early as either in the phase of activation or in that of nucleation of actin protofibril formation.

The data of our recent work (Karsanov et al. 1988) showed that the sites of tropomyosin and α - and β -actinin interaction of F-actin are not changed in heart failure. Hence, the conformation of the microenvironment of these sites is not changed either. This means that alterations of tyrosyl and phenylalanyl optical activities, together with the change of tryptophanyl peak ellipticity, should reflect rearrangements of actin-actin and actin-myosin interaction sites. Hence, the absence of changes of phenylalanyl and tyrosyl optical activities in the range of 255–272 nm during polymerization of failing heart actin is indicative of the absence of corresponding conformational rearrangements in N- and C-terminal parts of actin polypeptide chain where the sites responsible for polymerization and myosin interaction are located. We believe that in heart failure these events underly the decrease in actin ability to polymerize (Karsanov et al. 1986), the weakening of bond strength between F-actin protomers (Karsanov et al. 1978) and the decline in hybrid actomyosin ability to generate force (Karsanov et al. 1985).

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