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The purification of cardiac myofibrils with Triton X-100

R. JOHN SOLARO, DAVID C. PANG and F. NORMAN BRIGGS

Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pa., and the Department of Physiology, Medical College of Virginia, Richmond, Va. (U.S.A.)

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SUMMARY

Cardiac myofibrils treated with 1% Triton X-100 are virtually free of contamination by mitochondrial, sarcolemmal and sarcoplasmic reticular membranes. The ATPase activity of such myofibrils corresponds closely to that reported for cardiac actomyosin and the sensitivity of this ATPase activity to calcium control is normal.

Although myofibrils have proved useful for the study of the contractile proteins of skeletal muscle, cardiac myofibrils have not been very satisfactory because of contamination by mitochondria, sarcolemma and sarcoplasmic reticulum[1,2]. Since these contaminants are membrane structures and since Triton X-100 has been shown to solubilize such membranes[3-5] and not to affect the intrinsic viscosity of myosin or tropomyosin[6], we have attempted to use this nonionic detergent to purify cardiac myofibrils.

Cardiac myofibrils were prepared as follows: Dog hearts, removed from animals anesthetized with pentobarbital, were immediately washed with 0.9% NaCl at 4°. Fat and connective tissue were trimmed away, and the muscle was cut into small pieces for homogenization in a Sorvall Omnimixer. This and all subsequent steps were performed at 0–4°. The muscle was homogenized with 4 volumes of 0.3 M sucrose containing 10 mM imidazole (pH 7.0) for 1 min. The homogenate was centrifuged at 17 300 x *g* for 20 min. The pellet from this spin was resuspended to the original homogenate volume using a salt solution of the following composition: 60 mM KCl, 30 mM imidazole, pH 7.0, and 2 mM $MgCl_2$ (standard buffer solution). During this and subsequent resuspensions, care was taken to leave behind the coarse bottom layer of debris. This suspension was then homogenized as before and centrifuged for 15 min at 750 x *g*. This sequence of resuspension, homogenization and centrifugation was repeated 4 more times. The homogenate obtained after this series of steps was light brown in color and termed washed myofibrils. After this series of

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid.

steps the myofibrils were suspended again in standard buffer solution containing 2 mM ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA) and centrifuged at 750 × *g* for 15 min.

Further purification of the myofibrils was achieved by resuspending the EGTA-treated, washed myofibrils in 8 pellet volumes of standard buffer containing 1% Triton X-100. The myofibrils were then centrifuged at 750 × *g* for 15 min. This, and all subsequent homogenizations, were carried out using a teflon-glass hand homogenizer. The Triton X-100 treatment was repeated once. The resulting pellets were washed 4 times with 8 pellet volumes of standard buffer to remove the Triton X-100. The purified myofibrils were then resuspended in standard buffer to a protein concentration of 10–15 mg/ml. Myofibrillar ATPase was determined from the rate of release of inorganic phosphate in an incubation medium of 2 mM ATP, 2 mM $MgCl_2$, 50 mM KCl and 20 mM imidazole, pH 7.0 Azide-insensitive and EGTA-insensitive ATPases were measured in the presence of 10 mM sodium azide and 1.6 mM EGTA, respectively. $(Na^+ + K^+)$-ATPase was measured as described by Post and Sen[7], except that Na^+ and K^+ were present in all tubes. Inorganic phosphate was determined by the method of King[8]. In the experiments with quinidine, the alkaloid was added to the standard phosphate solutions. Cytochrome oxidase was assayed spectrophotometrically by observing the rate of oxidation of ferrocytochrome *c* at 550 nm[9] All experiments were completed within 24 h.

The object of this investigation has been to determine if the nonionic detergent

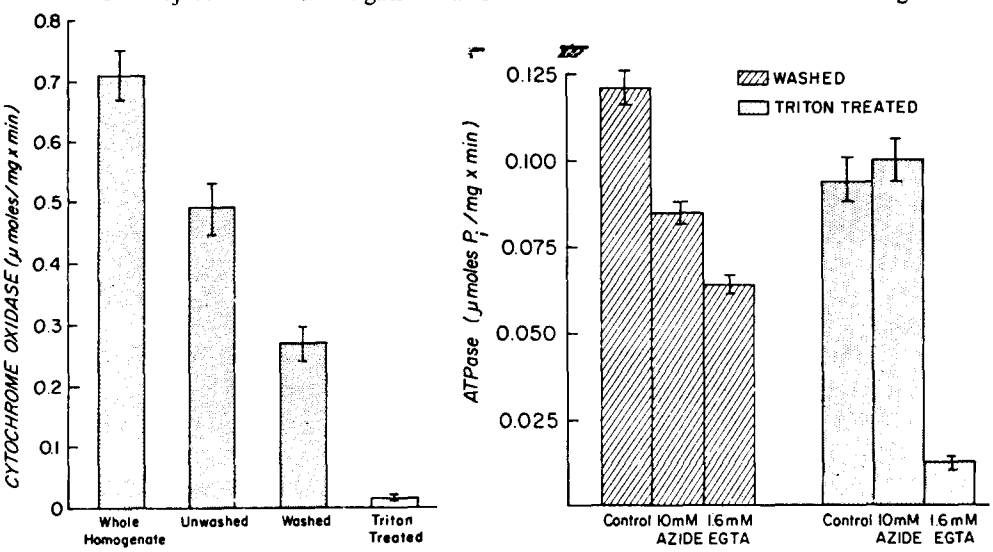

Fig. 1. Cytochrome oxidase activity of cardiac myofibrils. The protein fractions (0.1–0.2 mg/ml) were incubated in the medium containing 0.5 μM ferrocytochrome *c* and 0.01 M potassium phosphate buffer, pH 7.0, at 37°. The oxidation of the substrate was followed by observing the decrease in absorbance of the incubation medium at 550 nm every 15 sec for 5 min. The values are expressed as means ± S.E.

Fig. 2. Effect of azide and EGTA on cardiac myofibrillar ATPase activity. The protein fractions (1–2 mg/ml) were incubated in a medium containing 2 mM ATP, 2 mM $MgCl_2$, 50 mM KCl and 20 mM imidazole, pH 7.0, at 27°. Azide-insensitive and EGTA-insensitive ATPases were measured in the presence of 10 mM azide and 1.6 mM EGTA, respectively. The values presented are expressed as means ± S.E.

Triton X-100 can be used to remove the contaminating membranous materials present in cardiac myofibrils prepared by classical methods. The extent of mitochondrial contamination of myofibrils (as indicated by cytochrome oxidase activity) is shown in Fig. 1. Even after 8 washings with standard buffer solution, cytochrome oxidase activity was still detectable in the washed myofibrils (0.27 μmole/mg per min). Treatment with Triton X-100 reduced this enzyme activity to nearly immeasurable levels (0.01 μmole/mg per min). Since the cytochrome oxidase activity of a cardiac mitochondrial fraction measured in our laboratory was 4.68 ± 0.42 μmoles/mg per min, one can estimate that the level of contamination by mitochondrial protein in the Triton-treated myofibrils was only 0.3%. This low level of contamination is also confirmed by the observation that Triton X-100 treatment completely removes all azide-sensitive ATPase activity[10] from myofibrillar preparations (Fig. 2).

A second type of membrane which might be expected to contaminate cardiac myofibrils is the sarcolemma, since the cell surface to cell volume ratio in cardiac muscle fibers is much greater than in skeletal fibers. The $(Na^+ + K^+)$-ATPase activities of the treated and nontreated myofibrils are shown in Table I. Triton X-100 reduced this ATPase activity by 95%. Using the specific activity of purified sarcolemmal $(Na^+ + K^+)$-ATPase given by Schwartz *et al.* (14.0 ± 0.7 μmoles/mg per h)[11], one can calculate that the extent of contamination by this membrane enzyme before Triton was 8.6% while after treatment the contamination was reduced to 0.4%.

TABLE I

$(Na^+ + K^+)$-ATPase ACTIVITY OF CARDIAC MYOFIBRILS

The protein fractions (1–2 mg/ml) were incubated in a medium containing 3 mM ATP, 100 mM NaCl, 29 mM KCl, 5 mM $MgCl_2$, 0.5 mM H_2Na_2EDTA, 20 mM imidazole, pH 7.0, and 20 mM glycylglycine, pH 7.0, with and without 0.166 mM ouabain for 10 min at 37°. The values presented are expressed as means ± S.E.

Myofibrils	*$(Na^+ + K^+)$-ATPase (μmoles P_i/mg per min)*
Washed	0.020 ± 0.001
Triton-treated	0.001 ± 0.001

In an unpublished study, we have found that about 60% of the cardiac sarcoplasmic reticulum is associated with the unwashed myofibrillar fraction. Thus we also wished to determine if this impurity is eliminated by Triton X-100. Since the ATPase activity of the sarcoplasmic reticulum is inhibited by quinidine[12], we tested its effect on the ATPase activities of both washed and Triton-treated myofibrils. Although the washed myofibrils were inhibited 44% by quinidine, the ATPase activity of the treated fibers was uninfluenced by quinidine (Table II). Since quinidine blocks the ATPase of mitochondria as well, one may conclude from this result that the ATPase of both the sarcoplasmic reticulum and mitochondria are removed by Triton. The above finding is also in accord with that reported by McFarland and Inesi[5], stating that Triton X-100 solubilizes the Ca^{2+}-dependent ATPase of the sarcoplasmic reticulum from skeletal muscle.

TABLE II

EFFECT OF QUINIDINE ON MYOFIBRILLAR ATPase

The protein fractions (1–2 mg/ml) were incubated in the medium containing 2 mM ATP, 2 mM $MgCl_2$, 50 mM KCl and 20 mM imidazole, pH 7.0, with and without quinidine (1 mM) at 22°. The values presented are expressed as means ± S.E.

Myofibrils		*Myofibrillar ATPase (μmoles P_i/mg per min)*	*Inhibition (%)*
Washed	Control	0.092 ± 0.007	44
	+ 1 mM quinidine	0.051 ± 0.003	
Triton-treated	Control	0.065 ± 0.006	−6
	+ 1 mM quinidine	0.069 ± 0.012	

Although the contamination of myofibrils by membranes appears to be eliminated by Triton X-100, the effect of this detergent on the contractile proteins needed to be tested. To test the activity of the myofibrils after detergent treatment, the ATPase activity and the sensitivity of the myofibrils to the calcium chelator EGTA were measured. The ATPase activity of the Triton-treated myofibrils was 0.094 μmole/mg per min at 27° (Fig. 2) and 0.065 μmole/mg per min at 22° (Table II). These values are comparable to those reported by Tada (0.064 μmole/mg per min at 25°)[13] and Honig (0.09 μmole/mg per min at 30°)[14] on canine cardiac myosin B. Fig. 2 also shows the EGTA sensitivity of the Triton-treated myofibrils. The 88% inhibition of myofibrillar ATPase observed with EGTA is also in good agreement with 78% and 80% inhibition obtained by Fanburg *et al.*[1] on cardiac actomyosin and skeletal myofibrillar ATPase, respectively. These results indicate that the contractile proteins are not inactivated by Triton X-100.

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