COMPARATIVE STUDIES OF MYOFIBRILS, MYOSIN, AND ACTOMYOSIN FROM RED AND WHITE RABBIT SKELETAL MUSCLE*

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The well known differences in the speed of contraction and relaxation (Ranvier, 1874) between red (tonic or slow) and white (phasic or fast) muscles may be related to the lack of calcium uptake by a microsomal fraction (grana) obtained from red muscles resulting in an impairment of relaxation (Sreter et al., 1964; Sreter and Gergely, 1964). The investigations reported here show that differences exist also with regard to the contractile apparatus - myofibrils and myofibrillar proteins - in the two types of muscles.

The soleus, semitendinosus, crureus, and intertransversarius were used as red muscles; gastrocnemius, adductor magnus, and vastus lateralis as white. Natural actomyosin and myosin were prepared by the method described earlier (Gergely et al., 1959). Since myofibrils prepared from red muscle by a modification of the method of Perry (1952) as described previously (Seidel and Gergely, 1964) with the use of 0.08 M KCl and 0.02 M histidine, pH 7.0, contained few, or no, cross-striated fibrillar structures under the phase contrast microscope, the following modifications were introduced. Special care was used to remove fat and connective tissue. Muscle was minced finely with scissors, homogenized in a Waring blendor in 30 volumes of 0.15 M KCl, 0.01 M imida-

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zole, and 0.0005 M EDTA, pH 6.5. The homogenate was then diluted three-fold with the above solution. Myofibrils were separated by differential centrifugation similar to that described by Perry (1952), washed four times with 0.15 M KCl, 0.01 M imidazole, pH 6.5, and used within 5 days. Red myofibrils prepared by this modified method showed typical cross-striation. White myofibrils showed the same morphological and enzymatic characteristics regardless which method was used for preparing them.

Protein was determined by the biuret method, and inorganic phosphate according to Fiske and SubbaRow (1925).

Myofibrils or natural actomyosin from red muscle had 10 to 20% of the ATPase activity of white preparations in the presence of 5 mM $MgCl_2$ and 10^{-4} M $CaCl_2$ at low ionic strength. This suggests a similarity between

TABLE I

Activation of ATPase Activity of Red and White Muscle Preparations by Ca or Mg

Preparations	Source	ATPase Activity ΔP_i µmoles/mg of protein/min		Ratio of Ca activation to Mg activation
		10 mM $CaCl_2$	5 mM $MgCl_2$ +0.1 mM $CaCl_2$	
Myofibrils	red	0.17	0.10	1.7
	white	0.42	0.60	0.7
Natural actomyosin	red	0.13	0.04	3.1
	white	0.68	0.58	1.2

Conditions of Assay: 5 mM ATP, 0.025 M KCl, 0.025 M Tris-HCl, pH 7.5, 25°C, 0.5 mg of protein per ml.

actomyosin from red skeletal muscle and from cardiac muscle (Fanburg, et al., 1964), and in comparison with recent data on smooth muscle actomyosin (Filo et al., 1963) - a correlation between velocity of muscle contraction and actomyosin ATPase activity. The lower ATPase activity of red actomyosin and myofibrils conceivably could be due to the presence of an inhibitor. The following facts, however, made this view unlikely:

(i) there was a linear relationship between the initial rate of hydrolysis of ATP by red myofibrils and the myofibrillar protein concentration and, (ii) the ATPase activity of a mixture of red and white actomyosins was equal to the sum of the activities of the two actomyosins measured separately.

Another difference between the white and red actomyosin systems emerges from a comparison between the effects on ATPase activity of Mg (with trace amounts of Ca), with that of Ca as the chief divalent metal (Table I). While Mg or Ca were about equally effective in activating the ATPase of myofibrils or actomyosin from white muscles, in the case of red muscle Ca was 1.7 to 3 times more effective than Mg.

The differences between red and white muscles with respect to activation of ATPase by Ca and Mg do not depend on differences in the amount of Ca or Mg present in the isolated actomyosins, since the Mg content of red and white actomyosin was 5.8 and 6.2 μmoles per g of protein, respectively, and the Ca content 5.0 and 5.4 μmoles per g of protein, respectively.

The differences between actomyosins from the two types of muscle is evidenced by their viscosity characteristics in 0.6 M KCl. At the identical protein concentration of 3 mg per ml the reduced viscosity of actomyosin from red muscle was less than half of that of the actomyosin from white muscle. Although the viscosity of both actomyosins dropped on the addition of MgATP, the ATP-sensitivity (Weber and Portzehl, 1952) of actomyosin from red muscle was lower than that from white muscle. The

Table II

Effect of MgATP on Viscosity of Red and White Actomyosin

Additions	Reduced Viscosity		ATPase Sensitivity	
	Red	White	Red	White
None	6.7	15.0		
+ 1 mM MgATP	2.0	2.0	134	287

Conditions of Assay: 0.6 M KCl, 3 mg of protein per ml. Viscosities were measured in an Ostwald viscometer having an outflow time of 30 seconds for water. Temperature 25°C.

reduced viscosity after the addition of ATP was the same for red and white muscle preparations.

In contrast to the differences described above, the percentile inhibition by EGTA* of red myofibrillar or actomyosin ATPase at low ionic strength was the same as the corresponding inhibition of white preparations (Table III).** This inhibition of ATPase was paralleled by an in-

Table III

Effect of EGTA and EDTA on ATPase Activity of Myofibrils, Myosin, and Natural Actomyosin of Red and White Muscle

	ATPase Activity ΔP_i, μmoles per mg of protein per min.	
	Red	White
Myofibrils -	0.074	0.43
+ 1 mM EGTA	0.007	0.02
Actomyosin -	0.045	0.46
+ 1 mM EGTA	0.012	0.05
Actomyosin, 0.6 M KCl	0.04	0.13
+ 2 mM EDTA	0.15	0.36
+ 2 mM EGTA	0.05	0.09
Myosin		
+ 10 mM $CaCl_2$	0.08	0.42
+ 10 mM $MgCl_2$	0.01	0.01

Conditions of Assay: (i) Myofibrils: 5 mM ATP, 5 mM $MgCl_2$, 10^{-4} M $CaCl_2$, 0.05 M KCl, 0.05 M Tris-HCl, pH 7.5, (ii) Actomyosin at low ionic strength: 2 mM $MgCl_2$, 2 mM ATP, 10^{-5} M $CaCl_2$, 0.025 M KCl, 0.025 M Tris-HCl, pH 7.5, (iii) Actomyosin at high ionic strength: 5 mM ATP, 0.6 M KCl, 0.05 M Tris-HCl, pH 7.5, (iv) Myosin: 0.05 M KCl, 5 mM ATP, 10 mM $MgCl_2$ or 10 mM $CaCl_2$, 0.1 M Tris-HCl, pH 7.0. Protein concentrations and incubation (25°) times were so adjusted that not more than 10-20% of the ATP present was hydrolyzed.

hibition of superprecipitation, in the case of myofibrils and actomyosin from either red or white muscle. However, since microsomal fractions of

*Ethylene glycol bis (β-aminoethylether)-N,N'-tetra-acetic acid or 1, 2-bis-(2-dicarboxymethylaminoethoxy)-ethane.

**EGTA inhibition of myofibrils from red muscle prepared by the earlier procedure (Seidel and Gergely, 1964) could not be observed consistantly (Sreter, Seidel, and Gergely, 1964).

red muscle show little or no ability to accumulate calcium (Sreter and Gergely, 1964), the mechanism of in vivo regulation of the intracellular Ca concentration, and hence the mechanism of regulation of contraction and relaxation may differ in red muscle from that in white, and perhaps cardiac muscle.

At high ionic strength (0.6 M KCl) the hydrolysis of ATP catalyzed by actomyosin from either red or white muscle was activated by EDTA (but not by EGTA) (Table III). However, the EDTA-activated ATPase activity of red actomyosin was also less than 50% of that of white actomysin. In view of the evidence for the dissociating effect of ATP on actomyosin at high ionic strength (Szent-Györgyi, 1951; Weber, 1956; Gergely, 1956) and the activation of myosin ATPase by EDTA (Friess, 1954) but not by EGTA (Martonosi and Meyer, 1964), these results indicate that the ATPase activity of myosin from red muscle is less than that of white myosin. The Ca-activated ATPase of myosin isolated from white muscle had indeed considerably higher activity than that from red muscle. The Mg-inhibition of both supports the absence of contaminating Mg-activated ATPases (Table III).

These differences between red and white myosin, actomyosin, or myofibrils with regard to i) maximal ATPase activity, ii) activation of ATPase by Ca or by Mg, and iii) viscosity and ATP-sensitivity may represent differences in the structure of the proteins involved in contraction, they may be due to the differences in the ratio of actin and myosin in different types of muscle, or finally they may be attributable to the differences with respect to other components of red and white muscle. Work is in progress to resolve these problems.

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