ON THE NATURE OF THE CROSS-STRIATION OF BODY MUSCLE

by

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Body muscle is called "cross-striated" because it is built of alternating lighter "I", and darker and denser "A" disks. If such a muscle is extracted with salt solution of low ionic strength, *e.g.* 0.05 *M* KCl, globular proteins are extracted and the striation remains unchanged; the optical difference between the two discs even becomes accentuated. If fresh muscle is extracted with 0.6 *M* KCl, then, as a result of the combined action of KCl and ATP, myosin is dissolved. However, if the muscle is first extracted by glycerol, then, owing to the absence of ATP, only a little myosin is dissolved by 0.6 *M* KCl. HASSELBACH[1], as well as HANSON AND H. E. HUXLEY[2], found that if ATP or pyrophosphate is added to the salt of higher ionic strength, myosin is dissolved and, at the same time as the cross-striation disappears, the refractive indices of the discs become equalized. They concluded herefrom that myosin is located in the A band only and is responsible for the latter's higher refractive index. This assumption is difficult to reconcile with the known elastic and contractile properties of muscle, and so it may be asked whether these observations are not open to a different interpretation.

A priori the higher density of the A bands could be explained in two ways. One could assume, as did the authors quoted, that myosin is located in the A band; but one could also assume that, as hitherto supposed, myosin is equally distributed in the two discs and that the A band owes its density to some additional material, which is then eluted along with myosin by the solvents used for extraction. MACALLUM[3] and SCOTT[4] have shown that glycogen and salts are located preferentially in the A band; their quantity, however, is insufficient to account, in itself, for the difference in refraction. The problem is whether, after removal of the globular proteins with low KCl concentration, there are greater quantities of another protein eluted together with myosin on extraction with KCl-ATP or KCl-pyrophosphate. If such an extra protein approximated in quantity to half the myosin, one could account for a difference in the ratio of 1 : 2 in the refractive indices. HASSELBACH showed that his extract contained myosin and very little actin; he failed, however, to study his extracts for the presence of other proteins. Nor did HANSON AND HUXLEY extend their studies along this line. For this reason we have repeated the extraction of glycerinated muscle, after removal of the globular proteins, and have analysed our extracts for myosin, actin and protein nitrogen. The

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experiments showed that the extracts contained other protein besides myosin in relatively large quantities. The concentration of this protein approximated to half of that of the myosin.

EXPERIMENTAL

Glycerol-extracted psoas muscle was broken down into fibrils 1–2 μ in diameter with the aid of a Serval-Omnimixer. The fibrils were washed with about 20 volumes of 0.04 M KCl, containing 0.0067 M neutral phosphate buffer, for five minutes, centrifuged and resuspended. The bulk of the globular proteins was removed in the first and second washings. After the fifth extraction no more protein was eluted. The fibres were washed 7 times in all. This treatment did not cause any significant change in the phase-contrast microscopic appearance of the fibrils (Fig. 1a and b). Storage of the fibrils for two or more days in the solution of low ionic strength failed to extract any more nitrogen-containing material.

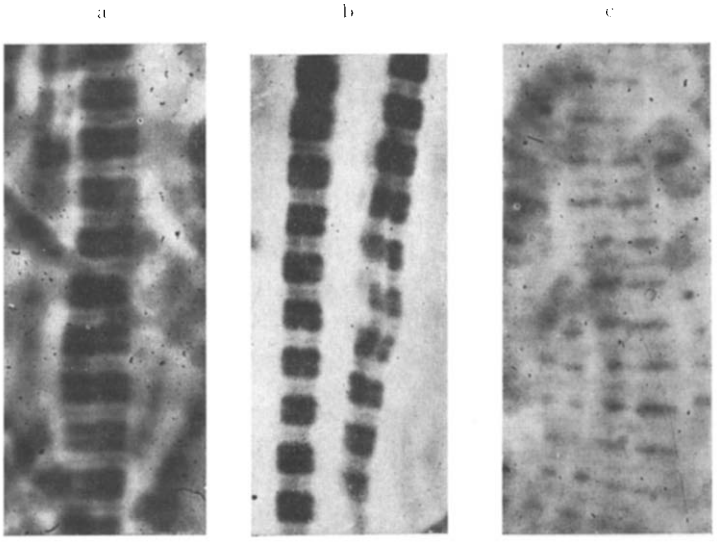

Fig. 1. Phase-contrast photomicrographs of glycerol-treated rabbit psoas muscle: a. unextracted; b. extracted with dilute KCl; c. extracted with strong KCl-pyrophosphate-Mg.

For the removal of the A band the experimental conditions of HANSON AND H. E. HUXLEY were imitated as closely as possible, especially in regard to the speed of the operations. The fibers were checked in parallel experiments under identical conditions under the phase-contrast microscope. The extraction with GUBA-STRAUB[5] solution with added ATP in $8 \cdot 10^{-4} M$ concentration and the HASSELBACH-SCHNEIDER[6] pyrophosphate solution containing $1 \cdot 10^{-3} M$ $MgCl_2$ was completed within 1–2 minutes, the residue was obtained by centrifugation at 25,000 g, and the extract was analysed after overnight storage in the refrigerator for total protein and myosin content. In agreement with HANSON AND HUXLEY the A band was removed rapidly from fiber preparations, especially in the case of the pyrophosphate treatment; the preparations, after treatment, looked fairly homogeneous (Fig. 1c).

The amount of myosin was estimated in the following way:

(a) viscosimetric measurements, using a factor of 0.0864[7] for the logarithm of the

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relative viscosity for 1 mg per ml concentration at 23° C, after addition of ATP. The difference in viscosity with and without ATP served as a measure of the amount of actin present, which was negligible and in agreement with HASSELBACH's experiments.

(b) measuring the amount of protein which precipitates at low salt concentrations and dissolves again in higher concentrations. The extract was dialysed overnight in the cold against 0.04 *M* KCl containing 0.0067 *M* neutral phosphate buffer, centrifuged, redissolved and brought to the original volume with 0.6 *M* KCl. The viscosity and total nitrogen were measured again.

Tables I and II show a representative series of experiments. Table III summarises the result of the different extractions. Myosin accounted for only 65–75% of the total protein extracted from washed muscle, which corresponds to about 50% of the protein present in this preparation. The amount of myosin extracted was thus 25–30% of the total protein present in whole muscle, which is a considerable part of the myosin the muscle contains. Similar results were obtained repeating the experiments at pH 7.1, storing the fibers in glycerol for 4–30 days before homogenization, or storing the fibrils after homogenization for three days.

TABLE I

REMOVAL OF GLOBULAR PROTEINS FROM GLYCEROL-EXTRACTED MUSCLE HOMOGENATE WITH REPEATED WASHINGS FOR 5 MIN WITH 20 VOLUME OF 0.04 *M* KCl CONTAINING 0.0067 *M* NEUTRAL PHOSPHATE BUFFER. PREPARATION CONTAINED 1,810 mg PROTEIN

	Extracted protein	
	in mg	*in %*
1st washing	610	33.7
2nd washing	109	6.0
3rd washing	24	1.3
4th washing	11	0.6
5th and 6th washing	< 3	
Total extracted	754	41.6
Residue	980	54.2

TABLE II

SHORT EXTRACTION OF WASHED RESIDUE WITH GUBA-STRAUB-ATP AND HASSELBACH-SCHNEIDER-PYROPHOSPHATE-$MgCl_2$ SOLUTION

	Protein in mg Kjehldahl	*Myosin in mg viscosimetric*	*Myosin in % of protein extracted*	*Non-myosin protein in % of myosin extracted*
Washed residue	702			
KCl-ATP extract	473	339	72	39
KCl-pyrophosphate extract	478	332	70	43
KCl-ATP extract dialysed precipitate redissolved	295	298		
KCl-pyrophosphate extract dialysed, precipitate redissolved	311	305		2

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TABLE III

		Myosin in % of the total protein extracted	*Myosin in % of the total protein in washed muscle*	*Non-myosin protein in % of myosin extracted*
6.24.54	ATP	70.3	31.5	42
7. 8.54	ATP	73	63	37
7. 8.54	PP	72	47	30
7.26.54	ATP	65	57	54
	ATP	69	57	45
	PP	75	54	33
	PP	80	58.5	12
8.16.54	ATP	72	51	39
	ATP	72	48	39
	PP	70	47	43
	PP	71	28.5	41
9.10.54	PP	77	*ca.* 50	30

The nature of the protein extracted in addition to myosin has not yet been studied extensively. It can be precipitated by trichloroacetic acid. It has a low viscosity and is soluble at any [KCl]. It slowly passes through the dialysing membrane. It is possible that it is kept in the structure by the myosin and this is why extraction of myosin is necessary for its removal. The accompanying picture shows that the M and Z bands remain intact during extraction, suggesting that the extra material comes from the A band.

SUMMARY

After the removal of globular proteins, solvents used for extraction of myosin remove from glycerol-pretreated body muscle a considerable amount of protein other than myosin, the quantity of which is sufficient to account for the differences in refraction between the A and I bands.

RÉSUMÉ

Après l'enlèvement des protéines globulaires, les solvents utilisés pour l'extraction de la myosine extraient du muscle traité préalablement à la glycérine une quantité considérable de protéine différente de la myosine. Cette quantité est suffisante pour expliquer les différences de réfraction entre les bandes A et I.

ZUSAMMENFASSUNG

Nach der Entfernung der Globulin-Proteine, extrahieren Lösungsmittel welche zur Extraktion des Myosins angewendet werden, aus dem mit Glyzerin vorbehandelten Muskel eine bedeutende Menge Protein, das vom Myosin verschieden ist. Die Menge dieses Proteins ist genügend, um die Unterschiede zwischen der Refraktion der A- und I-Banden verursachen zu können.

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