THE ADENOSINETRIPHOSPHATASE ACTIVITY OF LIPOPROTEIN GRANULES ISOLATED FROM SKELETAL MUSCLE

by

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INTRODUCTION

Investigation of the adenosinetriphosphate (ATP) splitting action of myofibrils isolated from rabbit psoas muscle showed that 97% of the enzymic activity of the whole muscle homogenate in the presence of calcium and magnesium was associated with the myofibrillar fraction which could be centrifuged down in 15 min at 1500 g[1]. The adenosinetriphosphatase (ATPase) of the myofibrils was shown to be of the myosin type, but as no attempt was made to preserve the labile KIELLEY-MEYERHOF magnesium activated ATPase[2,3], the investigation did not provide any information as to the relation of this enzyme to the ATPase of the myofibril.

It was also noted in these studies[1] that the cloudiness appearing in the supernatant when the myofibrils had been centrifuged down was due to small granules which had been washed out of the muscle. In 1856 KOLLIKER[4] studied the granules of sarcoplasm and found that they consisted of fatty droplets and small interstitial particles which were resistant to caustic alkalis and acetic acid. A careful histological investigation made later by BULLARD[5] provided evidence that these smaller granules were of lipoprotein nature. More recently BRACHET AND JEENER[6] have isolated microsomes from a large number of tissues; but from muscle, fatty, and red blood cells however, they were able to isolate only small amounts of these components. KIELLEY AND MEYERHOF[2,3] isolated a magnesium activated ATPase from rat and rabbit muscle which they were not able to purify completely as the enzyme was associated with phospholipid material. The high lipid content of this preparation indicated that it might be of microsome origin and KIELLEY AND MEYERHOF[3] concluded that a choline-containing component of the phospholipid associated with the enzyme was essential for ATPase activity.

VAN THOAI, ROCHE, AND DE BERNARD[7] have studied the adenyl pyrophosphatase of carp and dog muscle and do not consider that it is necessary to assume that there is more than one enzyme. They concluded that the differences were due to variation in the amounts of activators found in the preparations they obtained.

In view of the lack of information as to the relation within the muscle cell of the myosin ATPase to the KIELLEY-MEYERHOF enzyme, an attempt has been made to clarify the position. Fractionation of the muscle into cytoplasmic components in the presence of low concentrations of potassium cyanide indicated that the magnesium activated ATPase was associated with the granular fraction of the sarcoplasm. The evidence presented in the following paper supports the view that this enzyme is not

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identical with the myofibrillar ATPase but that it is very probably similar in nature and function to the ATPase which is widely distributed in tissues and associated with the granular components of cytoplasm[8,9].

METHODS

Preparation of granules

All operations in this preparation and the others described in this paper were carried out at 0° C.

Muscle from a freshly killed rabbit was minced and homogenized in four volumes of either 0.08 *M* borate buffer pH 7.1, or in 30% sucrose (w/v). In the preparation of granules for enzyme experiments both solutions contained potassium cyanide which was neutralized before addition to bring the final concentration to 0.002 *M*. The muscle was homogenized in a Waring Blendor for four 40 sec periods with cooling between runs, centrifuged for 20 min at 600 *g* and the supernatant removed. After blending the residue again with three volumes of the medium the centrifugation was repeated, the supernatants combined and filtered through glass wool. To remove any remaining myofibrils the combined supernatants were centrifuged again at 600 *g* for 20 min and the turbid supernatant carefully removed to avoid contamination with the small amount of sediment. When a drop of this supernatant (which will be referred to hereafter as the turbid supernatant) was stained with methylene blue and examined with a high power objective it was seen to be free from myofibrils, but to contain large numbers of fine granules which tended to clump, particularly in the borate medium.

The turbid supernatant was centrifuged for 20 min at 14,000 *g*; the resulting supernatant, which was still slightly cloudy, discarded, and the yellow translucent pellet suspended in about 50 ml of medium per 100 g of muscle, care being taken to disperse the granules completely. The centrifugation was repeated, the supernatant again discarded and the granules resuspended in an appropriate volume of medium.

For analysis the granules and myofibrils were dialysed at 4° C against glass distilled water changed twice daily for four days. the contents of the dialysis bags were freeze-dried and the material obtained stored in a vacuum desiccator.

Preparation of myofibrils

Myofibrils were prepared essentially as described previously[1], with the slight modification that the myofibrillar fraction centrifuged down at 1500 *g* was separated from coarse elements by three centrifugations at 400 *g* for 3 min.

Preparation of muscle homogenates

Skeletal muscle tissue is not readily broken down to cell-free homogenates such as are easily made from liver, but a satisfactory preparation consisting entirely of granules, nuclei, and myofibrils as single units or in small bundles, was obtained in the following manner. Fresh rabbit muscle, preferably psoas, was sectioned at a thickness of 25 μ with the freezing microtome and the slices suspended in 15–20 volumes of borate buffer. The suspension was broken up with a small Waring Blendor-type homogenizer constructed from Perspex to hold from 40 ml to 80 ml of suspension, and in which the internal flutings were designed to give maximum agitation. A satisfactory homogenate was obtained after 2–4 min, care being taken to avoid any heating during this period by jacketing in ice.

Estimation of the ether-alcohol soluble fraction

About 30 mg of the freeze-dried material was extracted for 20 h with 25 ml of a mixture of three parts alcohol and one part ether. This was carried out in the micro-extraction apparatus designed by Dr P. MITCHELL of this department, details of which were kindly made available before publication.

Measurement of ATPase activity, and the preparation of myosin and ATP were carried out as described in a previous publication[1]. Nitrogen was estimated by the micro-Kjeldahl technique and phosphorus by the method of FISKE AND SUBBAROW.

RESULTS

Distribution of the magnesium activated ATPase within the muscle cell

When psoas muscle homogenates were made in 0.08 *M* borate buffer pH 7.1, containing 0.002 *M* potassium cyanide, and centrifuged for 20 min at 600 *g*, the turbid supernatant obtained contained no recognizable myofibrils or nuclei but showed

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magnesium activated ATPase activity (measured in borate or glyoxaline buffers at pH 7.0 and 0.005 *M* magnesium chloride) which amounted to 9–15% of the activity of the whole homogenate.

When the turbid supernatant was centrifuged for 30 min at 19,000 *g* a small yellow translucent pellet containing 50–60% of the ATPase activity in the presence of 0.005 *M* magnesium chloride, of the whole supernatant was obtained. After 90 min centrifugation 30% of the activity still remained dispersed. The possibility has not been excluded that part of the ATPase activity which was not sedimented under these conditions was apparent ATPase activity, such as would be obtained for example if soluble creatine phosphokinase produced creatine phosphate from the creatine present in the muscle extract and the ATP added. Under FISKE-SUBBAROW conditions of phosphate estimation creatine phosphate estimation would be measured as phosphate, *cf.* CORI[10].

After high speed centrifugation the supernatant was often still slightly turbid and granules could be seen when a smear was stained with methylene blue. On addition of ammonium sulphate to 35% saturation a precipitate possessing magnesium activated ATPase activity was obtained.

On resuspension of the pellet in borate-potassium cyanide medium, 95% of the ATPase activity could be centrifuged down in 45 min at 19,000 *g*. Centrifugation of the turbid supernatant obtained from a muscle homogenate in 30% sucrose for 150 min at 15,000 *g* left 70% of the activity in the supernatant, and much less material sedimented compared to that obtained when the homogenate was made in borate.

To determine the distribution of the magnesium activated ATPase in the granular components of the cell, the turbid supernatant obtained from a borate homogenate of rabbit muscle was centrifuged at various speeds for a fixed time interval. For this purpose about 200 g of fresh muscle was homogenised in the Waring Blendor to provide approximately 1000 ml of turbid supernatant. Equal portions of this were then centrifuged as is indicated in Table I, and the fraction of the ATPase activity of the original turbid extract left in each supernatant estimated. At the same time the pellet which sedimented was drained free of supernatant resuspended in borate, dialysed against glass distilled water for 4 days and finally freeze-dried. The whole process was carried out quantitatively so that an estimate of the amount of material sedimenting could be made. Table I shows that there was not much variation in the composition of the granules; the phosphorus content rose slightly and in general the amount of ATPase activity disappearing from the extract was approximately proportional to the amount of material sedimented.

The results of an experiment designed to wash out quantitatively the granular ATPase from the myofibrillar fraction of a muscle homogenate are shown in Table II. A psoas muscle homogenate, in which the cells had been completely broken down to myofibrils, was centrifuged for 20 min at 200 *g* (this low centrifugal force readily sedimented the myofibrils because they had been slightly shortened in the preparation, as sometimes happens if the muscle contains considerable amounts of ATP and the temperature rises a little above 0° C). The splitting of ATP in the presence of magnesium by the supernatant and the whole homogenate was compared; the supernatant was removed and the sedimented material resuspended in fresh borate so that the total volume was equal to that of the original homogenate. Centrifugation was repeated and the activity of the supernatant again measured. This procedure was carried out once more.

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TABLE I

COMPOSITION AND ATPase ACTIVITY OF RABBIT MUSCLE GRANULES SEDIMENTED IN DIFFERENT CENTRIFUGAL FIELDS

(ATPase activities measured in borate buffer pH 6.9 and 0.005 *M* $MgCl_2$. Centrifugations carried out for 20 min in each case)

Fraction	*1*	*2*	*3*
Volume of turbid supernatant (ml)	280	280	280
Centrifugal force (*g*)	2,400	6,900	14,200
Wt. of material sedimenting (mg)	41	85	130
Total nitrogen (%)	10.03	9.87	10.06
Total phosphorus (%)	1.10	1.17	1.24
Alcohol-ether soluble material (%)	38	34	35
ATPase activity in supernatant (as % of the total in the original turbid supernatant)	86	76	51

TABLE II

EFFECT OF LOW SPEED CENTRIFUGATION ON THE ATPase ACTIVITY OF A HOMOGENATE OF RABBIT PSOAS MUSCLE

(Centrifugations were carried out for 20 min at 200 *g*. Enzymic activity was measured in 0.1 *M* glyoxaline buffer, pH 7, and 0.0061 *M* ATP. Results are expressed as μg phosphorus liberated per ml suspension in 5 min at 37° C)

		Activator		
		nil	*0.005 M $MgCl_2$*	*0.005 M $CaCl_2$*
Original Homogenate	Whole homogenate	164	649	500
	Supernatant	13	55	10
	% activity of whole original homogenate in supernatant		8.5	2
Resuspension 1	Whole suspension			
	Supernatant	7	23	
	% activity of whole original homogenate in supernatant		3.5	
Resuspension 2	Whole suspension	155	477	
	Supernatant	8	23	
	% activity of whole original homogenate in supernatant		3.5	

If a correction is applied for the amount of supernatant left in the sedimented material each time it was resuspended, the total magnesium activated ATPase activity to be found in the supernatants amounts to 13% of that of the whole homogenate. On the other hand, if it is assumed that the difference in enzymic activities of resuspension 2 (Table II) and the original homogenate is due to granular enzyme which has been washed out, the latter is responsible for 27% of the ATPase activity of the whole homogenate. This assumption takes no account of any loss in activity of the myofibrillar ATPase during the resuspension and centrifugation. The difference shown in Table II

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is higher than that obtained in other similar experiments but is given here because in this case a shorter interval (90 min) elapsed between the death of the animal and the carrying out of the enzymic tests.

To arrive at an independent value for the contribution of the granules to the splitting of ATP by the whole muscle homogenate, experiments were carried out on the magnesium activated ATPase activity of the homogenate before and after treatment with 1 *M* potassium chloride. After such treatment the ATPase of the myofibrils is no longer activated by magnesium at pH 7 and in the presence of 0.1 *M* potassium chloride, whereas the granular enzyme is unaffected (see Table III). From these studies it was concluded that when the enzymic activity is measured in glyoxaline buffer, pH 7, in the presence of 0.005 *M* magnesium chloride and 0.13 *M* potassium chloride, the granules can account for 20–25% of the ATP splitting activity of whole rabbit psoas muscle.

TABLE III

EFFECT OF *M* POTASSIUM CHLORIDE ON THE MAGNESIUM ACTIVATED ATPASE OF GRANULES FROM RABBIT MUSCLE

(Enzymic activity was measured in 0.1 *M* borate buffer, pH 6.9, and is expressed in the table as μg phosphorus liberated per ml granule suspension in 10 min at 37° C)

Concentration of activator (M)		*Control granules*	*Granules treated with 1 M KCl*
KCl	*$MgCl_2$*		
0.1	nil	68	74
0.1	0.001	344	337
0.1	0.005	410	431

Properties of the granular ATPase

Granule preparations with Q_p values of 1000 and higher at pH 7 in the presence of 0.005 *M* magnesium chloride were readily obtained after two high speed centrifugations of the turbid supernatant. Fig. 1 shows that in borate buffer the magnesium activated ATPase of the granules has a pH optimum of 8, whilst at higher pH values the activity falls off in a marked fashion. In glyoxaline buffer the activity of the enzyme rises to a high value at 7.4 and increases slightly over the buffer range up to 7.8.

Calcium had little or no activating effect at pH 7.0, whereas at concentrations up to 0.005 *M*, magnesium activated strongly. This activating effect of magnesium fell off slightly with increasing concentration of the cation, although at 0.05 *M* the granules still split ATP at an considerable rate (Fig. 2).

It was shown in an earlier study[1] that after pretreatment of collagenase prepared myofibrils with 1 *M* potassium chloride, the magnesium activation of these structures measured in 0.1 *M* potassium chloride completely disappeared. Similar results have been obtained with myofibrils prepared without the use of collagenase; in these preparations homogenization alone was relied upon to break up the muscle cells with the result that the myofibrils were shorter than those obtained by the other method. Table III shows that the granular ATPase is not in any way affected by pretreatment with 1 *M* potassium chloride and hence indicates that it is an enzyme distinct from the myofibrillar ATPase.

Almost all the acid labile phosphate is split from ATP by the granules, and only when the amount of inorganic phosphate liberated approaches a value equivalent to

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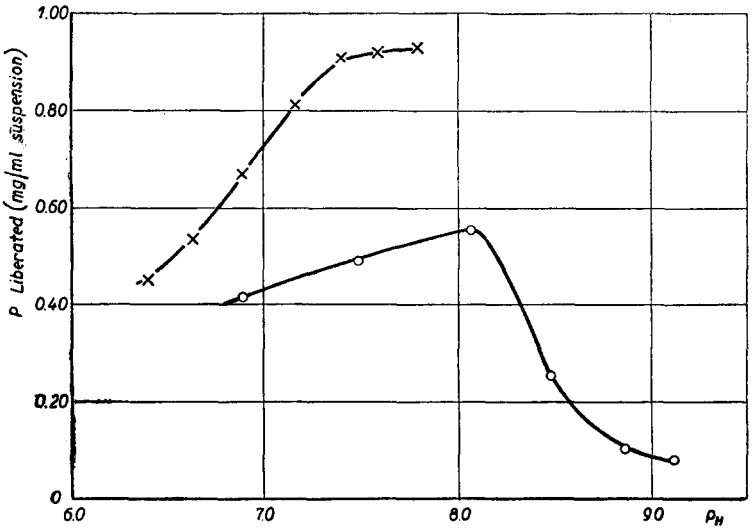

Fig. 1. Effect of pH on ATPase activity of granules isolated from rabbit muscle by the borate method. Incubation carried out in the presence of 0.005 *M* $MgCl_2$

O—O Preparation 8; 7 min at 37° C in 0.1 *M* borate buffer.
×—× Preparation 17; 5 min at 37° C in 0.1 *M* glyoxaline buffer

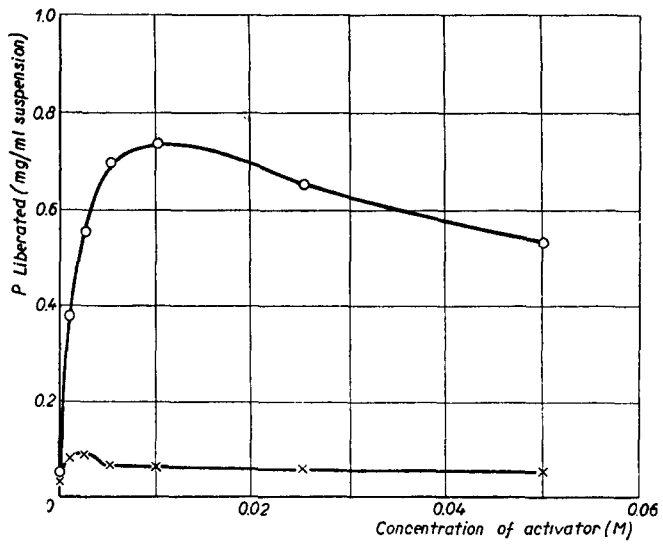

Fig. 2. Effect of calcium and magnesium on the ATPase activity of granules isolated in borate from rabbit muscle. Activity measured in 0.1 *M* glyoxaline buffer, pH 7.0, and 0.0061 *M* ATP; 0.038 m-equiv. potassium/ml. Incubated 10 min at 37° C.

×—× Calcium
O—O Magnesium

two phosphate groups per molecule of ATP does the rate of liberation fall off sharply. The third phosphate group of ATP is apparently not attacked.

The presence of myokinase in the granules was demonstrated as follows. Purified myosin was allowed to act on ATP until, in the presence of excess enzyme, no further splitting took place, *i.e.* all the ATP had been converted to adenosine diphosphate (ADP). Addition of the granules even if they had been previously acidified to pH 1 and heated for 2 min at 90° C, caused further liberation of inorganic phosphate in the myosin-ADP system. Myokinase is very closely associated with the granules; six additional washings by resuspension and centrifugation in fresh borate-cyanide failed to remove this enzyme. With this repeatedly washed preparation it was possible, however, to recognize a distinct break in the progress curve when about 50% of the acid labile phosphate had been split.

At pH 7 in the presence of 0.005 *M* magnesium chloride no splitting of β-glycerophosphate or disodiumphenyl phosphate was observed, but under these conditions the granules showed slight inorganic pyrophosphatase activity.

Relation of the ATPase of the granules to that of the myofibrils

As the myofibrillar ATPase showed certain similarities to its granular counterpart, and in view of the fact that the methods of preparation are such that without special care either component could be contaminated with the other, it was important to establish that the ATP splitting properties of these intracellular components arose from distinct and independent enzymes.

On the following grounds it is considered that the ATPases of the myofibril and the granules are different enzymes.

1. The ATPase of the granules is more labile than that of myofibrils.

2. Calcium and magnesium have different effects on the two systems. In particular, after treating with 1 *M* potassium chloride the granular enzyme, in marked contrast to that of myofibrils, is still activated by magnesium in the presence of 0.1 *M* potassium chloride.

3. If myosin, purified until it has neglible ATPase activity in the presence of magnesium, was precipitated from 0.5 *M* potassium chloride solution by dilution it carried down granules dispersed in the original solution and acquired magnesium activated ATPase activity. This latter activity was stable to 1 *M* potassium chloride.

4. It was possible to extract granules with 0.5 *M* potassium chloride for one hour and then sediment practically all the ATPase activity (97%) by high speed centrifugation. The small amount of activity remaining in the supernatant was magnesium activated. Any myosin in the granules would be extracted under these conditions.

5. After centrifugation of an extract of rabbit muscle in 0.5 *M* potassium chloride for 60 min at 19,000 *g* the supernatant had the same calcium activated ATPase activity as the original extract.

Liver granule ATPase

When rat liver homogenates were prepared in the same way as the turbid supernatant from rabbit muscle, 20 min centrifugation at 14,000 *g* sedimented 50% of the magnesium ATPase (measured at pH 7) of the whole homogenate whether the medium used was borate or sucrose. Very much larger yields per g of tissue were obtained and the granules appeared redder in colour than those from muscle.

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The preparation obtained from rabbit liver using the borate method was examined in more detail and found to consist of two distinct fractions; a yellowish translucent lower layer which was covered in the centrifuge tube by a flocculent light-brown sediment which could be decanted and washed off the tightly packed lower layer. The yellowish pellet closely resembled in appearance the granule preparation obtained from muscle and gave a creamy white suspension on redispersion. It possessed very slight magnesium activated ATPase activity whereas the flocculent layer had considerable activity in the presence of magnesium and calcium. The magnesium activation curve shown in Fig. 3 is very similar to that obtained with granules from muscle, but unlike the latter, liver granule ATPase was also activated by calcium to a considerable extent. Similar activation results were obtained with the whole granular fraction obtained from rat liver.

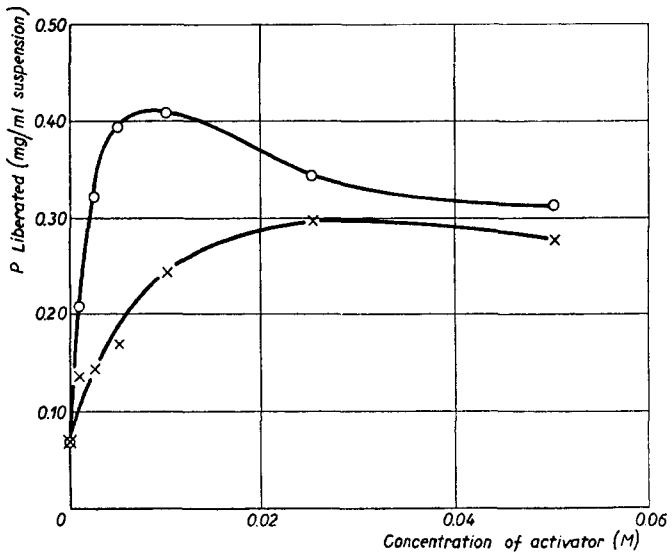

Fig. 3. Effect of calcium and magnesium on the ATPase of granules isolated in borate from rabbit liver. Activity measured in 0.1 *M* glyoxaline buffer, pH 6.9, and 0.0061 *M* ATP; 0.038 m-equiv. potassium/ml. Incubated 10 min at 37° C.

×—× Calcium
O—O Magnesium

*Composition of granules and myofibrils**

Table IV gives the analyses of granules isolated from rat and rabbit muscle by centrifugation of extracts for 20 min at 14,000 *g*. Granules isolated from rabbit muscle by the borate method have a high content of ethanol-ether soluble material. This fatty fraction is mainly phospholipid as it contains 2.26% total phosphorus and 2.16% nitrogen. Isolated in sucrose the granular fraction is smaller in amount and probably consists of the larger particles as sedimentation is slower in this medium. The granules contain less ethanol-ether soluble material and total phosphorus but more nitrogen. This would be expected if the average size of the particles is greater than that in the fraction isolated in borate under similar conditions, but as there is less preservation of structure

* The electron microscope study of some of the intracellular components whose analyses are presented in this section is described in an accompanying paper[11].

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TABLE IV

ANALYSIS OF GRANULES AND MYOFIBRILS FROM MUSCLE

(Figures marked with an asterisk are obtained from duplicated analyses on one preparation only. Isolation of granules involved two centrifugations at the speeds indicated)

Component	*Animal*	*Ether alcohol extractable material* (%)	*Total P* (%)	*Total N* (%)	*Ash* (%)	*Medium*	*Method of isolation*
Granules	rabbit	37	1.22	9.95	2–3	borate	20′ at 14,000 *g*
Granules (fat, T.C.A. extracted)	rabbit		0.21	14.35		borate	20′ at 14,000 *g*
Granules	rabbit	30*	0.98*	11.15*		sucrose	20′ at 14,000 *g*
Supernatant	rabbit	7*	0.07*	15.0 *		sucrose	150′ at 16,000 *g*
Granules (heavy)	rat	15*	0.28	12.78		sucrose	10 min at 800 *g*
Granules (light)	rat		0.58*	9.21*		sucrose	30′ at 14,000 *g*
Myofibrils	rabbit	4–7	0.10	14.1 *	0.5	borate	

in the latter medium the differences could, however, arise from the leaching out of nitrogen-containing compounds in the borate medium.

Rat muscle is richer in granules than the corresponding rabbit tissue, and centrifugation of extracts of the former brought down a fraction at quite low speeds (10 min at 800 *g*). This heavy fraction contained less lipid and phosphorus but more nitrogen than the normal preparation from the rabbit, but centrifugation at 14,000 *g* sedimented a fraction from rat muscle which was more comparable to that obtained from rabbit.

Application of the SCHMIDT AND THANNHAUSER[12] fractionation procedure showed that the trichloroacetic acid and fat extracted granules contained, on the average, 0.21% total phosphorus, about 65% of which could be accounted for as ribonucleic acid and phosphoprotein. The remaining 35% precipitates as deoxyribonucleoprotein. This seems to be a high figure for a sarcoplasmic component, particularly as frequent optical and electron microscope examinations of the supernatant from which this fraction was isolated did not show the presence of nuclei. It is possible that during isolation of the granules nucleoprotein was extracted from the nuclei and carried down with this fraction.

Myofibrils isolated by the collagenase method contain 4–7% of ethanol-ether soluble material and the total phosphorus content is less than 10% of that of granules isolated in borate from rabbit muscle. Total phosphorus estimations carried out on undialyzed myofibrillar suspensions gave higher values ranging from 0.1% to 0.5%, but in the trichloroacetic and fat-extracted freeze-dried material the figure was fairly constant and averaged 0.054%. This could practically all be accounted for as ribonucleic acid or phosphoprotein; the presence of deoxyribonucleic acid in the myofibrils is doubtful.

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DISCUSSION

Minor differences in properties exist between the magnesium-activated ATPase isolated by KIELLEY AND MEYERHOF and the ATP-splitting enzyme of the lipoprotein granules which can be obtained from skeletal muscle, but the circumstantial evidence very strongly suggests that the same enzyme is responsible for the activity of both preparations. The isolated granules will correspond fairly closely to the functioning units in the cell and it is not surprising that they show differences in enzymic behaviour from a preparation which has been obtained by the methods of protein chemistry. In the granules, the KIELLEY-MEYERHOF enzyme is associated with myokinase so that these particles liberate all the acid labile phosphate from ATP. Although myokinase is a water-soluble enzyme it is closely associated with both ATP-splitting systems of muscle, the myofibrils and the granules, and in each case, particularly in the latter, this enzyme is not readily removed by repeated centrifugation and resuspension.

In adult skeletal muscle the two enzyme systems appear distinct and presumably because of their precise localization within the cell they have different functions. The granular enzyme resembles the ATPase widely distributed in tissues which is associated with mitochondria and the oxidative enzyme systems of the cell. The belief of many investigators that in the myoblast, myofibrils arise from mitochondria is of considerable interest in any consideration of the relation between the ATPases of the muscle cell. This hypothesis is attractive because apart from the implication of the fact that some mitochondria exist in a filamentous form, the differentiation of the cell for special function would involve a modification of the existing granular ATPase for function in the contractile structures.

The granules obtained from rabbit muscle differ from mitochondria isolated from the liver of the rabbit or rat. In their properties and appearance the former resemble the microsome fraction but contain much less nucleic acid than is usually found in such particles. It is striking that the fraction isolated from liver which in appearance more nearly resembles the muscle granules has little ATPase activity.

The myofibrils contain much less total phosphorus and lipid than do the granules. Freeze-dried myofibrils, which have been extracted with trichloroacetic acid and fat solvents, contain about as much total phosphorus as was found by BAILEY[13] for myosin. This means in other terms that the maximum nucleic acid content of isolated myofibrils is approximately 0.5%, which, even if it all occurs as the N protein of GERENDAS AND MATOLTSY[14], does not seem adequate to compensate for the positive double refraction of the actomyosin in the I band, as has been postulated by these authors.

SUMMARY

1. High speed centrifugation of muscle homogenates, from which myofibrils and nuclei had been removed, sedimented lipoprotein granules possessing magnesium-activated ATPase activity.

2. Although slight differences exist between the properties of the ATPase associated with ths granules and the Kielley-Meyerhof enzyme preparation, it is concluded that the same enzyme is responsible for the activity of both preparations. This enzyme is not identical with myosin ATPase which is localized in the isolated myofibrils.

3. The granular ATPase accounted for 20–25% of the ATP-splitting activity of a cell-free homogenate of rabbit psoas muscle in the presence of magnesium at pH 7.

4. The magnesium activation of the ATPase of the granules, unlike that of the myofibrils, was not destroyed by 1 *M* KCl.

5. Figures are given for the composition of granules and myofibrils isolated from skeletal muscle.

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RÉSUMÉ

1. Par centrifugation à grande vitesse d'homogénats de muscle dont les myofibrilles et les noyaux avaient été enlevés des granules de lipoprotéine à activité ATPasique activée par le magnésium se déposèrent.

2. Bienqu'il existe de légères différences entre les propriétés de l'ATPase associée à ces granules et la préparation d'enzyme Kielley-Meyerhof, nous concluons que la même enzyme est responsable de l'activité des deux préparations. Cette enzyme n'est pas identique à la myosine-ATPase localisée dans les myofibrilles isolées.

3. L'ATPase granulaire était responsable de 20–25% de l'activité de scission d'ATP d'un homogénat exempt de cellules de muscle psoas de lapin en présence de magnésium et à pH 7.

4. Contrairement à celle des myofibrilles, l'activation par le magnésium de l'ATPase des granules n'était pas détruite par le KCl en concentration molaire.

5. Nous donnons des chiffres concernant la composition des granules et des myofibrilles isolées de muscle strié.

ZUSAMMENFASSUNG

1. Beim Hochtouren-Zentrifugieren von Muskelhomogenaten, aus welchen die Myofibrillen und Kerne entfernt worden waren, sedimentierten Lipoproteinkörner, welche eine durch Magnesium aktivierte ATPase-Aktivität besassen.

2. Obwohl zwischen den Eigenschaften der ATPase dieser Körner und dem Enzympräparat von Kielley-Meyerhof geringe Unterschiede bestehen, wird geschlossen, dass dasselbe Enzym für die Aktivität beider Präparate verantwortlich ist. Dieses Enzym ist nicht identisch mit der Myosin-ATPase, welche in den isolierten Myofibrillen lokalisiert ist.

3. Die ATPase der Körner ist für 20–25% der ATP-spaltenden Aktivität eines zellfreien Kaninchen-Psoasmuskel-Homogenates in Gegenwart von Magnesium und bei pH 7 verantwortlich.

4. Die Magnesium-Aktivierung der ATPase der Körner wurde, zum Unterschied von derjenigen der Myofibrillen, durch *M* KCl nicht zerstört.

5. Für die Zusammensetzung der aus Skelettmuskel isolierten Körner und Myofibrillen werden Zahlen angegeben.

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