LOCALIZATION OF THE MAGNESIUM-ACTIVATED APYRASE OF INSECT MUSCLE IN THE SARCOSOMES

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Summary

Sarcosomes of the fibrillar flight muscles of blowflies have been isolated in a reasonably pure state and shown to possess powerful apyrase activity (Q_p 2500-5000).

The evidence suggests that the Mg-activated apyrase of insect muscle previously described is located exclusively in the sarcosomes.

Only a negligible amount of apyrase activity appears in solution when the sarcosomes are suspended in a hypotonic medium.

The possible function of the apyrase is discussed.

I. INTRODUCTION

The Mg-activated ATP-ase of mammalian muscle, described by Kielley and Meyerhof (1948), has been shown by Perry (1952) to be associated with lipoprotein granules sedimenting at 14,000g, and has been related in function to the ATP-ase widely distributed in tissues, and associated with cytoplasmic granules. In insect muscle there is a Mg-activated apyrase which seems to correspond with the Kielley-Meyerhof enzyme (Gilmour and Calaby 1952). This apyrase is much more abundant in the indirect flight muscles than in other skeletal muscle. In considering the intracellular distribution of this enzyme, attention was directed to the sarcosomes, interfibrillar components of the muscle cells of insects, which have been shown to possess some of the enzymic properties of mitochondria (Watanabe and Williams 1951). The sarcosomes of insect muscle are most abundant and reach their largest size (up to 5 μ dia.) in the indirect flight muscles, particularly in the so-called "fibrillar" muscle of Diptera and Hymenoptera. Sarcosomes are present in the other skeletal muscles, but they are relatively few in number and of much smaller size. In this paper are described experiments which have shown that the Mg-activated apyrase is located in the sarcosomes.

Before the work reported here had been completed, the paper by Sacktor (1953) on the ATP-ase of the sarcosomes of *Musca* appeared. Sacktor demonstrated the presence in the sarcosomes of a Mg-activated ATP-ase, which, while not identified by him with the apyrase extracted from muscle, nevertheless had many of the properties of this enzyme. The present paper confirms and extends the observations of Sacktor.

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II. Experimental and Results

(*a*) *Apyrase and Phosphate Determinations*

The methods used in the determination of apyrase activity were the same as those described in the previous communication (Gilmour and Calaby 1952). Inorganic P was determined routinely by the Fiske and Subbarow (1925) technique. Since this method measures labile organic phosphate esters as inorganic P, and would thus mask the activity of enzymes transferring phosphate from ATP to an organic acceptor, the inorganic P determinations were checked by the Lowry and Lopez (1946) method. True inorganic P, by the Lowry and Lopez method, was approximately 90 per cent. of that measured by the Fiske and Subbarow technique. Figures for apyrase activity given have been corrected for this factor.

(*b*) *Fractionation of Blowfly Flight Muscle*

Sarcosome preparations were made from the fibrillar flight muscles of the adults of the blowflies *Calliphora stygia* (F.) and *C. fallax* Hardy, which were caught in traps at Canberra.

The thoracic muscles were dissected from a number of flies and dropped immediately into the ice-cold "Ringer" solution of Ephrussi and Beadle (1936), developed originally for use with *Drosophila* (NaCl 7.5 g, KCl 0.35 g, $CaCl_2$ 0.21 g per litre). The yield from 100 flies was about 1 g muscle. A glass rod was used to separate the muscle masses, breaking them up into small groups of fibrils, and releasing most of the sarcosomes. Gentle pressing and grinding against the side of the vessel was usually sufficient to effect this separation. The disintegrated muscle was then filtered through organdie with gentle pressure. The muscle fragments were resuspended and extracted twice more by this method. After the third extraction the volume of filtrate (S_1) was made up to 10 times the original weight of muscle. The muscle residue (R_1) was resuspended in Ringer solution (volume $\equiv$ 15 times the original weight of muscle). Microscopic examination of the two fractions showed the filtrate to be a very dense and practically pure suspension of sarcosomes with no visible muscle fragments. The residue consisted mostly of separated muscle fibrils with numbers of sarcosomes still attached. The sarcosomes, removed from suspension in S_1 by centrifuging at 500*g* for 15 min, formed an orange-coloured plug at the bottom of the centrifuge tube. This residue (R_2) was resuspended in the original volume of Ringer solution. The supernatant from this separation (S_2) was then centrifuged at 20,000*g* for 15 min. The residue (R_3) was resuspended in the original volume of Ringer solution. The final supernatant (S_3) was clear. These manipulations were carried out at 0°C. Apyrase activities of the fractions obtained are shown in Table 1.

It is apparent that more than half the total apyrase activity was present in the sarcosome fraction, which contained approximately one-third of the total protein. A more complete separation was not possible, since a more vigorous treatment of the muscle broke up the fibrils into fragments which sedimented with the sarcosomes. Moreover, the actomyosin-ATP-ase of the fibrils probably

contributed to the apyrase activity measured in R_1. Further centrifugations and resuspension of the sarcosome fraction revealed that 85-90 per cent. of the total activity was always associated with the particulate material sedimenting at 500*g* (see, for instance, Table 1, S_4 and R_4). Of the remaining 10-15 per cent. of activity, most remained in the supernatant even after centrifugation at 20,000*g* for 15 min. Thus, although a precise separation was not possible, it seems most likely that the Mg-activated apyrase activity was associated exclusively with the sarcosomes.

TABLE 1
FRACTIONATION OF BLOWFLY FLIGHT MUSCLE
Figures compounded from several experiments

Fraction	Weight of Protein per g Wet Weight Muscle (mg)	Total Apyrase Acitvity: Units (Q_p) per g Wet Weight Muscle
Muscle residue (R_1)	130	10,600
Muscle extract (S_1)	90	13,400
Residue after centrifuging S_1 at 500*g* for 15 min (R_2)	74	14,100
Supernatant after centrifuging S_1 at 500*g* for 15 min (S_2)	16	1,600
Residue after centrifuging S_2 at 20,000*g* for 15 min (R_3)	1	200
Supernatant after centrifuging S_2 at 20,000*g* for 15 min (S_3)	15	1,400
Residue after centrifuging R_2 (resuspended) at 500*g* for 15 min (R_4)	41	10,000
Supernatant after centrifuging R_2 (resuspended) at 500*g* for 15 min (S_4)	28	1,500

(*c*) *Experiments with Washed Sarcosomes*

By washing the sarcosomes several times with *Drosophila* Ringer solution, sedimenting at 500*g* on each occasion, it was possible to obtain a reasonably pure and active preparation, having a Q_p of from 2500 to 5000. The highest activities were always obtained by testing the preparation in 0.05M borate buffer at pH 8.0. In *Drosophila* Ringer solution, or in 0.16M NaCl, the activity was only about half as great, although the activity in Ringer could be increased by substituting Mg for Ca in the Ringer solution (Table 2). Substitution of Ca for Mg in the presence of borate buffer produced an almost complete inhibition, further confirming the identity of the sarcosome enzyme with the apyrase previously described (Gilmour and Calaby 1952).

It was thought that the higher activity in borate buffer might be accounted for by the rupture of the sarcosomes in hypotonic solution, followed by the release of apyrase into solution. It was observed through the microscope that sarcosomes in hypotonic solution tended to clump together, and that some were ruptured. Attempts to increase significantly the yield of apyrase in the super-

natant by treating the sarcosomes with either water or 0.05M borate buffer, however, were unsuccessful. This is illustrated by some examples in Table 3. Of the small amount of apyrase removed from the sarcosomes by this treatment, most was associated with fragments sedimenting at 20,000g, i.e. the reverse of the result obtained in the first washings of the sarcosomes, as illustrated in Table 1.

TABLE 2

RELATIVE APYRASE ACTIVITIES OF VARIOUS PREPARATIONS OF WASHED SARCOSOMES

Suspending Medium	Activating Metal Added at Final Concentration of 0·001M	Activity, Expressed as Percentage of Maximum Activity
Borate buffer, 0·05M, pH 8·0	Mg	100
	Ca	12
Drosophila Ringer solution, pH 8·0	Mg	52
Drosophila Ringer solution with Mg substituted for Ca, pH 8·0	—	74
NaCl, 0·16M, pH 7·0	Mg	55

TABLE 3

REMOVAL OF APYRASE FROM SARCOSOMES BY SUSPENSION IN HYPOTONIC MEDIA

All activities measured in presence of 0·001M Mg and 0·05M borate buffer at pH 8·0, and expressed as a percentage of the activity of the original sarcosome suspension

Treatment	Specific Activity (per unit wt. protein)	Total Activity
Washed sarcosome suspension	100	100
Sarcosomes suspended 24 hr in water, centrifuged at 500*g* for 15 min; supernatant tested	60	12
Sarcosomes suspended 24 hr in water, centrifuged at 20,000*g* for 15 min; supernatant tested	30	1
Sarcosomes suspended 1 hr in 0·05M borate buffer, centrifuged at 20,000*g* for 15 min; supernatant tested	12	<0·1

Sacktor (1953) has attributed the hydrolysis of two phosphate groups of ATP by sarcosomes to the simultaneous action of ATP-ase and adenyl kinase. Although it is not doubted that an adenyl kinase is present, the apyrase is itself capable of dephosphorylating ADP (Gilmour and Calaby 1952) and sarcosomes hydrolyse IDP slowly (at about 7 per cent. of the rate of splitting of ATP) in spite of the inactivity of adenyl kinase against IDP.

III. DISCUSSION

It seems most likely that the sarcosomes, which in the highly developed flight muscles of Diptera and Hymenoptera comprise a significantly large pro-

portion of the muscle protein, are concerned largely with the oxidative production of the energy needed to drive the muscle machine. Sarcosomes have been identified biochemically with mitochondria (Watanabe and Williams 1951) and, although so far it has not been possible to demonstrate oxidative phosphorylation in them (Sacktor 1953), their abundance in the flight muscles of insects fits in with the known rapid rate of oxidative metabolism during flight, and the very small oxygen debt of the flight muscles following exercise (Chadwick and Gilmour 1940; Krogh and Weis-Fogh 1951).

The role of the apyrase which is usually found associated with the oxidative enzyme systems in cell granules is not clear. It could act as a regulator, controlling the level of either ATP or inorganic P. Another possibility is that the enzyme is normally coupled with some other process, and that the destruction of ATP is an artefact induced by the separation procedure. It has been shown that an active ATP-ase can be extracted from liver mitochondria, which in the intact state have very little apyrase activity (Kielley and Kielley 1951, 1953). But the intact sarcosomes of insects, suspended in isotonic solution under apparently reasonably physiological conditions, still possess strong apyrase activity. If the apyrase of the sarcosome is coupled with any system to which the energy of the terminal phosphate of ATP can be transferred, it may not be unreasonable to look for this system outside the sarcosome rather than within it.

That the sarcosomes are in fact intimately concerned in the contraction in insect flight muscle can be deduced from the observations of Hanson (1952) on the ATP-contraction of isolated fibrils. Hanson found that contraction occurred only in fibrils to which some sarcosomes were adhering, and that when only a few sarcosomes were attached, the contraction wave could be seen to be starting at the point of attachment of the sarcosome. In other words, the unit of contraction was fibril plus sarcosomes. It is unlikely that the critical function of the sarcosomes was the production of phosphate bond energy, since ATP was present in excess. It may be that a more intimate role in the contraction process for the Mg-activated apyrase, which appears to be quantitatively the more important of the enzymes hydrolysing ATP in flight muscle (Gilmour and Calaby 1953), is to be found in this association of fibril and sarcosomes.

IV. References

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