A7

RELATIVE ACTIVITIES OF THE ADENOSINETRIPHOSPHATASE ENZYMES IN FROG AND PIG SKELETAL MUSCLE

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Introduction: It is well accepted that the rate of post-mortem glycolysis in pig skeletal muscle is a very variable quantity which is profoundly affected by physiological, genetical and environmental factors. Considerable evidence has been presented in the last decade indicating that the rate-determining step in post-mortem glycolysis is the hydrolysis of adenosine triphosphate by one or more of the several adenosinetriphosphatase enzymes (ATPases) present in the muscle fibre. The suggestion that control of the post-mortem glycolytic rate might be effected by an ATPase enzyme was first made by Bendall (1960), who considered that the sarcoplasmic ATPase enjoyed this role.

Kastenschmidt (1970) attributes much of the variability in the rates of post-mortem glycolysis in pig muscle to altered concentrations of such compounds as ATP, ADP, AMP, glucose-6-phosphate, inorganic phosphate and calcium ions. The utilisation of ATP by the various adenosinetriphosphatase enzyme systems from the time of death would in itself cause an increased glycolytic flux by changing the chemical equilibria involved in the maintenance of the resting ATP concentration. Of the products of ATP hydrolysis, inorganic phosphate acts as a substrate for phosphorylase and glyceraldehyde-3-phosphate dehydrogenase, and as an allosteric activator of phosphofructokinase (PFK) and hexokinase (vide review, Kastenschmidt, op.cit.). The rate-limiting reactions in the glycolytic cycle under anoxic conditions, according to Helmreich & Cori (1965), are those catalysed by phosphorylase and PFK.

Several workers have turned their attention to the role of the ATPase enzymes in regulating the rate of post-mortem glycolysis and to the identification of the specific ATPase involved(Quass & Briskey, 1968; Greaser et al., 1969; Heffron & McLoughlin, 1971). The importance of this goal is seen when it is realised that the conversion of muscle to meat is fundamentally the effect of the hydrolysis of ATP during the period from death to the development of rigor mortis (vide, Lister, 1970). Quass & Briskey found that the calcium-activated myosin ATPase activity was higher in stress susceptible animals while Greaser and co-workers showed that there was no difference in the sarcoplasmic reticulum (SR) magnesium-activated ATPase activity in stress susceptible and normal animals. These observations suggest that the myofibrillar ATPase may play a more significant role in determining the rate of post-mortem glycolysis than was heretofore realised.

It was of interest therefore, to examine the relative activities of the ATPase enzymes in pig muscle, and to compare the activity patterns of red and white muscle. A comparison is made with frog skeletal muscle which exhibits very low rates of pH change and ATP hydrolysis post-mortem.

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A7

Methods: Samples of the m. longissimus dorsi and the white and red fibre areas of the m. semitendinosus of male Landrace pigs (73-82 kg.) were subjected to differential centrifugation after homogenisation in nine volumes of 0.25M sucrose+ 0.025M Tris-HCl pH 7.3-7.4. The upper leg muscles of the frog, Rana temporaria, were treated similarly. Natural actomyosin (NAM) was prepared from each muscle as described by Heffron & McLoughlin (1971). Succinic dehydrogenase (SDH) was assayed with iodonitrotetrazolium or manometrically at 37°C. ATPase activities were measured as described elsewhere (Heffron & McLoughlin, op.cit.).

Results: Mg^{2+}-activated ATPase activity in the differential fractions from the sucrose homogenate of the pig longissimus dorsi was assayed in a medium containing 2mM ATP-tris, 2mM $MgSO_4$, ionic strength 0.05, pH 7.25 and 37°C. The ATPase and SDH activities of the isolated fractions are presented in Table 1.

Table 1 - Adenosinetriphosphatase and succinic dehydrogenase activities of subcellular fractions of the longissimus dorsi of the pig.

Fraction	*ATPase activity	%	†SDH activity	%
Homogenate	-	-	12.43	100.0
2,000xg/10 min.	15.0	91.2	8.69	70.0
10.000xg/20 min.	77.2	3.5	1.53	12.4
38,000xg/90 min.	32.5	2.0	0.37	3.0
Supernatant	1.6	3.3	0.00	-

*µmoles ATP hydrolysed/mg. protein/hour at 37°C. † Absorbance (490 nm)/gram wet tissue/hour at 37°C. Activities are average values for three fractionations.

The recovery of SDH activity in the isolated fractions was 85.4% of the total activity of the homogenate. 82% of the SDH activity is found in the so-called myofibrillar fraction and the mitochondrial fraction proper while only 3% remains in the 'heavy' microsomal fraction obtained at 38,000xg. The myofibrillar fraction contains almost all of the Mg^{2+}-activated ATPase activity as might be expected. The mitochondrial ATPase has the greatest specific activity though its apparent contribution to the total is only 3.5%. On the basis of the SDH activity being exclusively mitochondrial the calculated ATPase activity of the mitochondria is 24.4% of the total in the isolated fractions, and the amount of mitochondrial protein is 7.7 mg. per gram wet tissue. The specific activity of the microsomal ATPase is 18.4 µmoles/mg. protein/hour when the presence of mitochondria is accounted for (24%). Assuming that the ATPase activity of the supernatant is due to a lighter microsomal fraction (not sedimented at 38,000xg) the total microsomal ATPase activity is 5.3%, and the corresponding amount of microsomal protein is 4.3 mg. per gram wet tissue.

The semitendinosus muscle of the pig is differentiated into distinct white and red fibre areas; a separate study of the white and red areas was made. Fractionation of the homogenates of the separate fibre areas was carried out as for the longissimus dorsi, and the results are shown in Table 2. The white fibre area

A7

Table 2 - Adenosinetriphosphatase and succinic dehydrogenase activities of subcellular fractions of the white and red fibre areas of the semitendinosus muscle of the pig.

Fraction	White fibre area				Red fibre area			
	*ATPase activity	%	*SDH activity	%	*ATPase activity	%	*SDH activity	%
Homogenate	-	-	10.05	100.0	-	-	29.39	100.0
2,000xg/10 min.	15.0	90.2	7.20	71.6	9.8	83.6	21.20	72.1
10,000xg/20 min.	56.5	3.0	1.30	12.9	46.2	9.2	4.10	14.0
38,000xg/90 min.	29.6	2.6	0.30	3.0	27.5	3.3	0.50	1.7
Supernatant	2.0	4.3	0.00	-	1.6	3.9	0.00	-

*Enzyme activities expressed as in Table 1.

of the semitendinosus has a somewhat lower SDH activity (81%) than the longissimus dorsi while the red area contains three times as much as the white. The recovery of the SDH activity in the isolated fractions was the same for both fibre areas, being 88%. The distribution of the SDH activity in the fractions is similar to the longissimus dorsi. The myofibrillar and mitochondrial ATPase activities are greater in the white fibre area than the red by 53% and 22%, respectively. The mitochondrial content, calculated from the total SDH activity, is 8.9 mg. protein per gram wet tissue for the white area and 20.8 for the red area. Similar calculations to those carried out for the longissimus dorsi show that 21.9% and 57.7% of the total ATPase activity of the isolated fractions belong to the mitochondrial fractions of the white and red areas, repectively. The specific activity of the microsomal ATPase is 21.6 μmoles/mg. protein/hour in the white area and 25.0 in the red area. The corresponding amounts of microsomal protein are 6.0 mg. per gram wet tissue in the white and 4.4 in the red. It is notable that mitochondrial contamination of the red microsomal fraction is only 12% compared with 23% in the white

A complete fractionation of the homogenate derived from the upper leg muscles of the frog is shown in Table 3. The distribution of SDH activity in the fractions is similar to the pig muscles. The calculated mitochondrial ATPase activity is 17.3% of the total, and the amount of mitochondrial protein per gram wet tissue is 15.5 mg. The microsomal fractions have the greatest specific ATPase activity, the 'light' (200,000xg) fraction having a greater activity than the 'heavy' (75,000xg) fraction. The ATPase activity of the combined microsomal fractions is 18.6% of the total for the isolated fractions, assuming that the ATPase activity of the supernatant is due to residual microsomal material. It is calculated that 0.9 mg. of microsomal protein per gram tissue remains in the supernatant after centrifugation at 200,000xg, and that the total microsomal protein content is 4.5 mg. per gram wet tissue.

A7

Table 3 - Distribution of adenosinetriphosphatase and succinic dehydrogenase activities in subcellular fractions of frog leg muscle.

Fraction	mg.Protein /gram wet tissue.	*ATPase activity	%	†SDH activity %
Homogenate	-	-	-	100.0
2,000xg/10 min.	142.0	7.0	76.6	72.3
10,000xg/10 min.	3.9	14.5	4.4	24.4
75,000xg/20 min.	2.3	43.5	7.7	2.0
200,000xg/40 min.	1.6	58.0	7.2	0.0
Supernatant	22.0	2.4	4.1	0.0

*μmoles ATP hydrolysed/mg. protein/hour at 30°C. †measured manometrically at 37°C. Activities are average values for 3 (ATPase) and 4 (SDH) preparations.

ATPase activity of natural actomyosin: Natural actomyosins isolated by the same method from the longissimus dorsi, the white and red areas of the semitendinosus of the pig, and the leg muscle of the frog had the following activities when assayed in 2mM ATP-tris, 2mM $MgCl_2$, ionic strength 0.05, pH 7.3 and 30°C, respectively: 19.7, 18.0, 12.0 and 28.3 μmoles ATP hydrolysed per mg. protein per hour. The frog actomyosin was more active than any of the others while the red actomyosin showed the lowest activity.

Discussion: There is little difference in the amounts of myofibrillar material in the longissimus dorsi, the white and red areas of the semitendinosus of the pig, and the leg muscles of the frog. Of the muscle examined, the myofibrillar Mg^{2+}-activated ATPase activity of the longissimus dorsi was greatest. Natural actomyosin Mg^{2+}activated ATPase activity was greatest in the longissimus dorsi and frog leg muscle and least in the red fibre area of the semitendinosus.

Microsomal Mg^{2+}-activated ATPase showed greatest activity in the red fibre area of the semitendinosus under the assay conditions used; it was 34% greater than the longissimus dorsi enzyme and 16% greater than the enzyme from the white fibre area, but it was less than half as active as the corresponding frog muscle enzyme. The amount of microsomal protein was the same in all the muscles except the red fibre area of the semitendinosus which had 33% more protein. The latter had also the greatest amount of mitochondrial protein, as expected. The mitochondrial ATPase activity of the pig muscles was very high compared with with that of the frog muscles. However, in view of the latency of mitochondrial ATPase in liver tissue (Myers & Slater, 1957), it is likely that the rather high activity of isolated muscle mitochondria is due to disruption of the mitochondrial membrane by the homogenisation procedure, and that mitochondrial ATPase activity is of little importance in the intact tissue.

It was not possible to detect the Na^+-K^+-ATPase in the isolated fractions with the preparative procedure used. Its contribution to the total ATPase activity of

A7

the muscle fibre cannot be assessed until a reliable method is obtained for its preparation in an active form.

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