Nematode Metabolism: the Adenosine Triphosphatase of *Ascaris suum* Muscle

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The relationship between ATP hydrolysis and muscle contractility has been firmly established (Szent-Györgi, 1950; Davies, 1965), with the hydrolysis being catalysed by the myosin ATPase* activity of the actomyosin molecule (Huxley & Hanson, 1955). ATPase-like activity has been noted in *Ascaris lumbricoides* muscle (Kirby & Neuberger, 1938; Rogers & Lazarus, 1949), but no attempts were made to define its enzymic characteristics or to relate the activity to a physiological process.

Ascaris suum were obtained from a local slaughterhouse and were transported to the laboratory in Ringer solution (NaCl, 9g/l; KCl, 0.4g/l; CaCl₂·6H₂O, 0.25g/l; NaHCO₃, 0.2g/l). The muscle was stripped from the cuticle after removal of the digestive and reproductive tracts and placed in cold (2–4°C) Ringer solution. The muscle was washed twice and stored frozen in Ringer solution (10g of muscle/30ml). Before use the preparation was thawed and the muscle strips were washed twice with 0.2M-KCl. A 10g portion of muscle was then homogenized in 30ml of 0.2M-KCl in a Sorvall Omni-Mixer for three 1min intervals at full speed at 2°C. The homogenate was centrifuged at 49 500g for 1 h, the supernatant serving as the source of enzyme. Total protein was determined spectrophotometrically by using a modification (Langer *et al.*, 1967) of the method described by Layne (1957). The reaction mixture, consisting of 1ml of glycine-NaOH buffer, pH9.5 (0.2M), containing ATP (20nM) and CaCl₂ (10nM) and 1ml of enzyme (2–7mg of protein), was incubated for 60min at 37°C in a shaking water bath. Enzyme was added to the control tubes at the end of the reaction period and the reactions were stopped by the addition of 1ml of 15% (w/v) trichloroacetic acid. The coagulated protein was removed by centrifugation for 10min at 3100g. Then 0.5ml portions of the supernatant were removed for determination of P_i by the method of Fiske & SubbaRow (1925), as described by Leloir & Cardini (1957). Preliminary studies had shown that P_i production was a linear function of time and enzyme concentration over the ranges used in this study. The apparent Michaelis constants for ATP and Ca²⁺ were calculated from the slope and y-intercept of the line determined by least-squares analysis of the reciprocal-plot method of Lineweaver & Burk (1934). The effects of pH on enzymic activity were

studied by using tris-maleate, pH range 5.5–8.5 (0.2M), and glycine-NaOH, pH range 8.0–10.5 (0.2M), buffers. The inhibitory effect of Mg²⁺ was determined by incorporating the appropriate concentration of MgCl₂ into the buffer with the ATP and Ca²⁺. The studies showing Ca²⁺- and ATP-dependence and Mg²⁺ inhibition used an enzyme preparation that had been dialysed against four 1-litre changes of 0.2M-KCl over a 20h period.

The ATPase of *A. suum* muscle possesses a low activity (30–50% of maximal) in the pH range 5.5–8.5, reaching a minimum at pH6.5–7.0, followed by a sharp rise to a maximum at pH9.5 and rapid decline to near-zero activity at pH10.5 (Fig. 1). The reaching of a minimum at approximately neutral pH has been reported for the Ca²⁺-activated myosin ATPase of skeletal muscle (Katz, 1970). *A. suum* ATPase was activated by Ca²⁺ and inhibited by Mg²⁺ (Table 1), and in addition to hydrolysing ATP it was qualitatively determined that P_i was released from ITP and GTP. These observations are in good agreement

Fig. 1. Effect of pH on the activity of the ATPase of *A. suum* muscle

The line, as presented, results from computer analysis of the data. ●, Tris-maleate buffer; ○, glycine-NaOH buffer. Methods are described in the text.

* Abbreviation: ATPase, adenosine triphosphatase.

Table 1. *ATP- and Ca²⁺-dependence and Mg²⁺ inhibition of the ATPase of A. suum muscle*

Methods are described in the text.

Concn. of component in final reaction mixture (mM)			Specific activity (nmol of P _i formed/min per mg of protein at 37°C)
ATP	Ca ²⁺	Mg ²⁺	
—	5	—	0.15
10	—	—	2.69
10	5	—	9.66
10	5	0.005	9.51
10	5	0.05	8.88
10	5	0.5	5.92
10	5	5	3.58

with the properties of mammalian muscle myosin ATPase (Perry, 1955; Barman, 1969).

The apparent Michaelis constants, K_m , for ATP and Ca²⁺ were calculated to be 3×10^{-4} M and 4.7×10^{-4} M respectively. Our observed value for ATP is about 20 times that reported for rabbit skeletal muscle ATPase (Barman, 1969) and approx. 40% of the value observed for rat cardiac myosin ATPase (McCarl *et al.*, 1969). Direct comparison of the value calculated for the K_m for Ca²⁺ is not possible owing to the lack of a published value; however, an approximate K_m of 2.8×10^{-5} M has been calculated from data presented by Weber & Portzehl (1954). The K_m values observed with *A. suum* muscle ATPase appear to be within the ranges observed for mammalian myosin ATPase.

The findings suggest that the ATPase of *A. suum* muscle is a myosin ATPase (ATP phosphohydrolase, EC 3.6.1.3). This, in turn, is indicative that the mechanisms of muscular contraction utilized by organisms at various levels of the evolutionary scale are essentially the same, in that they appear to use the same substrates and enzymic processes.

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