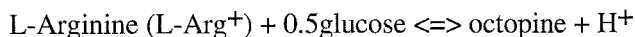
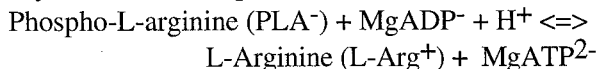


Studying the metabolic machinery of contracting squid mantle muscle *in vivo*: A time resolved ^{31}P -NMR study

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Introduction

The highly active, muscular squid of the pelagic rely on jet propulsion for locomotion by means of mantle muscle contractions. Continuous mantle activity is required for ventilation and gas exchange. The metabolic rate of this high strung molluscan muscle is the highest reported for marine invertebrates, and is supported by a high content of mitochondria. It makes maximum use of available oxygen by not only exploiting blood oxygen transport but also by oxygen uptake via the skin (1). At the anaerobic threshold (reached at a critical swimming speed) anaerobic energy production starts simultaneously in the cytosol and in mitochondria indicating that oxygen supply to mitochondria becomes limiting (2). This finding is quite opposite to the situation found in many other invertebrate and vertebrate species, where energy requirements in excess of aerobic energy production are covered by anaerobic metabolism, with the mitochondria remaining aerobic. Octopine is the end product of anaerobic glycolysis in squids, the muscular phosphagen is phospho-L-arginine, which releases arginine into octopine formation during anaerobic contraction and supports the extension of activity beyond the critical speed (3):



Considering this special metabolic situation in squid mantle muscle we studied the energetics of muscular contraction *in vivo* by use of ^{31}P -NMR.

Material and Methods

Squid (*Lolliguncula brevis*, 10-15 g body weight) were collected in the Galveston ship channel and maintained in aquaria with filtered, recirculated and well aerated sea water at ambient temperature (20° C) until experimentation. They were cannulated in the mantle cavity for continuous pressure recordings (via an UFI type 1050 pressure transducer and a MacLab system, ADI instruments, Hastings, UK) which reflect the contractions of the mantle. Cannulated squid were placed in a water bath containing continuously aerated sea water and were brought into a 2.5 cm diameter polyacrylic tube with an opening close to the water surface, such that a surface coil could be positioned close to the mantle. The catheter was fed through the wall of the tube and served to immobilize the squid. NMR experiments were performed in a Varian 40 cm horizontal bore magnet at 4.7T. *In vivo* ^{31}P -NMR spectra were acquired by gating the RF-pulses with pressure recordings. This allowed to monitor high energy phosphates and pH for various time windows during and between pressure pulses. Contractions and thus pressures varied during a more or less regular

cycling of spontaneous activity and allowed to monitor changes at different activity levels.

Results and Discussion

The recordings revealed an oscillatory use of the phosphagen, phospho-L-arginine (PLA), even during ventilatory mantle contractions at low pressure amplitudes as indicated by a slight, but significant drop in the ratios of inorganic phosphate levels over ATP and PLA concentrations. Intracellular pH was not affected during these slight oscillations but became more alkaline when contractions developed to be more vigorous. Ventilatory oscillations were without net effect on pH also when it was more alkaline in prestressed specimens. Spontaneous jetting at pressure amplitudes above critical (1.2 kPa) led to an alkalosis which was subsequently reversed. These data confirm previous, invasive analyses where levels of inorganic phosphate and phospho-L-arginine changed already at swimming speeds below critical (2).

In conclusion, the levels of these metabolites will fluctuate with any change in the workload of the muscle. However, these changes are reversible on a very short time scale as seen between ventilatory mantle contractions.

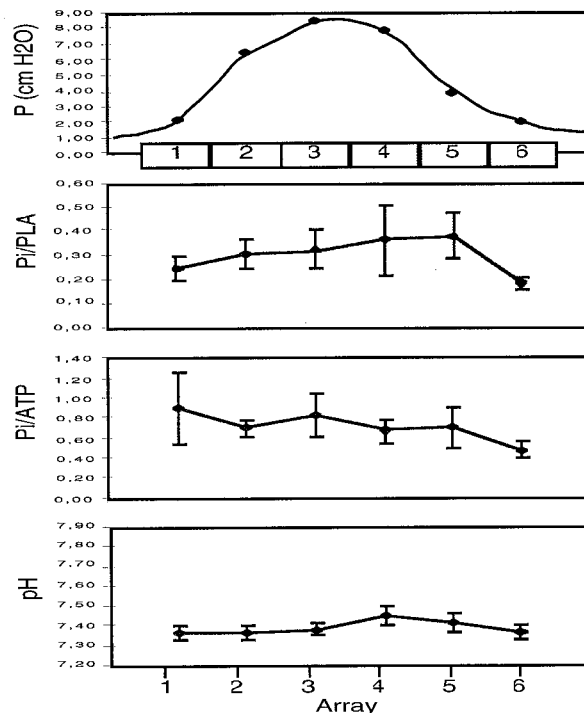


Figure 1: Changes in intracellular pH and the ratio of the contractions of inorganic phosphate (Pi) over the levels of phospho-L-arginine (PLA) or ATP monitored within 6 time windows during the course of a ventilatory pressure (P) pulse.

- (1) Pörtner, H.O. (1994) in "Physiology of cephalopod molluscs: lifestyle and performance adaptations" (ed. H.O. Pörtner, R.K. O'Dor, D. MacMillan, Gordon und Breach, Basel.
- (2) Finke, E., H.O. Pörtner, P.G. Lee, D.M. Webber (1996) J. exp. Biol. 199, 911-921.
- (3) Pörtner, H.O., E. Finke, P.G. Lee (1996) Am. J. Physiol. 271, R1403-1414.