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PYRUVATE KINASE AND PHOSPHOENOLPYRUVATE CARBOXYKINASE ACTIVITIES IN ADULT *ANGIOSTRONGYLUS CANTONENSIS*

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Hsiu-Hui Shih and Shiu-Nan Chen (1983) Pyruvate kinase and phosphoenolpyruvate carboxykinase activities of adult *Angiostrongylus cantonensis*. *Bull. Inst. Zool., Academia Sinica* **22**(2): 187-191. Pyruvate kinase (PK) (EC 2.7.1.40) and phosphoenolpyruvate (PEP) carboxykinase (EC 4.1.1.32) activities were measured for two subcellular fractions of *Angiostrongylus cantonensis* or *Ascaris suum* muscle.

The results showed that in adult *A. cantonensis* the activity of PK was 3.7-4.9 times higher than that of PEP carboxykinase. There were appreciable quantities of ethanol detected in the SUP and SED fractions of *A. cantonensis*. By contrast, in the muscle extract of *A. suum* PEP carboxykinase activity was significantly greater than PK activity.

In the study of glycolysis of *Angiostrongylus cantonensis*, Shih and Chen (1982) reported that this parasite possesses all the enzymes to operate the Embden-Meyerhof pathway. Under this metabolic pattern, pyruvate can be produced from phosphoenolpyruvate (PEP) catalyzed by pyruvate kinase (PK) (EC 2.7.1.40). However, whether *A. cantonensis* is capable of converting phosphoenolpyruvate to oxaloacetate (PEP $\xrightarrow{\text{PEP carboxykinase}}$ oxaloacetate) is still in doubt.

Bueding and Saz (1968) demonstrated that schistosomes possess a high PK and low PEP carboxykinase (EC 4.1.1.32) activities and excrete almost exclusively lactate. On the contrary, *Ascaris lumbricoides* (Bueding and Saz, 1968) and *A. suum* (Van Den Bossche, 1969) excrete very little or none lactate and large amounts of succinate and volatile acid following the utilization of glucose. Vaastrate (1969) reported that *Dictyocaulus viviparus* possessed PK and PEP carboxykinase with comparable activity.

To investigate the pattern of PEP degradation in *Angiostrongylus cantonensis*, the activity ratio of PK/PEP carboxykinase was elucidated. For comparison, muscle extract of *Ascaris suum* was also assayed for PK and PEP carboxykinase and served as positive control experiment.

MATERIALS AND METHODS

Adult *Angiostrongylus cantonensis* was obtained as described previously (Chen *et al.*, 1981) following 50 days post-infection of the 3rd stage larvae in rats. Adult *Ascaris suum* was obtained from local slaughter house.

All the experimental worms were washed in normal saline for several times and used for experiment freshly.

Preparation of worm fractions

Muscle of adult *A. suum* was obtained as described by Laser (1944) and Bueding (1950) by removing of visceral tissues and cuticles. The motile *A. cantonensis* and muscle strips of *A. suum* were then homogenized in three volumes of homogenizing media respectively,

using Bellco glass microhomogenizer (Vineland, New Jersey, USA) in an ice/water bath. The homogenizing media consist of 10 mM triethanolamine buffer, pH 7.6, containing 1 mM EDTA and 0.25 M sucrose for pyruvate kinase assay and 10 mM imidazole buffer, pH 7.0, containing 2 mM dithiothreitol and 0.25 M sucrose for phosphoenolpyruvate (PEP) carboxykinase assay.

The homogenates were then centrifuged for 15 minutes at $600\times g$ using Beckman J-21C centrifuge. The pellets were discarded and the supernatants were re-centrifuged at $10,000\times g$ for 10 minutes. Following centrifugation, the supernatants were collected and designated as SUP fraction and the pellets were resuspended in homogenizing media and designated as SED fraction. In the SED fractions mainly mitochondria were included.

Both of these two fractions were applied to Sephadex column chromatography and eluents were assayed of PK and PEP carboxykinase activities, respectively, as described in the following sections.

Column chromatography

SUP fraction obtained from either *A. suum* or *A. cantonensis* were applied to a Sephadex G-25 M column (PD-10 column, 15×5 cm, bed volume 9 ml, Pharmacia Fine Chemicals, AB Uppsala. Sweden) and with 1 mM imidazole buffer, pH 7.0 and 5 mM triethanolamine buffer, pH 7.6, alternatively. The activities of PEP carboxykinase and PK in the eluent were detected.

Enzyme assay techniques and identification of ethanol

PK activity was determined using the technique described by Shonk and Boxer (1964). The activity assay for PEP carboxykinase was based on the procedures of Bueding and Saz (1968).

All the enzyme assays were carried out at room temperature (approximately 25°C) with a Gilford Model 250 spectrophotometer. Quartz cuvettes with an optical path length of 1 cm and final volume of 1 ml were employed. The rate of oxidation of NADH was monitored with $\varepsilon=6220$ liter $\cdot$ mol^{-1} $\cdot$ cm^{-1} at A_{340}. One unit of enzyme is that amount causing the oxidation of 1 μmole of NADH per minute under described conditions.

PK and PEP carboxykinase activities were determined for homogenized materials and fractions from Sephadex G-25 M column.

The total protein in each fraction was determined by using BIO-RAD protein assay kit (BIO • RAD Laboratory, Richmond, Calif. USA).

The presence of ethanol in SUP or SED fractions was determined using spectrophotometric method as described by Lo and Reeves (1978).

The compounds used in the present experiment such as NAD, NADH, ADP, imidazole, sodium phosphoenolpyruvate, tris, dithiothreitol, yeast alcohol dehydrogenase, malate dehydrogenase and adenosine-5′-diphosphate were purchased from Sigma Chemical Co. (St. Louis, Mo. USA) and triethanolamine from Cambrian Chemicals (Crydon, UK).

RESULTS

The activities of PK and PEP carboxykinase in adult *A. cantonensis* and *A. suum* are presented in Table 1. Significant activities were observed in SUP and SED fractions of both *A. cantonensis* and *A. suum*. Either SUP or SED fraction of *A. cantonensis* exhibited higher activity of PK than that of PEP carboxykinase. The ratio of PK to PEP carboxykinase is 4.99 or 3.76 for *A. cantonensis* SUP and SED fractions respectively. On the contrary, greater activity of PEP carboxykinase was observed in *A. suum*. The PK/PEP carboxykinase for SUP and SED of *A. suum* is 0.11 and 0.15 respectively.

The peak fractions of *A. cantonensis* from Sephadex G-25 M column showed a significant PK activity which is 1.4 times higher than that from the SUP fraction. However, this peak fraction of *A. cantonensis* revealed only a very little activity of PEP carboxykinase (Table 1 and Fig. 1). Fig. 2 and Table 1 also show that the activity of PEP carboxykinase of *A. suum*

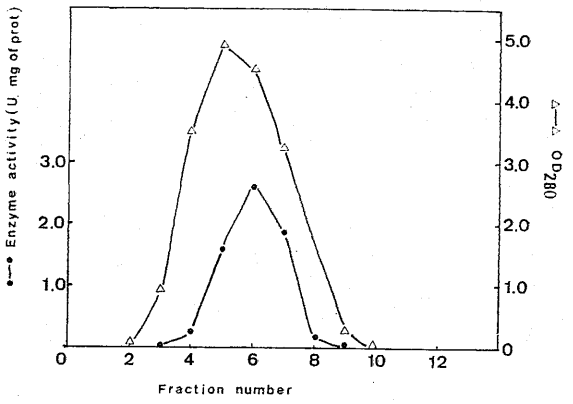


Fig. 1. Sephadex G-25 M chromatography of PK from SUP of *Angiostrongylus cantonensis*. The column was previously equilibrated with 5 mM triethanolamine buffer, pH 7.6. Elution was carried out with the same buffer and the fraction volume was 1 ml. Pyruvate kinase activity was measured as described in "Materials and Methods".

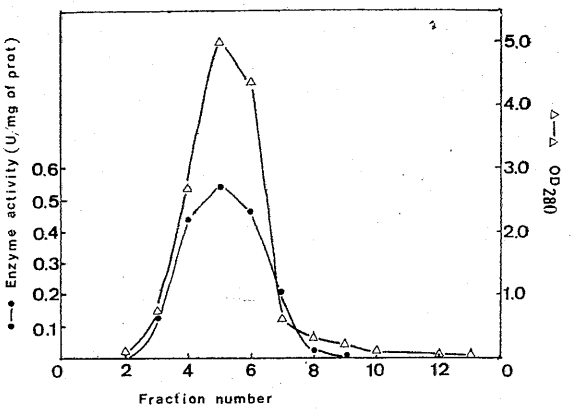


Fig. 2. Sephadex G-25 M chromatography of PEP carboxykinase from SUP of *Ascaris suum*. The column was previously equilibrated with 1 mM imidazole buffer, pH 7.0. Elution was carried out with the same buffer and the fraction volume was 1 ml. PEP carboxykinase activity was measured as described in "Materials and Methods".

TABLE 1

Pyruvate kinase and PEP carboxykinase activities[a] of various fractions of adult *Angiostrongylus cantonensis* and muscle of *Ascaris suum*

	Pyruvate kinase(1)	PEP carboxykinase(2)	Ratio(1)/(2)
Angiostrongylus cantonensis			
SUP	1.428±0.442[b] | 2.682±0.382[c]	0.286±0.096	4.99[b] | 6.19[c]
SED	0.673±0.126	0.179±0.014	3.76
Ascaris suum			
SUP	0.033±0.021	0.306±0.025[b] | 0.427±0.162[c]	0.11[b] | 0.08[c]
SED	0.021±0.008	0 140±0.030	0.15

SUP and SED fractions were obtained as described in the section of Materials and Methods.

a. Unit of specific activity of enzyme is μmole/min/mg protein (U/mg protein).

b. Results are the average of three experiments±the standard deviation.

c. Average of the activity of enzyme at the peak fraction following elution from Sephadex G-25 M column.

was enhanced following the elution of SUP fractions from Sephadex G-25 M column. In the same fraction very little activity of PK was observed. Using spectrophotometric method, 8.40 μmol/ml or 11.27 μmol/ml of ethanol was detected in the SUP or SED fraction of adult *A. cantonensis*.

DISCUSSION

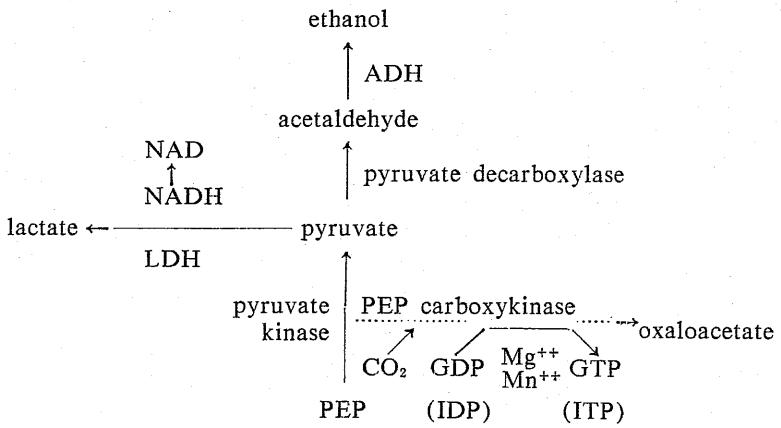
A number of studies have demonstrated that there were significant differences in pyruvate kinase and PEP carboxykinase activities in parasitic helminths (von Brand, 1973). Adult worms of *A. suum* (Bossche, 1969), *Hymenolepsis*

diminuta (Prescott and Campbell, 1965) and *Fasciola hepatica* (Prichard and Schofield, 1968) and the third stage larva of *Hoemonchus contortus* exhibited a higher activity of PEP carboxykinase to provide favorable conditions for oxaloacetate formation in these parasites. The similar results were also obtained in SUP and SED fractions of *A. suum* that were used as positive control in the present study. In contrast, *Schistosoma japonica* pyruvate kinase is significantly higher than PEP carboxykinase activity, suggesting the predominance of lactate over succinate formation in this parasite (Bueding and Saz, 1968).

The present study showed a marked difference between pyruvate kinase and PEP carboxykinase activity in both SUP and SED fractions of *A. cantonensis*. *A. cantonensis* pyruvate kinase activities is 3.7–4.9 times higher than PEP carboxykinase. It is suggested that the metabolism of PEP in *A. cantonensis* is directed towards its dephosphorylation for pyruvate formation by the catalysis of pyruvate kinase. This would also account for the presence of ethanol in *A. cantonensis*.

In the study of carbohydrate metabolism of *A. cantonensis*, Yanagisawa and von Brand (1965) reported that aerobically 76% or anaerobically 79–90% of absorbed glucose was degraded into lactate *in vitro*. They also reported that no succinic acid was formed in the metabolites of *A. cantonensis*. Little activity of PEP carboxykinase obtained in the present study further confirmed that PEP to oxaloacetate pathway is less important for PEP degradation of *A. cantonensis*.

In an earlier study (Shih and Chen, 1982) it was observed that the activities of lactate dehydrogenase, pyruvate decarboxylase and alcohol dehydrogenase were present in the extract of adult *A. cantonensis*. These results incorported with the greater activity of pyruvate kinase and the presence of ethanol in *A. cantonensis* may reflect that pyruvate to lactate and pyruvate to alcohol are possibly main metabolic pathways for adult *A. cantonensis*. (Scheme 1).



Scheme 1. Proposed phosphoenolpyruvate (PEP) degradation pathways in adult *Angiostrongylus* cantonensis.

REFERENCES

BUEDING, E. (1950) Carbohydrate metabolism of *Schistosoma mansoni*. *J. Gen. Physiol.* **33**: 475-495.

BUEDING, E. and H. J. SAZ (1968) Pyruvate kinase and phosphoenol-pyruvate carboxykinase activities of *Ascaris* muscle, *Hymenolepsis diminuta* and *Schistosoma mansoni*. *Comp. Biochem. Physiol.* **24**: 511-517.

LASER, H. (1944) The oxidative metabolism of *Ascaris suum*. *Biochem. J.* **38**: 333-338.

LO, H. and R. E. REEVES (1978) Pyruvate-to-ethanol pathway in *Entamoeba histolytica*. *Biochem. J.* **171**: 225-230.

PRESCOTT, L. M. and J. W. CAMPBELL (1965) Phosphoenol-pyruvate carboxylase activity and

glycogenesis in the flatworm, *Hymenolepsis diminuta*. *Comp. Biochem. Physiol.* **14**: 491-511.

PRICHARD, R. K. and P. J. SCHOFIELD (1968) Phosphoenolpyruvate carboxykinase in the adult liver fluke, *Fasciola hepatica*. *Comp. Biochem. Physiol.* **24**: 773-785.

SHIH, H. H. and S. N. CHEN (1982) Glycolytic enzymes in juvenile and adult *Angiostrongylus cantonensis*. *Southeast Asian J. Trop. Med. Pub. Hlth.* **13**(1): 114-119.

SHONK, C. E. and G. E. BOXER (1964) Enzyme patterns in human tissues. I. Methods for the determination of glycolytic enzymes. *Cancer Res.* **24**: 709-721.

VAN DEN BOSSCHE, H. (1969) Phosphoenolpyruvate carboxykinase activity in *Ascaris suum* muscle. *Comp. Biochem. Physiol.* **31**: 789-797.

VON BRAND, T. (1973) *Biochemistry of parasites*. 2nd ed. Academic Press, New York and London. 137 pp.

YANAGISAWA, T. and T. VON BRAND (1965) Carbohydrate metabolism in *Angiostrongylus cantonensis*. *J. Parasitol.* **51**(3): 418-423.

廣東住血線蟲 (*Angiostrongylus cantonensis*) 成蟲之丙酮酸激酶與磷烯丙酮羧酸激酶活性之研究

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本實驗乃在探測廣東住血線蟲中兩種酵素：丙酮酸激酶 (pyruvate kinase) 及磷烯丙酮羧酸激酶 (phosphoenolpyruvate carboxykinase) 之活性，並計算其比值，與其他線蟲比較之，以推測此線蟲對磷烯醇丙酮酸 (phosphoenolpyruvate) 之代謝途徑。

結果顯示，以 10,000×g 分離之廣東住血線蟲上層液與沉積物中皆有顯著之丙酮酸激酶與磷烯丙酮羧酸激酶之活性存在，而以上層液之活性較強。丙酮酸激酶/磷烯丙酮羧酸激酶之比值，上層液爲 4.99，沉積物爲 3.76。將上層液通過 Sephadex G-25 M column 後，以波長 280 μm 測出之吸光度尖峯之丙酮酸激酶活性增強，比值上升爲 6.19。此結果顯示，有關廣東住血線蟲之代謝，磷烯醇丙酮酸 —丙酮酸激酶→ 丙酮酸途徑較磷烯醇丙酮酸 —磷烯丙酮羧酸激酶→ 草酸爲重要。

本研究並以豬蛔蟲 (*Ascaris suum*) 爲對照組，實驗結果顯示其丙酮酸激酶/磷烯丙酮羧酸激酶比值在沉積物爲 0.15，上層液爲 0.11；通過 column 後，上層液之磷烯丙酮羧酸激酶之活性增強，酶之比值降爲 0.08。