FOLIA PARASITOLOGICA 34: 131–136, 1987.

MALIC ENZYME, MALATE DEHYDROGENASE, FUMARATE REDUCTASE AND SUCCINATE DEHYDROGENASE IN THE LARVAE OF TAENIA CRASSICEPS (ZEDER, 1800)

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Abstract. Malate dehydrogenase, malic enzyme, succinate dehydrogenase, and fumarate reductase activities have been studied in the cytoplasm and mitochondria of *Taenia crassiceps* larvae. The results show that these larvae contain enzymes for anaerobic acquisition of energy with terminal fumarate reductase, but some facts, as the high ratio of succinate dehydrogenase activity to fumarate reductase activity and the low proportion of fumarate reductase in the whole NADH oxidase activity in mitochondria, suggest that aerobic processes are also involved in the energy acquisition in this parasite.

The basic metabolic and bioenergetic processes in parasitic worms have been mostly studied only in some model species. The obtained results indicate that there are some common features characteristic of the metabolism of parasitic worms, which result from the parasitic way of living on the one hand and, particularly, from a certain stage of phylogenetic development on the other hand (Barrett 1981). Nevertheless, some peculiarities occur in individual species, which are characteristic of the species or group of species taxonomically related and with a similar way of living. It is therefore necessary to study the metabolism not only in individual helminth species but also in their individual developmental stages, as it is known that significant changes in the metabolism may occur in some species during their development.

The aim of the present studies was to obtain basic information on enzyme activities in the larvae (cysticercus) of *Taenia crassiceps*. The following enzymes, which are considered to be significant for the processes of energy acquisition in parasitic worms, have been studied: malate dehydrogenase (MDH) — L-malate: NAD oxidoreductase, EC 1.1.1. 37; malic enzyme (ME) — L-malate: NADP oxidoreductase (decarboxylating), EC 1.1.1. 40; succinate dehydrogenase (SDH) — succinate: oxidoreductase, EC 1.3.99.1; and fumarate reductase (FR). As to the last enzyme, it has not yet been decided whether a separate enzyme or fumarate reductase activity of succinate dehydrogenase was involved.

MATERIALS AND METHODS

The cysticerci of *T. crassiceps* used for the preparation of mitochondria and cytoplasmic fraction had been previously passaged with 6 months' intervals in rats in our laboratory. The cysticerci (90 ml) were washed six times with PBS, twice with 200 ml of mitochondrial medium (250 mM-sucrose containing 10 mM Tris and 2 mM EDTA, pH 7.5) and homogenized with 360 ml of mitochondrial medium using Potter homogenizer with teflon piston at 0 °C (700—1,000 RPM, 4 strokes). Then they were centrifuged at 500 × g for 10 min using Janetzki K 70 centrifuge. This and further processes were performed at 4 °C. The sediment was removed and the supernatant was centrifuged at 10,000 × g for 30 min. The supernatant (355 ml) was then stored and used as a cytoplasmic fraction in further studies of enzyme activities. The sediment was suspended in mitochondrial medium and further centrifuged at 11,740 × g for 30 min. The sediment (mitochondria) was then suspended in 20 ml of mitochondrial medium and its enzyme activities were studied. This suspension was sonicated in ice bath for 5 × 6 sec.

Assays of enzyme activities. All measurements were performed at 25 °C using a Specol 21 spectrophotometer with a recorder enabling to check on continually the absorbance changes. In all cases, the volume of the reaction mixture was 1 ml and the light path 1 cm. The composition of the reaction mixture was as follows:

MDH: 50 mM glycine buffer, pH 10.0, 30 mM malate Na, 2 mM NAD (absorbance increase monitored at 340 nm)

MDH (reduction of oxaloacetate): 100 mM phosphate buffer (Na, K), pH 7.5, 0.333 mM oxaloacetate, 1 mM NADH (absorbance decrease monitored at 340 nm)

ME: 100 mM Tris, pH 7.5, 30 mM malate Na, 1 mM NADP, 0.4 mM $MnCl_2$ (absorbance increase monitored at 340 nm)

SDH (after Boczon 1976): 100 mM pohosphate buffer (Na, K), pH 7.2, 1 mM $K_3Fe(CN)_6$, 10 mM KCN, 10 mM succinate (absorbance decrease monitored at 420 nm)

FR (after Boczon 1976): 400 mM phosphate buffer (Na, K), pH 7.0, 33 μM $CaCl_2$, 10 mM $MgCl_2$, 0.12 mM NADH, 10 mM fumarate (absorbance decrease monitored at 340 nm).

The concentrations of substrates were changed not only for the determination of K'_M, but also for the detection of the possible inhibition by the substrate and finding of optimum conditions of the reaction (see below). The activities of MDH with NADP and of ME with NAD as coenzyme were tested as well. The used amount of 20—50 μl of enzyme preparations corresponded to 28.3—70.8 μg of cytoplasmic proteins or to 33.4—83.5 μg of mitochondrial proteins. The values of molar extinction coefficient of reduced nucleotides, 6,220 M^{-1} cm^{-1}, and molar extinction coefficient of $K_3Fe(CN)_6$, 1,030 M^{-1} cm^{-1}, were used for the calculation. Protein was estimated after Lowry et al. (1951) employing bovine serum albumine as a standard. Kinetic constants were calculated from double reciprocal plots after Lineweaver and Burk, the interruption of the straight line was made by the method of smallest squares using TI-58c calculator.

RESULTS

Malate dehydrogenase. Results of the studies of this enzyme in the mitochondria and cytoplasm of *T. crassiceps* larvae are summarized in Table 1. The MDH activity was found to be 1.47 times (in relation to protein) higher in the cytoplasm than in mitochondria. In general, the MDH activity in the cytoplasmic fraction was 22 times higher than in mitochondria. The cytoplasmic enzyme differs from the mitochondrial

Table 1. Malate dehydrogenase in *T. crassiceps* larvae

Properties	Cytoplasmic MDH	Mitochondrial MDH
Specific activity (NAD as coenzyme) (mean of 4 measurements)	123.5 nmol substr./ min/mg protein	84 nmol substr./ min/mg protein
Activity with NADP	0	0
Inhibition by substrate (30 mM malate)	24.4 %	0 %
K'_M for malate (at 2 mM NAD)	$2.93 . 10^{-5}$ M*	$1.58 . 10^{-4}$ M
K'_M for NAD (at 30 mM malate)	$1.80 . 10^{-5}$ M	$9.45 . 10^{-5}$ M

* Tested at non-inhibiting malate concentration (up to 3 mM).

one in a higher affinity to the substrates and in that it is inhibited by higher malate concentrations. The K'_M values serve for the orientation in evaluating the reaction conditions and in comparing both MDH forms and a more precise determination of these values by means of purified enzymes will be necessary. The cytoplasmic MDH was further studied in a reversed reaction, in oxaloacetate reduction. While assessing

K'_M for NADH at oxaloacetate concentration of 0.333 mM it was found that the maximum reaction velocity was achieved already at the concentration of 0.143 mM and this concentration was further used. K'_M for NADH at 0.333 mM oxaloacetate was $2.53 . 10^{-5}$ M, while K'_M for oxaloacetate at 0.143 mM NADH was $4.5 . 10^{-5}$ M. Under conditions of maximum reaction velocity (0.143 mM NADH, 0.333 mM oxaloacetate) the activity of cytoplasmic MDH was 3 052.2 nmol substrate/min/mg protein. A comparison of the activities of cytoplasmic MDH in the direction malate oxidation — oxaloacetate reduction indicates that under optimum conditions, oxaloacetate reduction is 24.7 times more rapid than malate oxidation.

Table 2. Malic enzyme in *T. crassiceps* larvae

Properties	Cytoplasmic ME	Mitochondrial ME
Specific activity (mean of 4 measurements)	13 nmol substrate/ min/mg protein	61.2 nmol substrate/ min/mg protein
Effect of Mn^{2+}	activation	activation
K'_M for NADP (at 30 mM malate)	—	$5.78 . 10^{-6}$ M
Effect of inhibitors (at 30 mM malate)		
5 mM oxalate	74 % inhibition	89.20 % inhibition
0.167 mM oxalate	—	12.65 % inhibition

Malic enzyme. The properties of this enzyme are given in Table 2. Its activity was present mainly in mitochondria. In addition to the activity with NADP also the activity with NAD as coenzyme was measured, particularly in the cytoplasm. This activity, however, was independent of Mn^{2+} ions and strongly decreased during the reaction. Consequently, it is considered to be a MDH activity exhibited outside pH optimum. Under these conditions, the reaction is not supported by H^+ ions removal, as it is the case with pH optimum of malate oxidation of MDH — pH 10.0, and therefore the reaction velocity decreases and the reaction gets into a balance. Since ME is NADP-dependent, it is placed to the group EC 1.1.1. 40, which will include most probably also ME from other cestode species (e.g., a detail study of ME of *H. diminuta* was published by Li et al. 1972).

Table 3. Succinate dehydrogenase in *T. crassiceps* larvae

Properties	Cytoplasmic SDH	Mitochondrial SDH
Specific activity (mean of 4 measurements)	0	51.3 nmol succinate/ min/mg protein
K'_M for succinate	—	$5.72 . 10^{-5}$ M
Effect of inhibitors (at 10 mM succinate)		
5 mM malonate	—	94.3 % inhibition
5 mM oxaloacetate	—	100 % inhibition

Succinate dehydrogenase. The properties of this enzyme are summarized in Table 3. The fact that SDH was not found in the cytoplasm suggests the participance of this enzyme in the Krebs cycle (bound to mitochondria), as detected by us. However, these results differ from those of Dubinský and Ryboš (1982) who found comparable SDH activities in the mitochondrial and cytoplasmic fractions of *A. suum* organs. As to the activity of this enzyme (in relation to protein) in the studied *T. crassiceps* mitochondria, it is comparable with that in *T. spiralis* mitochondria (Boczoń 1976) in which the activity was 61 nmol succinate/min/mg protein.

With regard to the fact that the SDH activity in *T. crassiceps* mitochondria is relatively high and that it is much higher than the FR activity, it may be supposed that aerobic processes are involved in the total energy acquisition of the parasite.

Table 4. Fumarate reductase in *T. crassiceps* larvae

Properties	Cytoplasmic FR	Mitochondrial FR
Specific activity (mean of 4 measurements)	29 nmol NADH/min/ mg protein	5.2 nmol NADH/min/ mg protein
Effect of rotenone 1.25 μg/ml	no effect	100 % inhibition

Fumarate reductase. The properties of this enzyme are summarized in Table 4. The cytoplasmic FR activity is not inhibited by rotenone and therefore it does not seem to be involved in the energy production FR measuring in mitochondria revealed that the total NADH oxidase activity was 12 nmol NADH/min/mg protein in the presence of oxygen and fumarate, but 6.8 nmol NADH/min/mg protein in the absence of fumarate. The difference between these two values, 5.2 nmol NADH/min/mg protein, should be ascribed to fumarate reductase activity. Consequently, the FR activity represents only a part of the total NADH oxidase activity. In view of the fact that FR in mitochondria can be inhibited by rotenone, it can be assumed that it is involved in energy production, like in many other helminths.

DISCUSSION

The MDH activity in *T. crassiceps* larvae is 1.47 times greater in the cytoplasm than in mitochondria. For a comparison, Barrett and Beis (1973) state that in adult *Ascaris suum*, where the sequence oxaloacetate-malate-fumarate-succinate is basic in saccharide metabolism and energy production, only 3 % of MDH is present in mitochondria. According to Dubinský and Ryboš (1981) the ratio between MDH activities in the cytoplasm and mitochondria was 8.45 : 1 (in relation to protein) in muscles of *A. suum* females. According to Rotmans (1978) this ratio is reverse (1 : 9.25) in *Schistosoma mansoni*, since the type of saccharide metabolism and terminal production of lactate are different in this species. The author found that 30—40 % of the total MDH activity is present in mitochondria of *S. mansoni*. The values for *T. crassiceps* larvae lie between these two limit cases.

The velocity ratio in the direction oxaloacetate reduction — malate oxidation was reported by Prichard and Schofield (1968) to be 19.6 : 1 in *Fasciola hepatica*, while Rotmans (1978) recorded 12.3 : 1 in *S. mansoni*.

As to the effect of malate on the cytoplasmic and mitochondrial MDH, Zee and Zinkham (1968) found that both enzymes are inhibited in the same degree by higher concentrations of malate. Rotmans (1978), on the contrary, assessed that in *S. mansoni*, the mitochondrial MDH is more sensitive to malate inhibition than the cytoplasmic MDH. As regards the substrate inhibition, MDH from both the cytoplasm and mitochondria of *T. crassiceps* behaved similarly as MDH from chicken hearts (Kitto 1969).

The high activity of ME in *T. crassiceps* mitochondria compared to that in the cytoplasm agrees with the supposed role of this enzyme in the process of saccharide catabolism and energy production. Barrett (1981) supposes that ME in the intermembrane space of mitochondria of the so-called CO_2-fixing helminths oxidizes a part of malate to pyruvate and that the reduction equivalents arisig at the same time are then transferred to fumarate originating from the remaining part of malate as a result of fumarase effect. Therefore the activities in mitochondria are higher, which is in agreement with our results. The malic enzyme of the vertebrates, on the contrary, is localized mainly in the cytoplasm.

The SDH activity in *T. crassiceps* mitochondria is 2.3 times higher than that measured by Dubinský and Ryboš (1982) in females of *A. suum*. The authors used fenazin metosulphate and dichlorphenolindophenol for the detection of SDH so that the comparison is only approximate. The SDH activity in mitochondria of *T. crassiceps* was 2.48 times lower than that recorded by Boczoń (1976) in mitochondria from rat hearts.

The increase in NADH oxidase activity after addition of fumarate (1.76 times in *T. crassiceps* mitochondria) can be compared, e.g., with the results obtained by Bryant and Bennet (1983) in *Haemonchus contortus* (1.136 times) and by Fioravanti (1982) in *Hymenolepis diminuta* (5 times).

As concerns the character of metabolic processes associated with the degradation of sugars and formation of ATP in *T. crassiceps* larvae, it can be said that there are conditions for anaerobic acquisition of energy in the fumarate reductase system (presence of fumarate reductase, activity and distribution of MDH and ME), but that on the basis of some facts (active SDH, high ratio of its activity to FR activity, small proportion of FR activity in NADH oxidase activity) it can be assumed that also aerobic processes are involved in the total energy acquisition.

ДЕКАРБОКСИЛИРУЮЩАЯ МАЛАТДЕГИДРОГЕНАЗА, МАЛАТДЕГИДРОГЕНАЗА, ФУМАРАТРЕДУКТАЗА И СУКЦИНАТДЕГИДРОГЕНАЗА У ЛИЧИНОК *TAENIA CRASSICEPS* (ZEDER, 1800)

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Резюме. Изучали активность декарбоксилирующей малатдегидрогеназы, малатдегидрогеназы, фумаратредуктазы и сукцинатдегидрогеназы в цитоплазме и митохондриях личинок *Taenia crassiceps*. Результаты показывают, что у личинок *T. crassiceps* находятся ферменты для анаэробного получения энергии с терминальной фумаратредуктазой, но что на основании некоторых данных, как напр. высокого отношения между активностями сукцинатдегидрогеназы и фумаратредуктазы и небольшой доли фумаратредуктазы в общей NADH оксидазовой активности митохондрий можно считать, что также аэробные процессы участвуют в получении энергии у этого паразита.

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Received 4 April 1985

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