FOLIA PARASITOLOGICA 34: 233–241, 1987.

CHARACTERISTICS OF MALIC ENZYME FROM THE CYSTICERCI OF TAENIA CRASSICEPS (ZEDER, 1800)

J. ŽENKA and J. PROKOPIČ

Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice

Abstract. Kinetic parameters of partly purified malic enzymes (NADP+: L-malate oxidoreductase (decarboxylating) EC 1.1.1.40) from the cytoplasm and mitochondria of *Taenia crassiceps* cysticerci were compared. The cytoplasmic malic enzyme differed from the mitochondrial malic enzyme in the K'_M for malate; other studied properties were identical. The chromatographies of both the cytoplasmic and mitochondrial enzymes were identical. They exhibited a hyperbolic dependence of the activity on malate concentration, sigmoidal kinetics was not observed. The effects of succinate and fumarate known as positive modulators of the activity of malic enzymes from other sources were not observed either in cytoplasmic or mitochondrial enzymes from *T. crassiceps*. It was found that the two enzymes can be partly inhibited by ATP. Molecular weight of the malic enzyme from *T. crassiceps* cysticerci was determined by chromatography on Sephadex G-200 (Mw = 116,700) and a four-step purification of the enzyme was performed. The properties of malic enzymes from the cytoplasm and mitochondria of *T. crassiceps* are compared with those of the malic enzymes from other parasitic worms and higher organisms.

In a previous study (Ženka and Prokopič 1985), malic enzyme (NADP+: L-malate oxidoreductase (decarboxylating) EC 1.1.1.40) was found in the mitochondria and cytoplasm of *T. crassiceps*. The activity of this enzyme was dependent on Mn^{2+} ions and its specific activity was higher in the mitochondria than in the cytoplasm. Since malic enzyme is supposed to play an important role in carbohydrate metabolism of parasitic worms (Barrett 1981), we decided to study in detail the properties of this enzyme and, particularly, the possibility of its regulation. Malic enzyme is present at the site of branching of metabolic ways and it may be therefore supposed that it could be regulated. It was really found that malic enzyme from *A. suum* mitochondria which reacts specifically with NAD (Landsperger and Harris 1976) and is therefore of a different type (EC 1.1.1.39) exhibits sigmoidal kinetics and it can be positively regulated by fumarate. It was of interest to assess what is the kinetic behaviour of the enzyme studied by us and what is its molecular weight, as Li et al. (1972) demonstrated a surprisingly low molecular weight (approximately 120.000) of malic enzyme in *Hymenolepis diminuta*, which is a member of the same class (Cestoda) as *T. crassiceps*. This molecular weight was by one half lower than that of malic enzymes from other sources (for details see Discussion).

MATERIALS AND METHODS

Biological material. Cysticerci of *T. crassiceps* (strain KBS) passaged at 6-month intervals in rats in our laboratory were used in the experiments. They were washed out from the abdominal cavity of rats using PBS and processed as described below.

Determination of molecular weight of malic enzyme. The molecular weight of this enzyme was determined by column chromatography on Sephadex G-200 column (1.6×120 cm) equilibrated with elution buffer — 0.1 M phosphate buffer, pH 7.2, 1 mM EDTA, and 1 mM mercaptoethanol.

The supernatant of homogenate from whole cysticerci (2 ml) was applied to the column. The cysticerc were washed 6 times with elution buffer, homogenized at the ratio of 1 : 1 (v/v) with the same buffer for 2 × 2 min using MSE knife homogenizer (8,000—10,000 rpm), and centrifuged at 20,000 × g for 30 min. The supernatant contained 5.75 mg protein/ml. The elution was made with elution buffer (flow 5 ml/h). The column was calibrated by standards for gel chromatography (Pharmacia).

Preparation of cytoplasmic fraction and mitochondria. Always about 50—60 ml of cysticerci were washed 6× with PBS, 2× with mitochondrial medium (0.25 M sucrose in 0.01 M TRIS, 2 . 10^{-3} M EDTA, pH 7.5) and homogenized with 4-fold volume of mitochondrial medium using Potter homogenizer with teflon piston at 0 °C (700—1,000 rpm, 4 strokes). The homogenate was centrifuged at 500 × g for 10 min at 4 °C (the same temperature was used also for further process). The sediment was discarded and the supernatant was centrifuged at 10,000 × g for 30 min. The supernatant was then stored to be used in further studies as a cytoplasmic fraction. The sediment was suspended in mitochondrial medium and further centrifuged at 11,740 × g for 30 min. The sediment (mitochondria) was suspended in 8.5 ml of 0.1 M phosphate buffer, pH 6.5, 1 mM EDTA, and 1 mM mercaptoethanol and homogenized in MSE knife homogenizer at 8,000—10,000 rpm for 2 × 2 min. After centrifugation at 33,000 × g for 20 min the supernatant was used for the study of the malic enzyme.

Partial purification of malic enzyme from the cytoplasm and mitochondria of *T. crassiceps* cysticerci. The cytoplasmic fraction and supernatant of mitochondrial homogenate, prepared as described above, were separated on Sephacryl S-300 Superfine (1.6 × 100 cm) column equilibrated with 0.1 M phosphate buffer, pH 6.5, 1 mM EDTA, and 1 mM mercaptoethanol (flow rate 20 ml/h). The same buffer was used for the elution. Four ml of cytoplasmic fraction (= 18.2 mg of protein) or mitochondrial homogenate (= 21.2 mg of protein) were applied to the column. Fractions of 2.5 ml were collected and the activity of malic enzyme was determined. Fractions with maximum activities were combined and used for further study. Protein elution profile was continually registered by UV-2 monitor (Pharmacia). The column was calibrated by standards for gel chromatography (Pharmacia).

Determination of malic enzyme activity. This determination was used for the detection of malic enzyme in chromatographic fractions, as well as in the kinetic and inhibition studies of this enzyme. The growth of absorbance at 340 nm was followed continually in a Pye Unicam PU 8800 spectrophotometer. The volume of reaction mixture was 2 ml and optical path 1 cm. The reaction mixture contained the following final concentrations (optimalized for maximum reaction speed): 0.1 M TRIS, pH 7.5, 30 mM malate Na (or lower concentration for the study of enzyme saturation with substrate), 10^{-4} M NADP, 4 . 10^{-4} M $MnCl_2$, 30—100 µl of enzyme preparations and the substances the effect of which on malic enzyme activity was tested (if the properties of malic enzyme were studied). The temperature of the reaction mixture was 25 °C.

Four-step purification of malic enzyme from *T. crassiceps* cysticerci. Some steps described by Li et al. (1972) were applied for the purification. The cysticerci (50 ml) were homogenized in 50 ml of 0.1 M phosphate buffer, pH 6.5, containing 1 mM EDTA, and 1 mM mercaptoethanol for 2 × 2 min using MSE knife homogenizer at 8,000—10,000 rpm, and centrifuged at 12,000 × g for 20 min. Then $(CH_3COO)_2$ Mg was added to the supernatant to give a final concentration of 10 mM. After 10-min incubation at 58 °C, the mixture was centrifuged at 12,000 × g for 30 min, double volume of distilled water was added to the supernatant, and the pH was adjusted to 6.0. After binding on CM Sephadex C-50 equilibrated with 0.02 phosphate buffer, pH 6.0, containing 1 mM EDTA, and 1 mM mercaptoethanol (10 g of dry weight), double washing with the same buffer and elution with 270 ml of equilibration buffer containing 0.5 M NaCl were performed. Malic enzyme was then salted out with ammonium sulphate (68.5 % saturation) at 4 °C. After centrifugation at 12.000 × g for 30 min, the sediment was dissolved in 0.1 M phosphate buffer, pH 6.5, containing 1 mM EDTA, and 1 mM mercaptoethanol, and separated on Sephacryl S-300 Superfine column as described above. Fractions with the highest malic enzyme activities were combined.

Linear dependences in all determinations (column calibration, determination after Lineweaver and Burk, Hill's determination) were estimated by the method of smallest squares using TI-58-c computer.

Protein was determined by the method after Lowry et al. (1951) using BSA as the standard.

RESULTS

Determination of molecular weight of malic enzyme by gel chromatography on Sephadex G-200

Homogenate of whole *T. crassiceps* cysticerci (i.e. with malic enzymes from both the cytoplasm and mitochondria) was applied to the column. A single sharp peak appeared in the eluate at the activity determination. On the basis of a calibration of the column with four standards, the molecular weight of malic enzyme was assessed to be 116,700. The calibration curve and reading of molecular weight of malic enzyme are shown in Fig. 1.

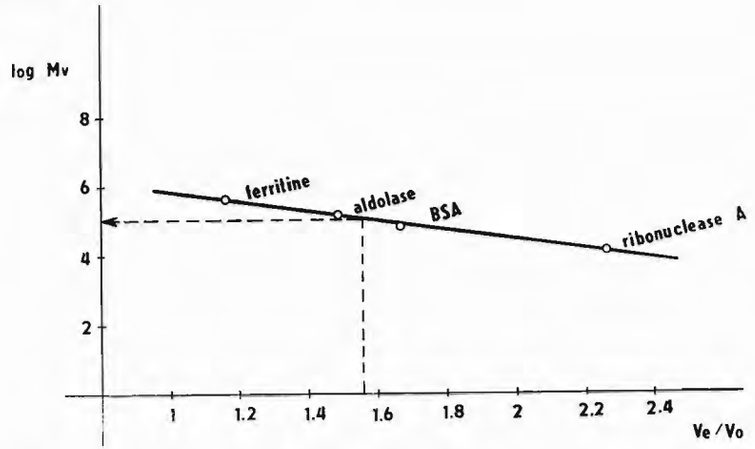

Fig. 1. Determination of molecular weight of malic enzyme from *T. crassiceps* larvae using gel chromatography on Sephadex G-200: calibration curve and reading of malic enzyme molecular weight.

Comparison of properties of partly purified malic enzyme from the cytoplasm and mitochondria of *T. crassiceps* cysticerci

Partial purification on Sephacryl S-300 Superfine column. Samples of cytoplasm and mitochondrial homogenate were separated on Sephacryl S-300 Superfine column. Fig. 2 shows the elution protein profiles and results of the detection of malic enzyme activities in both samples. The chromatography of both samples was identical. According to the calibration, the elution volume of malic enzyme in both samples corresponded to the molecular weight of 110.700. The part of the eluate which contained the highest activity of malic enzyme (fractions 33—36 in both samples) was used for further studies of kinetic parameters of this enzyme (about 10 ml).

Kinetic properties of malic enzyme from the mitochondria and cytoplasm of *T. crassiceps*

The properties of mitochondrial and cytoplasmic malic enzymes (partly purified on Sephacryl S-300 Superfine column) were compared. First of all, the dependence of the activity on malate concentration was studied using a wide range of substrate concentra-

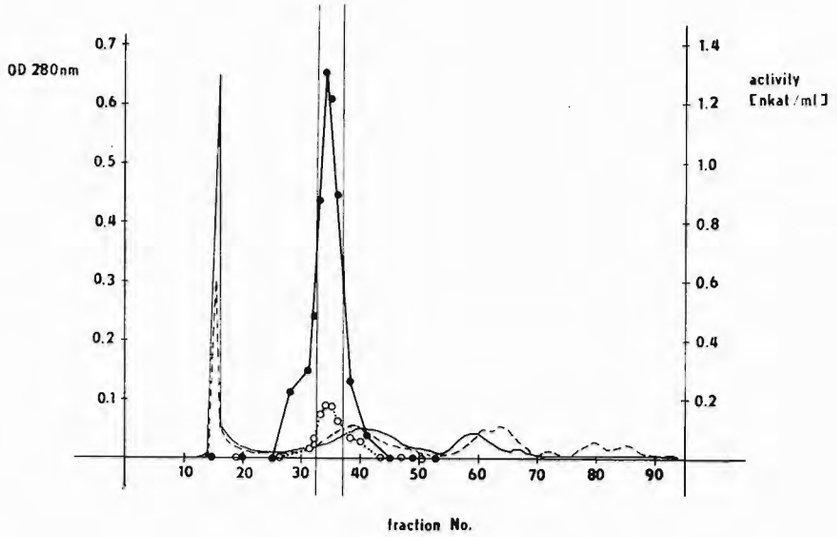

Fig. 2. Partial purification of malic enzyme from the cytoplasm and mitochondria of *T. crassiceps* using the chromatography on Sephacryl S-300 Superfine. —— protein, mitochondria, - - - protein, cytoplasm, —— • —— malic enzyme, mitochondria, - - - o - - - malic enzyme, cytoplasm. Vertical lines indicate a part of eluates with highest malic enzyme activities used for further kinetic parameters of this enzyme.

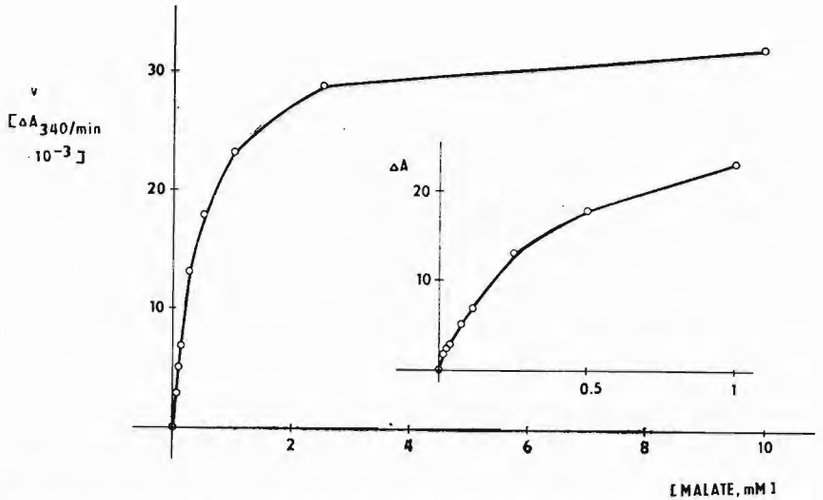

Fig. 3. Dependence of the activity of malic enzyme from *T. crassiceps* mitochondria on malate concentration. Plotting of the dependence on low malate concentrations.

tions including very low ones. Fig. 3 shows this dependence for the mitochondrial malic enzyme at low malate concentrations. The dependence has a hyperbolic course; sigmoidal course was not recorded even at very low malate concentrations. Fig. 4 shows the same dependence for the cytoplasmic malic enzyme. Even in this case the hyperbolic course was recorded.

Double reciprocal plotting of measured values after Lineweaver and Burk was made at malate concentrations of $7.14 \cdot 10^{-5}$ M — $1 \cdot 10^{-2}$ M. A linear dependence was obtained for both the cytoplasmic and mitochondrial enzymes. Michaelis constant for malate at 10^{-4} M concentration of NADP was $K'_M = 4 \cdot 10^{-4}$ M for mitochondrial malic enzyme, and $K'_M = 1.3 \cdot 10^{-4}$ M for cytoplasmic malic enzyme.

The values obtained in the previous determination of the dependence of the activity

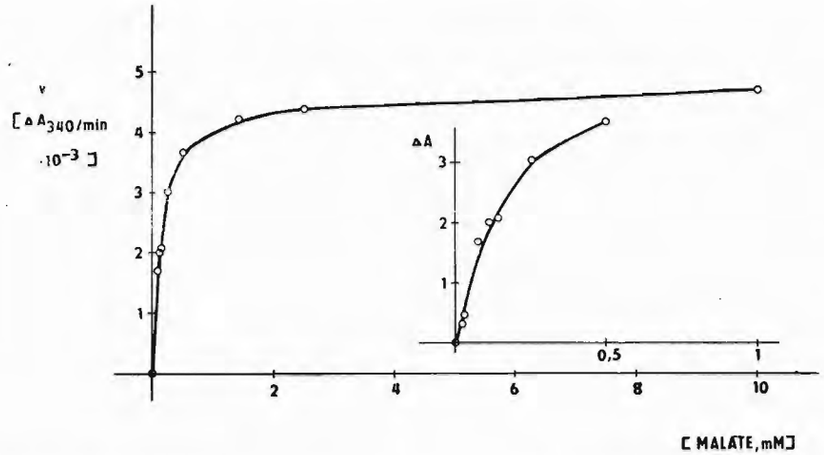

Fig. 4. Dependence of the activity of malic enzyme from *T. crassiceps* cytoplasm on malate concentration. Plotting of the dependence on low malate concentrations.

on malate concentration were plotted after Hill. In case of the mitochondrial malic enzyme, a linear dependence was obtained (correlation coefficient = 0.998857), according to the gradient of which Hill coefficient = 0.9665. In case of the cytoplasmic malic enzyme, the gradient of linear dependence (correlation coefficient = 0.9949045) gave Hill coefficient = 1.18. It is known if the values of Hill coefficient are close to one, as in our case, the cooperativeness of subunits is excluded and therefore the sigmoidal dependence of the enzyme activity on substrate concentration does not occur. Effects of succinate, fumarate, and ATP on the activity of partly purified malic enzyme. Results of the studies of the effects of these substances on the cytoplasmic and mitochondrial malic enzymes are summarized in Table 1. The effects of the studied substances were similar in both the enzymes.

Four-step purification of malic enzyme from *T. crassiceps* cysticerci

The method of purification was described in Materials and Methods and its result is shown in Table 2. The obtained enzyme was studied in the same way as the malic enzyme from the cytoplasm and mitochondria of *T. crassiceps* cysticerci. Also the

Table 1. The effect of succinate, fumarate, and ATP on malic enzyme activity

Substance	Malate concentration	% inhibition/activation	
		mitochondrial malic enzyme	cytoplasmic malic enzyme
10^{-2} M succinate	10^{-4} M	34.3 inhibition	30.9 inhibition
7 . 10^{-4} M fumarate	10^{-4} M	2.9 activation	0.75 activation
10^{-2} M fumarate	10^{-4} M	30.6 inhibition	40.1 inhibition
10^{-3} M ATP	10^{-3} M	36.1 inhibition	34.7 inhibition

Table 2. Four-step purification of malic enzyme from *T. crassiceps* cysticerci

Step	V (ml)	Protein (mg)	Total activity (μmol/min)	Specific act. (μmol/min/mg)	Purification rate	Purification rate compared to preced. step	Yield (%)
Original supernatant of the homog.	81	401	8.85	0.022	1	1	100
Heat denaturation	72	174	6.95	0.04	1.8	1.8	78.4
CM Sephadex C-50	272	24.7	4	0.162	7.3	4.07	45.1
Precipitation $(NH_4)_2SO_4$	5	2.14	1.1	0.512	23.2	3.16	12.3
Sephacryl S-300 Superfine	17	0.82	0.44	0.536	24.3	1.05	4.94

Table 3. The effect of succinate and fumarate on the activity of purified malic enzyme from *T. crassiceps* cysticerci (four-step purification)

Substance	Malate concentration	% inhibition
10^{-2} M succinate	10^{-4} M	27.2
7 . 10^{-4} M fumarate	10^{-4} M	9
10^{-2} M fumarate	10^{-4} M	47.1

dependence of its activity on malate concentration was studied in a wide range of substrate concentrations. The value of K'_M for malate (in the presence of 1—10^{-4} M NADP) was 3.8 . 10^{-4} M. The course of the dependence was hyperbolic. Table 3 shows the dependence of malic enzyme activity on the concentrations of succinate and fumarate.

DISCUSSION

The molecular weight of malic enzyme in *T. crassiceps* corresponds to that of malic enzyme in *Hymenolepis diminuta* determined by Li et al. (1972) to be 120,000 (as detected by the chromatography on Sephades G-200) and 115,000 (as detected by ultracentrifugation (sedimentation balance)). These values differ considerably from those given for this enzyme from other materials, as rat liver — 250,000 (Saito et al. 1971), chicken liver — 250,000 (Silpananta and Goodridge 1971), pigeon liver — 250,000—280,000 (Hsu and Lardy 1967, Saito et al. 1971), *E. coli* — 550,000 (Spina et al. 1970), and *A. suum* — 250,000 (Fodge et al. 1972). The determination of the molecular weight of malic enzyme in *T. crassiceps* was deliberately made on non-purified material in order to prevent splitting of the enzyme during some more drastic steps of preparation. In spite of this, our results are comparable with those obtained for *Hymenolepis diminuta* (Li et al. 1972), where the determination of the molecular weight was made after previous purification processes including heat denaturation of ballasts. The data recorded by some authors (Hsu and Lardy 1967, Silpananta and Goodridge 1971, Spina et al. 1970, Fodge et al. 1972) indicate that the malic enzyme consists of subunits with molecular weights of 60,000—65,000. It is possible that the malic enzyme of *T. crassiceps*, similarly as that of *H. diminuta*, exists in a dimer form.

A comparison with malic enzymes from the cytoplasm and mitochondria of higher organisms revealed that these enzymes have different properties. Frenkel (1972a), who studied malic enzyme from the cytoplasm and mitochondria from bovine brain, observed different electrophoretic and chromatographic behaviour and found a sigmoidal dependence of their activity on substrate concentration at low malate concentrations, but only in the mitochondrial enzyme. The same author (Frenkel 1972b) detected a sigmoidal dependence of malic enzyme activity on malate concentration in bovine heart in the mitochondrial malic enzyme and absence of cooperativeness in case of the cytoplasmic enzyme. A sigmoidal dependence was exhibited also by NAD^+-dependent malic enzyme (EC 1.1.1.39) present in the mitochondria of adrenal cortex (Sauer 1973) and mitochondria of *A. suum* (Landsperger and Harris 1976). In our case, no cooperativeness was found in the mitochondrial enzyme and it exhibited, like the cytoplasmic enzyme, a hyperbolic curve of malate saturation. Also the chromatography of the two enzymes was the same. They could be differentiated only on the basis of K'_M for malate.

The effect of succinate on malic enzyme from the mitochondria and cytoplasm of *T. crassiceps* was similar and was exhibited by a partial inhibition. The results obtained correspond to the data published by Frenkel (1972b), who studied the kinetics of the cytoplasmic malic enzyme isolated from bovine heart. A contrary phenomenon was described for the mitochondrial enzyme with sigmoidal dependence of the activity on substrate concentration. The 10 mM succinate acted as an allosteric activator, increased the reaction velocity and changed the sigmoidal course of the dependence to a hyperbolic one. Malic enzyme (EC 1.1.1.39) either cannot be affected by succinate (*A. suum* — Landsperger and Harris 1976) or can be affected only slightly (adrenal cortex — Sauer 1973).

Another substance, fumarate, did not produce any effect on NADP-dependent malic enzyme from bovine brain (Frenkel and Cobo-Frenkel 1973), but it was reported as a positive modulator of malic enzyme (EC 1.1.1.39) from mitochondria of adrenal cortex (Sauer 1973) and of *A. suum* (Landsperger and Harris 1976).

The inhibitory effect of ATP might contribute to the feedback regulation of the whole ATP-producing process, in which malic enzyme participates. In our opinion, however, the degree of malic enzyme inhibition detected for ATP is not high enough to be metabolically significant, since at normal course of metabolism the ATP concentration in cells does not reach a level sufficient for affecting the activity of the enzyme.

The properties of malic enzyme from *T. crassiceps* cysticerci purified by a four-step purification procedure to a higher degree (but not to a completely pure state) correspond to the data obtained by us previously.

It can be concluded that the malic enzymes present in the mitochondria and cytoplasm of *T. crassiceps* cysticerci are very similar and can be differentiated only on the basis of K'_M for malate. Neither the mitochondrial nor the cytoplasmic malic enzyme exhibited a sigmoidal course of dependence of the activity on substrate concentration. The properties of malic enzyme from *T. crassiceps*, including its low molecular weight, are different from those of malic enzymes from other organisms. It is of interest that it differs even from the malic enzyme from *A. suum*. Our results (unpublished) indicate that fumarat-reductase activity is present in *T. crassiceps* and that both aerobic and anaerobic processes participate in the total energy metabolism of *T. crassiceps* larvae. Malic enzyme plays a key role in the anaerobic energy metabolism (which occurs not only in *A. suum*, but was described in the majority of parasitic worms). It is therefore surprising that the malic enzymes of the two species, *T. crassiceps* and *A. suum*, are so different, though it may be supposed that their metabolic position and significance are the same.

ХАРАКТЕРИСТИКА МАЛАТДЕГИДРОГЕНАЗЫ ИЗ ЦИСТИЦЕРКОВ *TAENIA CRASSICEPS* (ZEDER, 1800)

Я. Женка и Я. Прокопич

Резюме. В работе сравниваются кинетические параметры частично очищенных малатдегидрогеназ (NADP+: L-malate oxidoreductase (decarboxylating), EC 1.1.1.40) из цитоплазмы и митохондрий цистицерков *Taenia crassiceps*. Цитоплазматическая малатдегидрогеназа отличается от митохондриальной малатдегидрогеназы только значением K'_M для малата; другие изучаемые свойства одинаковые, так же как и хроматография этих ферментов. Оба фермента проявляли гиперболическую зависимость их активности от концентрации малата, сигмовидная кинетика не наблюдалась. Сукцинат и фумарат, известные как положительные модуляторы активности малатдегидрогеназ из некоторых других источников, не оказывали влияния на малатдегидрогеназу из цитоплазмы и митохондрий *T. crassiceps*. Было обнаружено, что оба ферменты можно частично ингибировать с помощью АТФ. Определяли также молекулярный вес малатдегидрогеназы из цистицерков *T. crassiceps* с помощью хроматографии на сефадексе Г-200 (мв = 116 700) и проводили четырехступенчатую очистку этого фермента. В работе сравниваются свойства малатдегидрогеназы из цитоплазмы и митохондрий *T. crassiceps* со свойствами этого фермента из других паразитических червей и из высших организмов.

REFERENCES

BARRETT J., 1981: Biochemistry of parasitic helminths. MacMillan Publishers LTD, London and Basingstoke, 308 pp.

FODGE D. W., GRACY R. W., HARRIS B. G., 1972: Studies of enzymes from parasitic helminths. I. Purification and physical properties of malic enzyme from the muscle tissue of *Ascaris suum*. Biochim. Biophys. Acta 268: 271—284.

FRENKEL R., 1972a: Isolation and some properties of a cytosol and a mitochondrial malic enzyme from bovine brain. Arch. Biochem. Biophys. 152: 136—143.

FRENKEL R., 1972b: Allosteric characteristics of bovine heart mitochondrial malic enzyme. Biochem. Biophys. Res. Commun. 47: 931—937.

FRENKEL R., COBO-FRENKEL A., 1973: Differential characteristics of the cytosol and mitochondrial isozymes of malic enzyme from bovine brain. Effects of dicarboxylic acids and sulfhydryl reagents. Arch. Biochem. Biophys. 158: 323—330.

HSU R. Y., LARDY H. A., 1967: Pigeon liver malic enzyme II. Isolation, crystalization, and some properties. J. Biol. Chem. 242: 520—526.

LANDSPERGER W. J., HARRIS B. G., 1976: NAD+ — malic enzyme. Regulatory properties of the enzyme from *Ascaris suum*. J. Biol. Chem. 251: 3599—3602.

LI T., GRACY R. W., HARRIS B. G., 1972: Studies on enzymes from parazitic helminths. Arch. Biochem. Biophys. 150: 397—406.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L., RANDALL R. J., 1951: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265—275.

SAITO T., YOSHIMOTO A., TOMOITA K., 1971: Malic enzyme of Hyperthyroid rat liver. J. Biochem. 69: 127—135.

SAUER L. A., 1973: Mitochondrial NAD-dependent malic enzyme: A new regulatory enzyme. FEBS Lett. 33: 251—255.

SILPANANTA P., GOODRIDGE A. G., 1971: Synthesis and degradation of malic enzyme in chick liver. J. Biol. Chem. 246: 5754 to 5761.

SPINA J. Jr., BRIGHT H. J., ROSENBLOOM J., 1970: Purification and properties of L-malic enzyme from *Escherichia coli*. Biochemistry 9: 3794—3801.

ŽENKA J., PROKOPIČ J., 1987: Malic enzyme, malate dehydrogenase, fumarate reductase and succinate dehydrogenase in the Larvae of *Taenia crassiceps* (Zeder, 1800). Folia parazitol. 34: 131—236.

Received 26 February 1986

J. Ž., Parazitologický ústav ČSAV,
Branišovská 31,
370 05 České Budějovice, ČSSR