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TRANSHYDROGENASE ACTIVITIES IN THE MITOCHONDRIA OF TAENIA CRASSICEPS (ZEDER, 1800) CYSTICERCI

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Abstract. NADPH/NAD and NADH/NAD transhydrogenase activities have been demonstrated in the mitochondrial fraction of *Taenia crassiceps* cysticerci. These activities seem to result from the activities of two different enzyme systems. Both transhydrogenase activities exhibited a high heat resistence and they were completely abolished only by the temperatures higher than 100 °C. The activity of NADH/NAD transhydrogenase was rather high (116 nmol . min^{-1} . mg^{-1} protein), but it was found to exhibit a low affinity to NADH (K'_M = 1.43 . 10^{-4} M). The physiological significance of NADH/NAD transhydrogenase is discussed.

ATP-independent NADPH/NAD and NADH/NAD transhydrogenases have been described in several helminths. Among the members of Cestoda, *Hymenolepis diminuta* (Fioravanti and Saz 1976), *Spirometra mansonoides* (Fioravanti and Saz 1978) and *Hymenolepis microstoma* (Fioravanti 1982) have been reported to contain these two enzymes, while in *Ascaris suum* of Nematoda only NADH/NAD transhydrogenase has been recorded (Köhler and Saz 1976).

The importance of NADPH/NAD transhydrogenase consists in that it transfers hydrogen ions from NADPH arising during the oxidation of malate by NADP-dependent malic enzyme to NAD. NADH then serves as a source of reduction equivalents for the mitochondrial electron transport in which the terminal fumarate reductase plays an important role. This has been demonstrated by Fioravanti (1981) and Kelvey and Fioravanti (1984) in *Hymenolepis diminuta*.

A. suum contains NAD-dependent malic enzyme, which is localized mainly in the intermembrane space of mitochondria (Rew and Saz 1974). The role of NADH/NAD transhydrogenase in *A. suum* is to transfer hydrogen ions through the inner mitochondrial membrane to its matrix side. This transfer has been demonstrated by Köhler and Saz (1976) and Fioravanti and Saz (1976). The transferred reduction equivalents are then used for the mitochondrial electron transport. Since the malic enzyme in *Ascaris* is NAD-dependent, NADPH/NAD transhydrogenation is unnecessary and NADPH-specific transhydrogenase is really lacking, as it was said above.

In our laboratory, we are dealing with the study of the bioenergetics of *Taenia crassiceps* cysticerci. The presence of NADP-dependent malic enzyme in the mitochondria of this parasite was demonstrated in a previous paper (Ženka and Prokopič 1987). With regard to the above-mentioned relations between the properties of the malic enzyme and transhydrogenase activities we wanted to assess whether the occurrence of the NADP-dependent enzyme in *T. crassiceps* is associated with the occurrence of NADPH/NAD transhydrogenase. The transhydrogenase activities have been measured only in four helminth species and more experiments will be necessary for a general conclusion. In the present study, also NADH/NAD transhydrogenase activity and problems of the identity of NADPH/NAD and NADH/NAD transhydrogenase activities have been dealt with.

MATERIALS AND METHODS

T. crassiceps cysticerci have been passaged on rats at 3-month intervals in our laboratory. After homogenization of the cysticerci in a Potter's piston homogenizer (glass/teflon) the mitochondria were prepared by differential centrifugation at 10,000 × g as described previously (Ženka and Prokopič 1986). They were disintegrated in 250 mM sucrose with 10 mM Tris (pH 7.5) and 2 mM EDTA for 2 × 45 seconds using Ultrathorax type homogenizer at 20,000 RPM at 4 °C. The disintegrated mitochondrial fraction used for measuring the transhydrogenase activities contained 4.8 mg . ml^{-1} protein.

NADPH/NAD and NADH/NAD transhydrogenase activities were assayed after Stein et al. (1959) by measuring the accumulation of reduced acetylpyridine NAD at 375 nm or of reduced thionicotinamide NAD at 398 nm. The reaction mixture (300 µl) contained 100 mM K phosphate buffer (pH 7.5), 0.3 mM NAD/P/H, 0.6 mM acetylpyridine NAD or thionicotinamide NAD (final concentrations). The measurements were performed using 10—24 µl of mitochondrial homogenate. The absorbance change at 375 or 398 nm was registered by Pye Unicam PU 8 800 spectrophotometer. Proteins were determined after Lowry et al. (1959) using bovine serum albumine as a standard.

RESULTS

The values of transhydrogenase activities are given in Table 1. NADH/NAD transhydrogenase activities were comparable if both NAD derivatives were used. The ratio of acetylpyridine NAD activity to thionicotinamide NAD activity was 1.66. In case of NADPH/NAD transhydrogenase activity, however, there were great differences between the two derivatives, acetylpyridine NAD being a more suitable substrate. The ratio of acetylpyridine NAD activity to thionicotinamide NAD activity was 11.21. This suggests that the two transhydrogenase reactions are catalyzed by different enzyme systems.

Table. 1. Comparison of NADH/NAD and NADPH/NAD transhydrogenase activities in the mitochondria from *T. crassiceps* cysticerci

Transhydrogenase reaction	Detected activity (nmol of reduced derivatives NAD/min/mg protein)
NADH/acetylpyridine NAD	116
NADPH/acetylpyridine NAD	5.46
NADH/thionicotinamide NAD	70
NADPH/thionicotinamide NAD	0.487

The result of the heat denaturation of transhydrogenase activities is shown in Fig.1. It indicates as well that different enzyme systems are involved. The temperature of 60 °C applied for 15 min inactivated 50.3 % of NADH/NAD transhydrogenase and 88.2 % of NADPH/NAD transhydrogenase activity. Differences in the heat inactivation were observed also after the treatment with a hot water bath for 30 min. Both transhydrogenase systems exhibited some common features, as a great resistance to high temperatures. The temperatures of 80—90 °C induced a lower inactivation than the temperatures of about 60 °C in both systems. A complete inactivation was achieved only by boiling of the mixture, when the temperature of the solution is higher than 100 °C.

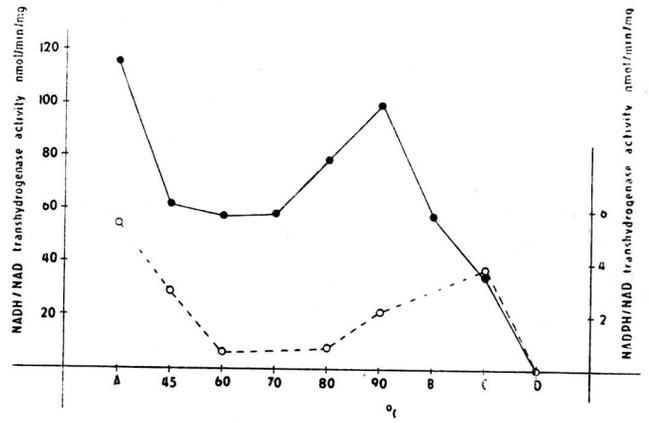

Fig. 1. Heat denaturation of NADH/NAD and NADPH/NAD transhydrogenases from mitochondria of *Taenia crassiceps* cysticerci (acetylpyridine NAD as substrate). — • — NADH/NAD transhydrogenase activity, — o — NADPH/NAD transhydrogenase activity. A — original activity, B — 15-min inactivation in boiling water bath, C — 30-min inactivation in boiling water bath, D — 10-sec. boiling of enzyme preparation.

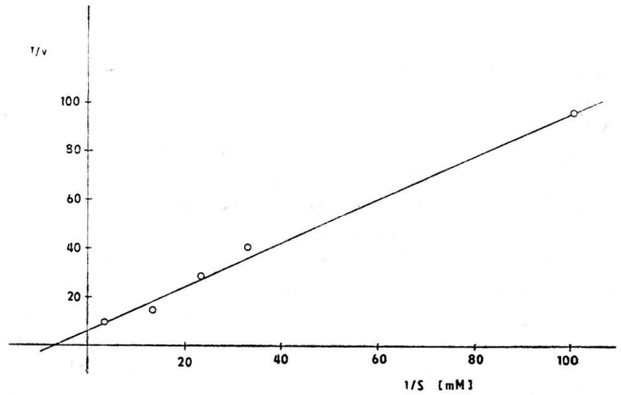

Fig. 2. Determination of K'_M for NADH NADH/NAD transhydrogenase activity in the mitochondria of *T. crassiceps* (acetylpyridine NAD as substrate).

Another problem under study was NADH/NAD transhydrogenase activity. The value of K'_M was 1.43 . 10^{-4}M for NADH if Lineweaver-Burk's plotting was used (Lineweaver-Burk 1934). Plotting of kinetic parameters is shown in Fig. 2.

Measurements of NADH/NAD transhydrogenase inhibition by various substances are summarized in Table 2. $HgCl_2$ was found to be an effective inhibitor of this enzyme. This indicates that cysteine residues are important for the activity of NADH/NAD transhydrogenase and for the conformation of its molecules.

The inhibitory effect of NAD can be explained by its competition with the NAD derivative. NADP has no inhibitory effect which indicates a substrate specificity of this enzymatic system.

Table. 2. Inhibition of NADH/NAD transhydrogenase activity (using acetylpyridine NAD).

Inhibitor	Inhibition (%)
10 mM succinate Na	0
10 mM malate Na	0
10 mM citrate Na	0
10 mM fumarate Na	23.2
10 mM pyruvate Na	0
0.02 mM $HgCl_2$	84.8
0.2 mM $HgCl_2$	100.0
1 mM NADP	0
0.1 mM NAD	18.2
0.333 mM NAD	35.3
1 mM NAD	80.7
1 mM ATP	34.5
1 mM $KAsO_2$	61.6

DISCUSSION

The presence of NADPH/NAD transhydrogenase activity was demonstrated in *T. crassiceps* mitochondria. NADPH arises ih the mitochondria of this parasite during the oxidation of malate by NADP-dependent malic enzyme (Ženka and Prokopič 1987). NADPH/NAD transhydrogenase enables the utilization of reduction equivalents by the mitochondrial electron transport (production of ATP).

The activities of NADH/NAD transhydrogenase with both NAD derivatives were comparable. This is in agreement with the results obtained by Fioravanti (1982) in *H. microstoma* and by Fioravanti and Saz (1978) in *Spirometra mansonoides*. Similarly as in *T. crassiceps*, also in these parasites the differences between NADPH/NAD transhydrogenase activity with acetylpyridine NAD and thionicotinamide NAD as substrates were very great, which indicates that the NADPH/NAD and NADH/NAD transhydrogenase activities are catalyzed by different enzymatic systems. Another evidence of this difference were the differences observed during the heat denaturation. A similar behaviour has been described in transhydrogenase systems of *Spirometra mansonoides* (Fioravanti and Saz 1978). The authors recorded a higher heat resistance of NADH/NAD transhydrogenase compared to NADPH/NAD transhydrogenase.

Surprising was the great heat resistance of both transhydrogenases in the mitochondrial fraction of *T. crassiceps*. A great heat resistance has been described in solubilized NADH/NAD transhydrogenase from *Ascaris suum* (Köhler and Saz 1976, Komuniecki and Saz 1979). However, in both papers the resistance concerned the temperature of 75 °C, whereas at 80 °C, the enzyme was markedly denatured. Komuniecki and Saz (1979) recorded a complete inactivation of the enzyme subjected to the effect of the temperature of 85 °C for 20 min. The decreased heat resistance of the enzyme from *Ascaris* might have been caused by the solubilization. A lower inactivation of transhydrogenases in *T. crassiceps* at the temperatures of 80—90 °C, as compared to 60 °C, may be explained by the combination of the denaturation of transhydrogenase and of surrounding membrane proteins. Evidently, at the temperature of about 60 °C

the proteins are so changed that the activity of transhydrogenase is decreased (steric inhibitions and others). Only higher temperatures change the proteins in such a way that they do not block the transhydrogenase activity. However, this hypothesis should be verified.

NADH/NAD transhydrogenase activity with acetylpyridine NAD was by one order higher than in the NADPH/NAD system. If thionicotinamide NAD was used, then the activity was higher even by two orders. Comparable results have been reported by Fioravanti (1982) in *H. microstoma*. However, it is not yet clear what is the effect of this high activity on the metabolism of the parasite. The study of this activity brought some interesting information. The value of K'_M for NADH detected in the present study ($1.43 \cdot 10^{-4}$M) was much higher than in *A.suum* ($9 \cdot 10^{-6}$M) (Komuniecki and Saz 1979). This might reduce the transhydrogenation abilities of this system under physiological conditions.

NADH/NAD transhydrogenase activity in *A. suum* is associated with lipoamid dehydrogenase activity of pyruvate dehydrogenase complex (Komuniecki and Saz 1979). This possibility has not been studied in *T. crassiceps*.

Experiments on the inhibition of NADH/NAD transhydrogenase showed that this enzyme is very sensitive to the inhibitors of sulphydryl groups, which corresponds to the behaviour of NADH/NAD transhydrogenase in *A.suum* (Köhler and Saz 1976). On the other hand, the inhibitory effect of ATP recorded in NADH/NAD transhydrogenase in *T. crassiceps* has not been demonstrated in this transhydrogenase in the mitochondria of *A. suum* (Köhler and Saz 1976). However, it should be said that not even in the transhydrogenase from *T. crassiceps* the inhibitory effect was pronounced.

The inhibition by NAD corresponds with the observed effect of NAD on the activity of both NADH/NAD transhydrogenase and NADPH/NAD transhydrogenase in *Spirometra mansonoides* (Fioravanti and Saz 1978) and is explained by the competition with an artificial acceptor for the hydride ion.

The effect of other metabolites was studied in order to assess the possibility of regulation of the NADH/NAD transhydrogenase activity. However, the effect of the studied substances was negligible.

The present study adds another species, *T. crassiceps*, to the parasitic worms in which the presence of mitochondrial transhydrogenase has been demonstrated and confirms other authors' conclusions that NADH/NAD and NADPH/NAD transhydrogenations are catalyzed by two independent enzymes.

АКТИВНОСТЬ ТРАНСГИДРОГЕНАЗ В МИТОХОНДРИЯХ ИЗ ЦИСТИЦЕРКОВ *TAENIA CRASSICEPS* (ZEDER, 1800)

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Резюме. В митохондриальной фракции цистицерков *Taenia crassiceps* обнаружена активность NADPH/NAD и NADH/NAD трансгидрогеназ. Эти активности, по-видимому, являются результатом действия двух разных энзиматических систем. Обе трансгидрогеназные активности обладали высокой термоустойчивостью и они полностью исчезли только после влияния температуры выше 100 °C. Активность NADH/NAD трансгидрогеназы была сравнительно высока (116 нмол . мин$^{-1}$. мг$^{-1}$ белка), но было обнаружено, что эта трансгидрогеназа обладает низкой аффинностью к NADH ($K'_M = 1{,}43 \cdot 10^{-4}$ M). Обсуждается физиологическое значение NADH/NAD трансгидрогеназы.

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