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TISSUE SPECIFICITY OF THE MITOCHONDRIAL FORMS OF MALIC ENZYME IN HERRING TISSUES

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Abstract—1. The activity of malic enzyme per gram wet weight or per milligram of mitochondrial protein in herring tissues showed significant tissue specificity.

2. The specific activity of herring malic enzyme per mg of mitochondria from testes was 30 times greater than that of malic enzyme found in mitochondria of ovaries.

3. The NAD(P)-dependent mitochondrial malic enzyme was present in all tissues tested and testis mitochondria contained the highest activity of this molecular form.

4. Herring skeletal muscle mitochondria contained nearly identical activities of both molecular forms, the NADP- and NAD(P)-dependent malic enzyme, respectively.

5. The results extend and support the proposal that mitochondria of some fish may contain two molecular forms of malic enzyme and have a unique intramitochondrial pathway for malate metabolism.

INTRODUCTION

Fish and crustacean muscle mitochondria utilize malate as one of the major respiratory fuels (Skorkowski *et al.*, 1977, 1984). In a previous paper we suggested that the regulatory malic enzyme that is present in the mitochondrial matrix space of crustacean and fish muscle may function in the provision of intramitochondrial pyruvate (Świerczyński *et al.*, 1980; Skorkowski *et al.*, 1984). Depending on the species and type of tissue, various molecular forms of malic enzyme may occur, both in the cytosol and in mitochondria (Frenkel, 1975). Sauer (1973) and Lin and Davis (1974) were the first to show that mitochondria isolated from calf adrenal gland and rabbit heart have two different molecular forms of malic enzyme, one specific for NADP only as the coenzyme (NADP-dependent malic enzyme) and the other utilizing both coenzymes but preferring NAD [NAD(P)-dependent malic enzyme]. The mitochondria isolated from cod and salmon trout hearts and herring skeletal muscle also have two forms of malic enzyme (Skorkowski *et al.*, 1984, 1985; Biegniewska and Skorkowski, 1986) and the regulatory properties of herring skeletal muscle mitochondrial NAD(P)-dependent malic enzyme have been reported recently (Skorkowski and Storey, 1988). In the present study, we examined the tissue specificity of malic enzyme in mitochondria from herring skeletal muscle, liver, testis and ovary for the presence or absence of the NADP- and NAD(P)-dependent forms of malic enzyme. Malic enzyme was present in relatively high activity in herring testes mitochondria with the NAD(P)-dependent form dominating; by contrast, activity was extremely low in herring ovary mitochondria.

MATERIALS AND METHODS

Reagents

L-Malic acid, Tris, NAD, NADP and Triton X-100 were obtained from Serva (FRG). DEAE–Sephacel was from Pharmacia Fine Chemicals AB. All other chemicals were of the highest reagent quality obtainable.

Animals

Fresh herring (*Clupea harengus*), kept ice-cold, were purchased from local fishing boats on the Gdańsk Bay or from local suppliers on the eastern Atlantic coast of Canada during the winter and spring seasons.

Preparation of mitochondria

All operations were carried out at 1–4°C. For the preparation of herring skeletal muscle mitochondria, we adopted the method described previously for isolation of crayfish abdomen muscle mitochondria (Skorkowski *et al.*, 1976). Herring skeletal muscle was removed and immersed in ice-cold 0.15 M KCl solution. About 200 g of muscle tissue was washed three times in cold 0.15 M KCl solution and then filtered through a polyethylene sieve to remove the KCl solution. The muscle was then suspended in about 5 vols of cold isolation medium containing: 0.21 M mannitol, 0.07 M sucrose, 0.01 M ethylenediaminetetra-acetate (EDTA), 0.01 M Tris–HCl (pH 7.4) and was mixed for 15 sec in an omnimixer. The fatty layer that rises to the top was skimmed from the homogenate. The homogenate was centrifuged for 10 min at 600 *g*. The remainder of the fatty layer was skimmed off, and the supernatant fluid was carefully decanted and centrifuged again for 10 min at 600 *g*. The resulting supernatant was filtered through cotton gauze and the filtrate was centrifuged for 10 min at 14,000 *g*. Supernatant was discarded. The crude mitochondrial pellet was resuspended in approximately 20 ml of isolation medium. The suspension was centrifuged at 600 *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 8000 *g* for 10 min. The average yield was 1 mg

of mitochondrial protein per 1 g of muscle fresh weight. The mitochondrial pellet was suspended finally in the 2% Triton X-100 in buffer A (10 mM Tris–HCl + 2 mM EDTA, pH 7.8) to obtain a protein concentration of approximately 10 mg/ml, and was then centrifuged at 22,000 *g* for 30 min.

Liver, testis and ovary mitochondria from herring were separated by differential centrifugation. Tissues were cut into small pieces and washed three times in cold isolation medium containing: 0.25 M sucrose, 0.01 M Tris–HCl pH 7.4, and 0.01 M EDTA. Then the tissue was suspended in approximately 10 vols of cold isolation medium and homogenized manually with a Potter–Elvehjem homogenizer. The homogenate was centrifuged for 10 min at 600 *g*. The resulting supernatant was filtered through cotton gauze and the filtrate centrifuged for 10 min at 14,000 *g*. The supernatant was discarded. The pellet was suspended in the isolation medium and centrifuged for 10 min at 600 *g*. The pellet was discarded and the supernatant was centrifuged at 8000 *g* for 10 min. The mitochondrial pellet was finally suspended in the 2% Triton X-100 in buffer A to obtain protein concentration of approximately 10 mg/ml and centrifuged at 22,000 *g* for 30 min.

DEAE–Sephacel chromatography

The supernatant fluid (mitochondrial extract) was filtered on a DEAE–Sephacel column (1.5 × 30 cm) equilibrated at 4°C with buffer A. Ten-milliliter fractions were collected. A linear gradient of KCl (300 ml of buffer A: 300 ml 0.5 M KCl in buffer A) was used to elute and separate the malic enzyme forms (Fig. 1).

Enzyme and protein assays

Malic enzyme activity was followed spectrophotometrically with a Specord UV–VIS recording spectrophotometer by observing the appearance of NADH or NADPH at 340 nm and 30°C. The standard reaction mixture (final vol 1 ml) contained 50 mM Tris–HCl pH 7.5, 1 mM $MnCl_2$, 0.25 mM NAD or NADP, 10 mM L-malate and the enzyme in amounts which caused an increase in absorbancy at the range 0.1–0.2. Enzyme activities were calculated using EmM/340 = 6.22 for NADH and NADPH in a 1-cm light path quartz cell. Protein concentration was determined by the Coomassie Blue method (Spector, 1978).

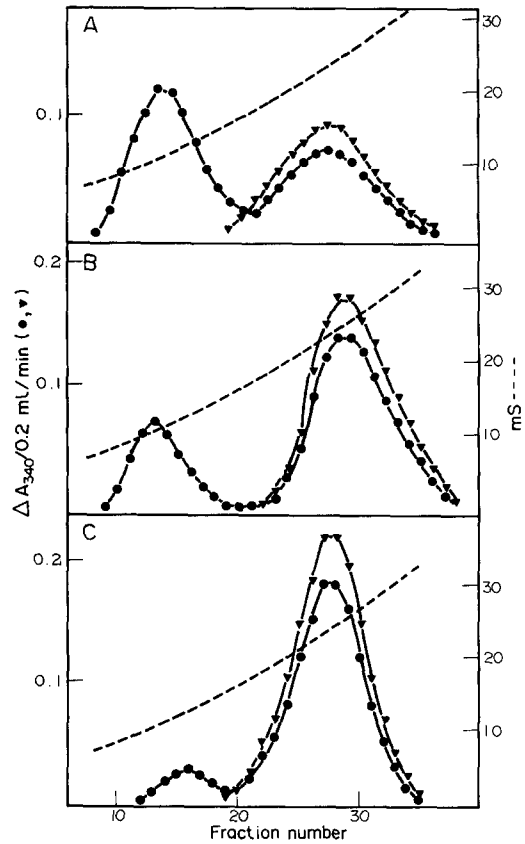

Fig. 1. Elution profiles of NADP- and NAD(P)-dependent malic enzyme activities of the mitochondrial fraction from herring: (A) skeletal muscle, (B) liver and (C) testes following DEAE–Sephacel chromatography. The column (1.5 × 30 cm) was equilibrated with buffer A. A linear KCl gradient (------) formed by mixing 300 ml of buffer A with 300 ml 0.5 M KCl in buffer A was used to elute malic enzyme activities. Effluent fractions (10 ml) were analyzed for both the NADP- and NAD(P)-dependent malic enzyme activities with NADP (●——●) and NAD (▼——▼) as described in Materials and Methods.

Table 1. Maximum activity of the $NADP^+$-linked malic enzyme in herring tissues, cytosol plus mitochondrial enzymes

Tissue	Enzyme activity (μmol NADPH min^{-1} g^{-1} fresh tissue)
Ovaries	0.58 ± 0.08 (3)
Testes	2.64 ± 0.38 (4)
Liver (male)	5.54 ± 0.62 (3)
Liver (female)	5.84 ± 0.52 (3)
Skeletal muscle	1.85 ± 0.25 (4)

The values represent the $NADP^+$-linked activities of malic enzyme and ±SD. Numbers in parentheses indicate the number of different preparations. Only the $NADP^+$-linked activity is reported due to interference in the NAD^+-linked assay in unpurified tissue extracts from lactate and malate dehydrogenases.

Table 2. Activities of mitochondrial malic enzyme in herring tissues

Source of mitochondria	Specific activity (nmol NADPH min^{-1} mg^{-1} mitochondrial protein)
Ovaries	8.4 ± 2.2 (3)
Testes	256.3 ± 21.2 (4)
Liver (female)	70.7 ± 9.8 (3)
Skeletal muscle	74.8 ± 10.8 (5)

The values represent the $NADP^+$-linked specific activities of both mitochondrial forms of malic enzyme and ±SD. Numbers in parentheses indicate the number of different preparations. Only the $NADP^+$-linked activity is reported due to interference in the NAD^+-linked assay at this step from lactate and malate dehydrogenases.

RESULTS AND DISCUSSION

Table 1 shows the maximum activities of all malic enzyme forms in both the cytosol and mitochondria from several herring tissues; activity is expressed as μmol of NADPH produced per min per g wet wt. Total activity of malic enzyme in the liver tissue of female herring was 10 times higher than that found in ovaries. On the other hand, the activity of malic enzyme occurring in herring skeletal muscle was very close to that observed in blue marlin white and red skeletal muscle (Suarez *et al.*, 1986). The results show that there are significant tissue differences as far as malic enzyme activity is concerned.

Table 2 shows the distribution of malic enzyme activity in mitochondria isolated from different

Table 3. Activities of the mitochondrial forms of malic enzyme in herring tissue

Source of mitochondria	$NADP^+$-dependent malic enzyme activity	$NAD(P)^+$-dependent malic enzyme activity	
		NAD^+ as coenzyme	$NADP^+$ as coenzyme
Liver	3.081	9.709	7.454
Skeletal muscle	5.645	5.419	4.203
Testes	0.806	10.258	6.029

Mitochondria were prepared from fresh tissue of 50 g liver, 200 g skeletal muscle and 50 g testes. Approximately 15 μmol NADPH/min of mitochondrial malic enzyme activity was applied on DEAE–Sephacel columns. Enzyme activities are given in μmol of pyridine nucleotide reduced per min per activity peak after DEAE–Sephacel chromatography and were determined as described under Materials and Methods.

herring tissues in the presence of the coenzyme NADP. Here again, the activity of malic enzyme in mitochondria isolated from ovaries was the lowest of the herring tissues tested. By contrast, mitochondria isolated from herring testes had an unusually high specific activity of malic enzyme. In this case, the specific activity of malic enzyme per mg of testis mitochondrial protein was more than 30 times greater than that of malic enzyme in ovarian mitochondria and more than three times that of malic enzyme activity in liver or skeletal muscle mitochondria. Herring testis mitochondrial malic enzyme activity is comparable in activity to malic enzyme of mitochondria isolated from mucosa of canine small intestine (Nagel and Sauer, 1982).

Both NADP and NAD-linked malic enzyme activities were identified in mitochondrial fractions isolated from herring tissues, although an exact measure of the NAD-linked activity was influenced and lowered due to interference in the NAD-linked assay from lactate and malate dehydrogenases. These dehydrogenases can, however, be removed by DEAE–cellulose chromatography (Skorkowski and Storey, 1988). Using this column, malic enzyme activity from fish heart mitochondria was separated into two distinct peaks, by using a linear gradient of KCl concentration (Skorkowski *et al.*, 1984). The first peak contained NADP-dependent activity only and the second peak had the NAD(P)-dependent activity. Figure 1 shows the separations achieved when mitochondrial fractions from herring skeletal muscle, liver and testis, containing both mitochondrial malic enzymes, were subjected to DEAE–Sephacel chromatography. The NADP- and NAD(P)-dependent malic enzyme activities in the effluent from the column were measured as described in Materials and Methods. Total activity was estimated by adding the activity of the fractions constituting the NADP- and NAD(P)-dependent malic enzyme peaks. The activities of the two mitochondrial malic enzymes in the tissues examined are listed in Table 3. The characteristic mitochondrial malic enzyme content of each tissue as shown in Fig. 1 was consistently observed, indicating that the distribution was tissue-dependent. Testes mitochondria contained the highest activity of the NAD(P)-dependent malic enzyme and the lowest activity of the NADP-dependent malic enzyme. On the other hand, herring skeletal muscle mitochondrial fraction contained nearly identical activities of both molecular forms. In the mitochondria of rat tissues such as testes and lungs, Nagel *et al.* (1980) showed the presence of two forms, one NADP-dependent mitochondrial form found in rat brain, heart, kidney and skeletal muscle and one NAD(P)-dependent mitochondrial form found in rat intestines, spleen and thymus. In respect of malic enzyme distribution in mitochondria, only herring testis has shown some similarities with rat tissue. Rat skeletal muscle has only one mitochondrial form (NADP-dependent) and normal rat liver mitochondria did not contain the malic enzyme (Nagel *et al.*, 1980).

The two mitochondrial forms of fish malic enzyme have different kinetic properties, e.g. in the relative rates of malate decarboxylation versus pyruvate carboxylation (Biegniewska and Skorkowski, 1986; Skorkowski, 1988). This ratio was decidedly higher for the fish NAD(P)-dependent mitochondrial enzyme, and we suggested earlier that the NAD(P)-dependent cod heart malic enzyme was responsible for pyruvate formation from malate in isolated mitochondria; thus, these mitochondria can readily oxidize malate as the only added substrate (Skorkowski *et al.*, 1984). On the other hand, for fish mitochondrial NADP-dependent malic enzyme the relative rates of malate decarboxylation versus pyruvate carboxylation depend much more on the assay pH. At pH 7.0 the rate of pyruvate carboxylation is equal to the rate of malate decarboxylation (Biegniewska and Skorkowski, 1986, 1987), suggesting an anaplerotic function for this form during the oxidation of fatty acids or pyruvate (Billinski and Jonas, 1964; Bilinski, 1974; Walton and Cowey, 1982; Mourik, 1983).

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