Biochimica et Biophysica Acta, 612 (1980) 1—10

BBA 68923

ISOLATION AND REGULATORY PROPERTIES OF MITOCHONDRIAL MALIC ENZYME FROM RAT SKELETAL MUSCLE

JULIAN ŚWIERCZYŃSKI, ANDRZEJ STANKIEWICZ, PIOTR ŚCISLOWSKI and ZENON ALEKSANDROWICZ

Department of Biochemistry, I.B.M. Medical School Gdańsk, Debinki 1, 80-211 Gdańsk (Poland)

(Received June 5th, 1979)

Key words: Malic enzyme; Regulatory properties; Pyruvate carboxylation; (Skeletal muscle mitochondria)

Summary

Mitochondrial malic enzyme (L-malate:$NADP^+$ oxidoreductase (oxaloacetate-decarboxylating), EC 1.1.1.40) has been isolated from rat skeletal muscle by $(NH_4)_2SO_4$ fractionation, chromatography on DEAE-cellulose and Ultrogel AcA 34. Specific activity of the purified enzyme was 25 μmol/min per mg of protein which corresponds to about 840-fold purification.

The enzyme was shown to carboxylate pyruvate in the presence of high concentrations of $KHCO_3$ and pyruvate at about 15% of the rate of the forward reaction. The K_m values determined at pH 7.2 for malate, NADP and Mn^{2+} were 0.33 mM, 6.8 μM and 7.1 μM, respectively. The K_m values for pyruvate, NADPH and $KHCO_3$ were 8.3 mM, 19.6 μM and 24.4 mM, respectively.

Purified enzyme showed allosteric properties at low concentration of malate and this characteristic can be modified by succinate and fumarate which do not affect the maximum velocity of the reaction.

The pH optimum for decarboxylation reaction was between 7.2 and 8.4.

Possible metabolic role of mitochondrial malic enzyme in skeletal muscle is discussed.

Introduction

It is well established that two different forms of malic enzyme (L-malate: $NADP^+$ oxidoreductase (oxaloacetate-decarboxylating), EC 1.1.1.40) are present in most of the mammalian tissues. One of these isozymes is located within the mitochondrial matrix, whereas the other in the cytosol [1—7]. With regard to the activity levels in mitochondria and cytoplasmic fraction the

tissues might be divided into two groups [3]. One is represented by liver adipose tissue and adrenal medulla in which the bulk of activity is present in the cytoplasmic fraction. The tissues of the second group as heart, brain, kidney and adrenal cortex, exhibit a considerable activity of malic enzyme in mitochondria. Lin and Davis [8] showed that two distinct forms of malic enzyme are present in rabbit heart. One of them catalyses the oxidative decarboxylation of malate in the presence of either NAD or NADP, whereas the other is specific for NADP. Malic enzyme which is reactive with NAD was also found in the heart mitochondria of guinea-pig and pigeon [8]. A NADP-specific malic enzyme in bovine [4] and pig [5] heart mitochondria was also described. Opie and Newsholme [9] found the activities of malic enzyme in homogenates of skeletal muscle from some species. Nolte et al. [10] showed that malic enzyme in different skeletal muscle is present both in the cytosol and mitochondrial fraction. Unusually high activity of NAD-linked malic enzyme in flight muscle mitochondria of the *Popillia japonica* was reported by Hansford and Johnson [11]. Hoek et al. [12] found high activity of NAD-linked malic enzyme in mitochondria isolated from flight muscle of the tsetse fly and other insects.

Recently we have shown high activity of NADP-linked malic enzyme in mitochondria isolated from abdomen muscle of the crayfish *Orconectes limosus* [13]. The above data indicate that malic enzyme is widely distributed in the skeletal muscle of different species. However, the metabolic role of the malic enzyme in skeletal muscle is not yet clear. Garber et al. [14], Goldstein and Newsholme [15] and Davis and coworkers [8,16] have proposed recently that malic enzyme in skeletal muscle functions in pyruvate production from citrate cycle intermediates.

In the present paper purification and some properties of mitochondrial malic enzyme from rat skeletal muscle are described.

Materials and Methods

Chemicals. L-Malate, sodium pyruvate, NADH, NAD, NADP were obtained from Sigma Chem. Co.; NADPH was from Boehringer GmbH, Mannheim; antimycin A was from Calbiochem; Triton X-100 was from Serva; Ultrogel AcA-34 was from LKB, Sweden; DEAE-cellulose was from Whatman Biochemicals Ltd. All other chemicals were of the highest purity available commercially.

Male Wistar rats maintained on a commercial complete rat diet were used for experiments.

Preparations of rat skeletal muscle mitochondria. Rat skeletal muscles (both red and white) obtained from the hind legs immediately after decapitation were freed of connective and adipose tissues, minced finely with scissors and rinsed thoroughly with isotonic KCl. Mitochondria were prepared as described previously [17], except that the last centrifugation was done at 14 000 $\times g$ for 10 min. In this manner both heavy and light mitochondria were obtained.

Protein concentrations were determined by the biuret method or after extensive purification of the enzyme by the method of Spector [18].

Enzyme assays. The enzyme activities at all purification steps were followed spectrophotometrically with an Unicam SP800 recording spectrophotometer

by observing the appearance of NADPH (for malic enzyme assay) and disappearance of NADH (for lactate and malate dehydrogenase assays) at 340 nm and 30° C. The standard reaction mixture for malic enzyme assay (final volume 1 ml) contained: 50 mM Tris-HCl (pH 7.4), 1 mM $MnCl_2$, 0.5 mM NADP, 10 mM L-malate, and the enzyme preparation in amounts which caused increase of absorbance at the range 0.1—0.2. The medium was supplemented with 2 μg/ml antimycin A when disrupted mitochondria were used as a source of malic enzyme.

Lactate dehydrogenase was assayed in the medium containing 50 mM Tris-HCl, pH 7.4, 1 mM pyruvate and 0.2 mM NADH.

Malate dehydrogenase was measured in the medium containing: 50 mM Tris-HCl, pH 7.4, 1 mM oxaloacetate and 0.2 mM NADH.

Results

Purification of mitochondrial malic enzyme

For purification of mitochondrial malic enzyme both light and heavy mitochondria were used. These mitochondria incubated in the presence of malate and Mn^{2+} did not reduced exogenous NADP. When mitochondria were solubilized by the addition of Triton X-100, NADP started to be reduced in a malate-dependent manner if an inhibitor of the respiratory chain was present in the incubation medium (not shown). These data suggest that the enzyme activity is located within the intramitochondrial compartment. Thus first step of purification of mitochondrial malic enzyme was solubilization of mitochondria. It was done by suspending the mitochondria in 10 mM Tris-HCl, pH 7.8, containing 2 mM EDTA and 2% Triton X-100 (about 2.5 g mitochondrial protein in 150 ml of this buffer). The pH of the suspension was adjusted to 7.8 with concentrated Tris/base and it was centrifuged at 20 000 × *g* for 60 min. The resulting supernatant was used for the purification of malic enzyme as described below.

$(NH_4)_2SO_4$ fractionation

The solution was adjusted to 35% saturation of $(NH_4)_2SO_4$ by gradual addition of 20.9 g solid $(NH_4)_2SO_4$/100 ml of suspension. After stirring for 30 min the solution was centrifuged for 20 min at 20 000 × *g* and the precipitate discarded. The supernatant fluid was then brought to 70% saturation of $(NH_4)_2SO_4$ by the gradual addition of 23.8 g of solid $(NH_4)_2SO_4$/100 ml of solution. During addition of $(NH_4)_2SO_4$ the pH was kept constant at 7.8 by the addition of 5 M NH_4OH. The suspension was stirred for 30 min and subsequently centrifuged for 20 min at 20 000 × *g*. The precipitate was dissolved in a solution containing 10 mM Tris-HCl, pH 7.8, and 2 mM EDTA (approx. 0.1 vol/1 vol. of the original mitochondrial suspension). The resulting suspension was dialyzed overnight against 200 vols. of the same buffer. The dialyzed solution was centrifuged at 20 000 × *g* for 20 min in order to remove insoluble material.

Chromatography on DEAE-cellulose

The dialyzed 35—70% fraction was applied to a column (2 × 30 cm) of

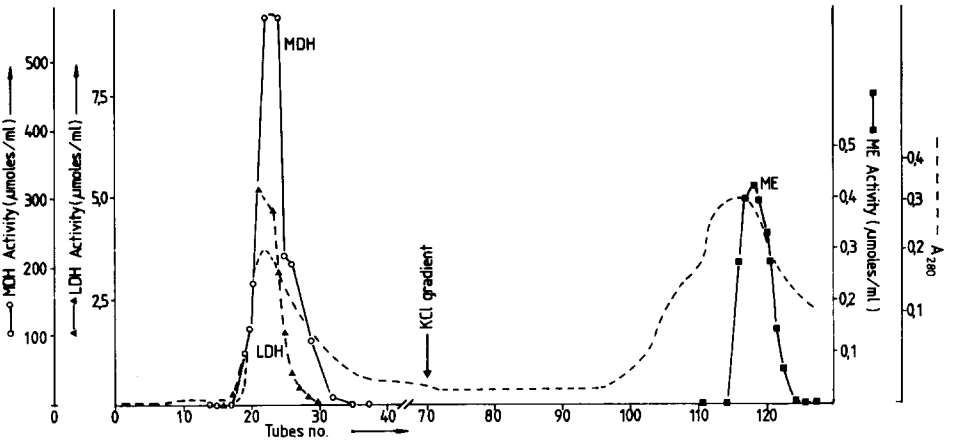

Fig. 1. DEAE-cellulose chromatography of mitochondrial malic enzyme from rat skeletal muscle. Fractions (5 ml) were collected from the column at the rate of 25 ml/h. Protein was measured spectrophotometrically at 280 nm using Uvicord II (Ultraviolet Absorptiometer Detector, LKB). The enzyme activities were measured as described under Materials and Methods. ------, absorbance at 280 nm; ○———○, malate dehydrogenase activity; ▲———▲, lactate dehydrogenase activity, and ■———■, malic enzyme activity.

DEAE-cellulose. The DEAE-cellulose (type DE 23) was washed with 20 vols. of 1 M Tris/base, and then repeatedly with deionized water and equilibrated by washing with a solution containing 10 mM Tris-HCl, pH 7.8, and 2 mM EDTA. The elution was started with 10 mM Tris-HCl, pH 7.8, and 2 mM EDTA and continued until a protein peak, devoided of malic enzyme activity, but containing lactate dehydrogenase and malate dehydrogenase activities, emerged from the column (Fig. 1). Malic enzyme activity was eluted by a 30—400 mM KCl gradient plus 10 mM Tris-HCl and 2 mM EDTA. As illustrated in Fig. 1 enzyme activity was eluted as a single symmetrical peak included in a large protein fraction. The active fractions from this column were pooled and concentrated with collodion bag apparatus (Schleicher and Schuell).

TABLE I

PURIFICATION OF MITOCHONDRIAL RAT SKELETAL MUSCLE MALIC ENZYME

Experimental conditions see text.

Step	Volume (ml)	Total protein (mg)	Specific activity (nmol · min^{-1} · mg^{-1})	Total activity (μmol · min^{-1})	Yield (%)	Purification (-fold)
Solubilized mitochondria	150	1425	29.7	42.3	100	1
Soluble protein recovered	140	840	47.0	39.5	93.4	1.6
$(NH_4)_2SO_4$ 35—70% fractionation	20	200	153.0	30.6	72.3	5.2
DEAE-cellulose chromatography	40	8	2112.5	16.9	40	71.1
AcA-34 chromatography	31	0.44	25 000.0	11.0	26	841.2

Ultrogel AcA-34 chromatography

Concentrated eluate from DEAE-cellulose was applied to a column of Ultrogel AcA-34 (2 × 90 cm) equilibrated with 10 mM Tris-HCl (pH 7.8) and 2 mM EDTA. Elution was performed with the solution containing 10 mM Tris-HCl (pH 7.8) and 2 mM EDTA at the rate of 5 ml/h. The washing procedure was continued untill a protein peak appeared from the column. Malic enzyme activity was eluted as a single symmetrical peak (not shown). 3-ml fractions were collected.

Table I summarizes the purification of the malic enzyme from rat skeletal muscle mitochondria. At a yield of about 26% the enzyme was purified 840-fold. Specific activity was similar to that reported by Bartholome et al. [5] for mitochondrial malic enzyme from pig heart, purified about 1000-fold. The enzyme preparation was free of lactate dehydrogenase, isocitrate dehydrogenase (both NAD and NADP linked) and fumarase activities, but contained traces of malate dehydrogenase activity, which did not interfere with the assay of malic enzyme activity. This was confirmed by the finding that pyruvate was formed stoichiometrically with NADPH. Polyacrylamide gel electrophoresis of the purified enzyme in the presence of sodium dodecyl sulfate showed one main band and three faint bands stained for protein (not shown). The purified enzyme was stable for two weeks when stored at 4°C in the elution medium and concentrated to about 1 mg of protein/1 ml.

Properties of malic enzyme from rat skeletal muscle mitochondria

Fig. 2 shows the effect of pH on the activity and Michaelis constant of malic enzyme from mitochondria in the decarboxylation direction. The pH optimum of this activity was broad, the maximum activity being essentially unchanged between pH 7.2 and 8.4. The apparent Michaelis constant for malate varied with pH (Fig. 2), the values calculated from Eadie-Hofstee plots were increasing with the raising pH.

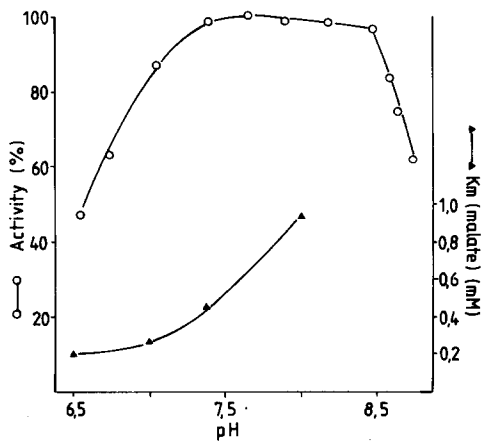

Fig. 2. Effect of pH on mitochondrial malic enzyme activity and K_m for malate. Reaction mixture contained: 50 mM Tris-HCl, 20 mM imidazole-HCl, 10 mM L-malate, 0.5 mM NADP and 1 mM $MnCl_2$. For estimation of K_m for malate the same reaction mixture was used with malate varied in concentration. K_m values were calculated from Eadie-Hofstee plot. o———o, enzyme activity; ▲———▲, K_m values.

TABLE II

KINETIC CONSTANTS FOR THE MITOCHONDRIAL MALIC ENZYME FROM RAT SKELTAL MUSCLE

Reaction rate for decarboxylation was measured at pH 7.2 in a medium containing: 50 mM Tris-HCl, 20 mM imidazole-HCl, 10 mM malate, 0.5 mM NADP, 1 mM $MnCl_2$ and 0.4 μg of the enzyme. The rate of reductive carboxylation was measured at pH 7.2 in a medium containing: 50 mM Tris-HCl, 20 mM imidazole-HCl, 20 mM pyruvate, 0.15 mM NADPH, 1 mM $MnCl_2$, 50 mM $KHCO_3$ and 4 μg of the enzyme. For estimation of K_m, all components required were present in concentration mentioned above, with the tested component varied in concentration. The K_m values were calculated from Eadie-Hofstee plots.

Decarboxylation		Carboxylation	
Reaction rate	20.6 μmol · min^{-1} · mg^{-1}		3.22 μmol · min^{-1} · mg^{-1}
K_m (malate)	0.33 mM	K_m (pyruvate)	8.3 mM
K_m (NADP)	6.8 μM	K_m (NADPH)	19.6 μM
K_m (Mn^{2+})	7.1 μM	K_m ($KHCO_3$)	24.4 mM

It has been reported that NADP-linked malic enzyme from heart [8] and brain [19] catalysed the reductive carboxylation of pyruvate at lower rate than the rate of the reverse reaction. If the pyruvate concentration was very high (20 mM) the enzyme isolated from rat skeletal muscle mitochondria catalysed the reductive carboxylation of pyruvate in the presence of 10 mM $KHCO_3$ at the rate up to 1/14 of the reverse reaction and in the presence of 50 mM $KHCO_3$ at the rate equal 1/6 of the malate decarboxylation (Table II). It has been proved that the true reductive carboxylation was taking place both by following the disappearance of NADPH at 340 nm and by enzymic determination of malate after the reaction had been completed. Every time the oxidation of NADPH was accompanied by an equivalent amount of malate produced.

Kinetic parameters

To characterize further the malic enzyme from rat skeletal muscle mitochondria, some kinetic measurements were performed at pH 7.2 both for carboxylation and decarboxylation reaction. Table II summarizes apparent K_m values for different substrates. The K_m values presented do not differ essentially from the K_m values reported for mitochondrial malic enzyme from other sources [5,7,19].

The effect of divalent cations

Malic enzyme from various sources requires some divalent cations for expressing the activity. We studied the effect of divalent cations on malic enzyme from rat skeltal muscle mitochondria. The order of effectiveness was as follows: $Mn^{2+} > Mg^{2+} > Cd^{2+} > Ni^{2+}$. Ca^{2+} was without effect, Co^{2+} and Zn^{2+} inhibited malic enzyme activity (not shown).

An interesting feature of the enzyme investigated revealed the dependence of the reaction rate on the concentration of malate at three different pH values. Double-reciprocal plots of velocity versus malate concentration at pH 7.5 and 8.0 yielded curves that were generally concave upwards (Fig. 3). This indicates that malic enzyme from rat skeletal muscle mitochondria showed allosteric properties under these conditions. The allosteric kinetics at pH 7.5 and 8.0 was

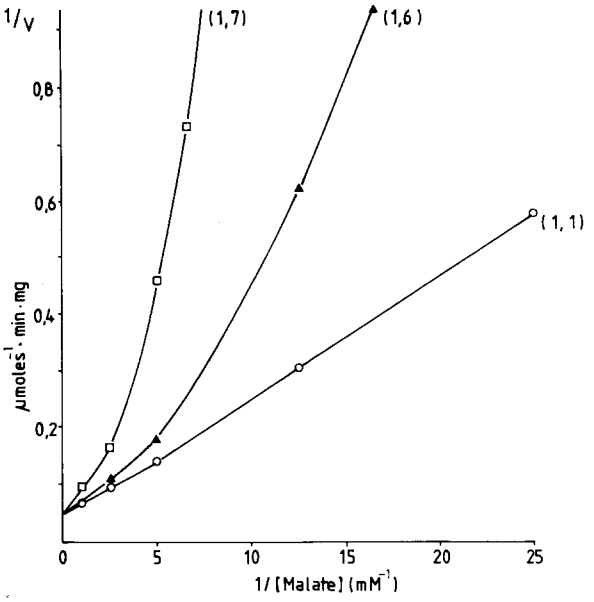

Fig. 3. Lineweaver-Burk plot of mitochondrial malic enzyme from rat skeletal muscle, using L-malate as variable substrate. The same conditions as indicated in Fig. 2 were used, at pH 7.0 (○———○), 7.5 (▲———▲), and 8.0 (□———□). The number of parentheses are the Hill coefficient.

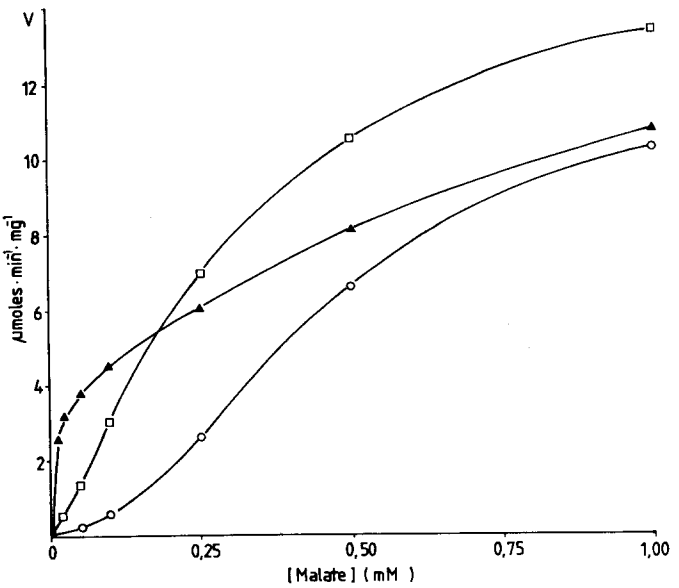

Fig. 4. Effect of succinate and fumarate on the oxidative decarboxylation of malate catalysed by the mitochondrial malic enzyme from rat skeletal muscle. The reactions were measured as indicated in Fig. 2 (pH 8.0) in the absence of any effector (○———○), in the presence of 10 mM succinate (□———□), and in the presence of 10 mM fumarate (▲———▲).

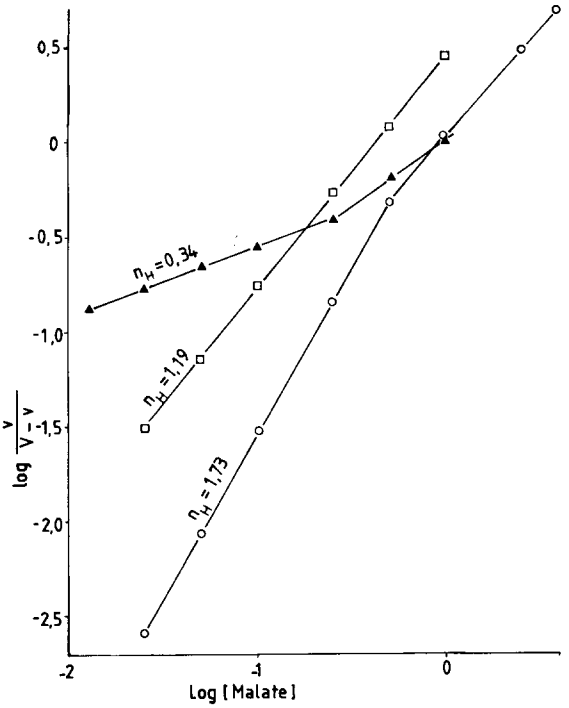

Fig. 5. Hill plot for the rat skeletal muscle mitochondrial malic enzyme in the absence of any effector (○———○), in the presence of 10 mM succinate (□———□) and in the presence of 10 mM fumarate (▲———▲). Enzyme activity assays as in Fig. 2 (pH 8.0).

also disclosed by calculating the Hill coefficients which were 1.7 at pH 8.0 and 1.6 at pH 7.5. At pH 7.0 double-reciprocal plot became a straight line and the Hill coefficient was 1.1. Frenkel has shown that NADP-linked malic enzyme from heart [20] and brain [7,19] mitochondria also shows allosteric properties and that this characteristics can be modified by some dicarboxylate anions. Therefore we studied the effect of succinate and fumarate on malic enzyme activity from rat skeletal muscle mitochondria. Fig. 4 shows the rates of the reaction at low concentrations of malate in the absence of effectors and in the presence of 10 mM succinate or 10 mM fumarate. Here again, control curve shows a distinct sigmoidicity. The addition of succinate or fumarate affects considerably the reaction kinetics. At low malate concentrations a clear stimulation of the reaction was observed when succinate or fumarate were added. It should be pointed out that none of these dicarboxylic anions caused NADP reduction in the absence of malate. At higher concentration of malate, addition of succinate or fumarate, showed no effect on the enzyme activity (not shown). The apparent activation constants for succinate and fumarate at pH 8.0 in the presence of 0.1 mM malate were 2.2 and 0.5 mM, respectively. As succinate and fumarate stimulated malic enzyme activity at low concentrations of malate, but appear to be completely ineffective at higher malate levels, this seems to be the K_m type of activation. The Hill plots presented on Fig. 5 show that in the absence of both succinate and fumarate two different slopes were

observed. The Hill coefficient at lower substrate concentrations was 1.7 and changed to about 1.0 at higher concentrations of malate. This may indicate that the allosteric site is able to bind not only succinate and fumarate but also malate. The addition of succinate caused a decrease of the Hill coefficient to 1.18 whereas in the presence of fumarate the coefficient was as low as 0.34. Thus although fumarate has been shown to be a positive allosteric effector of skeletal muscle mitochondrial malic enzyme, in its presence the enzyme seems to display negative cooperativity. The results presented above are somewhat similar to those reported by Frenkel, who studied malic enzyme from heart [20] and from brain [7,19] mitochondrial. However, this author did not observe any effect of fumarate on malic enzyme activity from brain mitochondria [7]. In our experiments fumarate appears to be more potent modulator of malic enzyme from rat skeletal muscle mitochondria than succinate.

Discussion

The activity of malic enzyme in the mitochondria from rat skeletal muscle is about 30 nmol/min per mg of mitochondrial protein, which may be compared with the activity of 25 nmol/min per mg of mitochondrial protein reported by Bartholome et al. [5] in pig heart mitochondria, with the range 4—21 nmol/min per mg of mitochondrial protein (depending on the presence of divalent cations and coenzyme) reported by Lin and Davis [8] in rabbit heart mitochondria and with the activity of 17.6 nmol/min per mg of mitochondrial proteins as reported by Sauer [21] in adrenal cortex mitochondria. The purification procedure employed for mitochondrial malic enzyme, the general outline of which is that used for isolation of this enzyme from other tissues, yields specific activity about 25 μmol/min per mg of protein. This is comparable with the activity of 28 μmol/min per mg of protein reported by Bartholome et al. [5] for mitochondrial malic enzyme from pig heart.

The data reported in this paper indicate that malic enzyme from rat skeletal muscle mitochondria shows allosteric properties and this characteristic can be modified by succinate and fumarate. These regulatory properties are similar to those described for the mitochondrial malic enzyme obtained from bovine heart [20] and bovine brain [7,19]. It is likely, therefore, that the NADP-linked mitochondrial malic enzyme displays regulatory kinetic properties. It is worth noting at this point that sigmoid kinetics were also observed by Sauer and coworkers with NADP malic enzyme from adrenal cortex mitochondria [22,23] and mouse ascites tumor mitochondria [24].

At present time the precise physiological role of mitochondrial malic enzyme in skeletal muscle is unknown. Chang and Goldberg [25] have shown that about 50% of the carbon chains of the aspartic acid, asparagine, glutamic acid, isoleucine and valine enter the tricarboxylic and cycle in skeletal muscle and are converted to CO_2 (less than 20%) or to lactate (less than 30%) and alanine (less than 2%) which are released from the muscle. The complete degradation of tricarboxylic acid cycle intermediates requires their conversion to pyruvate and then to acetyl-CoA. Tricarboxylic acid cycle intermediates are converted to lactate or alanine also via pyruvate. Conversion of tricarboxylic acid cycle intermediates to pyruvate requires either phospho*enol*pyruvate carboxykinase

which catalyses the conversion of oxaloacetate to phospho*enol*pyruvate, or malic enzyme catalysing the conversion of malate to pyruvate. High specific activity of malic enzyme in rat skeletal muscle mitochondria and relatively low K_m for malate and NADP suggest that malic enzyme may play an essential role in the conversion of tricarboxylic acid cycle intermediates to pyruvate. The activation of malic enzyme by succinate and fumarate is consistent with this suggestion. Taking into consideration that succinate and fumarate are metabolites present in the mitochondrial matrix, the activity of malic enzyme may increase rapidly under conditions which would favor accumulation of tricarboxylic and cycle intermediates.

The suggestion of Brdiczka and Pette [3] that one function of the mitochondrial malic enzyme might be replenishing the dicarboxylic acid pool in order to maintain the operation of the citric acid cycle during fatty acid oxidation does not appear to be likely, in view of the very low activity of this enzyme for reductive carboxylation of pyruvate even at very high pyruvate concentration. A precise definition of the physiological role of malic enzyme in skeletal muscle mitochondria requires further investigation.

Acknowledgements

We are indebted to Professor M. Żydowo and Professor L. Żelewski for a critical reading and discussion of the manuscript. This work was supported by the Polish Academy of Science within the project II.1.2.6.

References

1 Henderson, N.S. (1966) Arch. Biochem. Biophys. 117, 28—33
2 Simpson, E.R. and Estabrook, R.W. (1969) Arch. Biochem. Biophys. 129, 384—395
3 Brdiczka, D. and Pette, D. (1971) Eur. J. Biochem. 19, 546—551
4 Frenkel, R. (1971) J. Biol. Chem. 246, 3069—3074
5 Bartholome, K., Brdiczka, D. and Pette, D. (1972) Hoppe-Seylers' Z. Physiol. Chem. 353, 1487—1495
6 Pfeiffer, D.R. and Tchen, T.T. (1975) Biochemistry 14, 89—96
7 Frenkel, R. and Cobo-Frenkel, A. (1973) Arch. Biochem. Biophys. 158, 323—330
8 Lin, R.C. and Davis, E.J. (1974) J. Biol. Chem. 249, 3867—3875
9 Opie, L.H. and Newsholme, E.A. (1967) Biochem. J. 103, 391—399
10 Nolte, J., Brdiczka, D. and Pette, D. (1972) Biochim. Biophys. Acta 284, 497—507
11 Hansford, R.G. and Johnson, R.N. (1975) Biochem. J. 148, 389—401
12 Hoek, J.B., Pearson, D.J. and Olembo, N.K. (1976) Biochem. J. 160, 253—262
13 Skorkowski, E.F., Świerczyński, J. and Aleksandrowicz, Z. (1977) Comp. Biochem. Physiol. 58B, 297—301
14 Garber, A.J., Karl, J.E. and Kipnis, D.M. (1976) J. Biol. Chem. 251, 836—843
15 Goldstein, L. and Newsholme, E.A. (1976) Biochem. J. 154, 555—558
16 Lee, S-H. and Davis, E.J. (1979) J. Biol. Chem. 254, 420—430
17 Świerczyński, J., Aleksandrowicz, Z. and Żydowo, M. (1975) Int. J. Biochem. 6, 757—763
18 Spector, T. (1978) Anal. Biochem. 86, 142—146
19 Frenkel, R. (1972) Arch. Biochem. Biophys. 152, 136—143
20 Frenkel, R. (1972) Biochem. Biophys. Res. Commun. 47, 931—937
21 Sauer, L.A. (1973) FEBS Lett. 33, 251—255
22 Sauer, L.A. (1973) Biochem. Biophys. Res. Commun. 50, 524—531
23 Mandella, R.D. and Sauer, L.A. (1975) J. Biol. Chem. 250, 5877—5884
24 Sauer, L.A. and Dauchy, R.T. (1978) Cancer Res. 38, 1751—1756
25 Chang, T.W. and Goldberg, A.L. (1978) J. Biol. Chem. 253, 3685—3695