Phosphoenolpyruvate shuttle – transport of energy from mitochondria to cytosol

Zdeněk Drahota, Hana Rauchová, Marie Miková, Phool Kaul and Arnošt Bass

Institute of Physiology, Czechoslovak Academy of Sciences, Prague 4, Czechoslovakia

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Brown-adipose tissue mitochondria of hamster and rat contain phosphoenolpyruvate carboxykinase (EC 4.1.1.32). In the presence of ketoglutarate and malate, phosphoenolpyruvate is formed and exported from mitochondria. Phosphoenolpyruvate formation is inhibited by 1,2,3-benzenetricarboxylate. It is proposed that phosphoenolpyruvate carboxykinase together with pyruvate carboxylase and pyruvate kinase forms a phosphoenolpyruvate shuttle through which energy produced by the Krebs cycle in mitochondria may be exported to cytosol.

Phosphoenolpyruvate *Brown fat mitochondria* *Phosphoenolpyruvate carboxykinase* *Energy transformation*

1. INTRODUCTION

Phosphoenolpyruvate carboxykinase (EC 4.1.1.32) has unique species-dependent variation in its intracellular localization. In most species it is evenly distributed between cytosol and mitochondria. However, in rat and mouse it is localized in cytosol only and in avian liver all enzyme activity is in mitochondria [1]. It has been demonstrated that the cytosolic form of the enzyme plays an important regulatory role in glucogenesis [2]. Although there are many reports concerning the intracellular concentrations and distribution of phosphoenolpyruvate carboxykinase [3] the functional, structural and genetic relationships between the enzymes localized in cytosol and in mitochondria still remain obscure, data accumulate indicating that these two forms of the enzyme represent two different protein molecules [4].

Here we report data that showed the presence of phosphoenolpyruvate carboxykinase in mitochondria of the brown adipose tissue and we conclude from our experiments that in brown adipose tissue the mitochondrial phosphoenolpyruvate carboxykinase represents a part of the shuttle through which energy produced by the Krebs cycle may be transported from mitochondria to cytoplasm.

2. MATERIALS AND METHODS

Syrian hamsters and 10-day-old rats were used for experiments. Liver mitochondria were isolated according to Schneider and Hogeboom [5] and brown-fat mitochondria according to Hittelman et al. [6]. Isolated mitochondria were suspended in 100 mM KCl, 10 mM Tris–HCl and 1 mM EDTA (pH 7.4). Particle-free fraction of cytosolic soluble proteins was prepared by 60 min centrifugation of post-mitochondrial supernatant at 100000 × *g* using a Beckman L-50 centrifuge. Mitochondrial membranes were prepared by sonication (3 × 30 s) and centrifugation (60 min, 100000 × *g*).

Phosphoenolpyruvate carboxykinase was determined according to Chang and Lane [7] in the presence of 0.5% Triton X-100. In experiments where formation of phosphoenolpyruvate was determined, mitochondria were incubated for 10 min at 30°C in an incubation mixture containing 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 75 mM Tris–HCl buffer, 5 mM K-malate, 1 mM

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Na-oxoglutarate (pH 7.4). When 1,2,3-benzenetricarboxylate (50 mM) was added to the reaction medium, corresponding amounts of Tris–HCl were omitted. The incubation was terminated by addition of cold perchloric acid and after its precipitation by $KHCO_3$, phosphoenolpyruvate was determined in the supernatant according to Bücher et al. [8]. Protein was determined according to Lowry et al. [9].

Pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27), ADP and NADH used for phosphoenolpyruvate assay were purchased from Boehringer (Mannheim), 1,2,3-benzenetricarboxylate was a generous gift of Dr F. Palmieri. All other chemicals were of analytical grade purity.

3. RESULTS AND DISCUSSION

The activity of phosphoenolpyruvate carboxykinase and its modification by various hormones was studied in brown adipose tissue only in cytosol [10]. There are no data about the localization of this enzyme in brown-fat mitochondria of various animals. As demonstrated in table 1, in brown adipose tissue of hamster and rat phosphoenolpyruvate carboxykinase is localized in both compartments; i.e., in cytosol and in mitochondria. The specific activity of brown-fat mitochondria is quite high in comparison with the activity of liver mitochondria and also the quantity of this enzyme in the mitochondrial fraction of brown fat is much higher than that of the liver (table 1). The phosphoenolpyruvate carboxykinase activity is localized only in the soluble matrix of mitochondria. When brown-fat mitochondria were disrupted by sonication, specific activity of isolated membranes was 10% (3.4 U/g protein) of that of intact mitochondria (34.9 U/g protein). Activity of soluble proteins in these experiments was similar to that of original mitochondria (36.4 U/g protein) which indicates that during the sonication and centrifugation procedure the enzyme activity is partially inactivated.

In mitochondria containing phosphoenolpyruvate carboxykinase, export of phosphoenolpyruvate was found to be partially inhibited by inhibitors of the tricarboxylic acid carrier and adenine nucleotide translocator [11]. As shown in table 2, in brown-adipose-tissue mitochondria phosphoenolpyruvate formation is inhibited by 50% by 1,2,3-benzenetricarboxylate, an inhibitor of the tricarboxylate-transporting system which indicates that also in brown-adipose-tissue mitochondria the tricarboxylic acid anion carrier participates in the export of phosphoenolpyruvate from mitochondria.

Mitochondrial localization of phosphoenolpyruvate carboxykinase is an important factor that helps in understanding the molecular mechanisms participating in maintaining the high metabolic rate of adipocytes during their functional activity. This enzymic reaction consuming GTP and producing GDP is an important link between very active substrate-level phosphorylation [12] and

Table 1

Distribution of the phosphoenolpyruvate carboxykinase activity in liver and brown adipose tissue

	Hamster liver		Hamster brown adipose tissue		Rat brown adipose tissue	
	s.a.	t.a.	s.a.	t.a.	s.a.	t.a.
Homogenate	10.2 ± 0.58	10.2	33.4 ± 5.05	33.4	31.2 ± 3.18	31.2
Mitochondria	5.0 ± 0.67	0.9	39.9 ± 3.63	11.6	22.1 ± 0.82	3.1
Soluble protein	26.1 ± 2.87	10.9	60.0 ± 9.04	23.4	65.3 ± 3.86	22.1

s.a. = specific activity of phosphoenolpyruvate carboxykinase expressed in U/g protein

t.a. = the total activity of the enzyme in the given fraction expressed in U/g total protein in homogenate

Values are the means ± standard error for 5 expt

Table 2

Production of phosphoenolpyruvate by brown adipose tissue mitochondria

Additions	(nmol phosphoenol-pyruvate/mg protein)	(%)
–	0.106 ± 0.005	100
1,2,3-Benzenetri-carboxylate (50 mM)	0.057 ± 0.002	51

Values are the means ± standard error for 7 expt

cytosolic energy-consuming processes. Especially during the catecholamine-induced heat dissipation when ATP production by oxidative phosphorylation is strongly depressed [13] mitochondrial phosphoenolpyruvate formation, using GTP produced by substrate-level phosphorylation, could be a very important source of energy for cytoplasmic reactions. If we take into consideration also the fact that during the functional activity of adipocytes oxaloacetate formation is very high and that the function of pyruvate carboxykinase is required for the hormone-induced heat dissipation [14], a metabolic cycle for export of energy produced by substrate-level phosphorylation to cytoplasm may be proposed (fig.1). This energy-transporting shuttle requires two molecules of GTP for one molecule of phosphoenolpyruvate exported to cytosol, because ATP is necessary for pyruvate carboxylation. This requirement may be easily fulfilled because also in uncoupled conditions substrate-level phosphorylation can maintain a high level of intramitochondrial ATP [12,15].

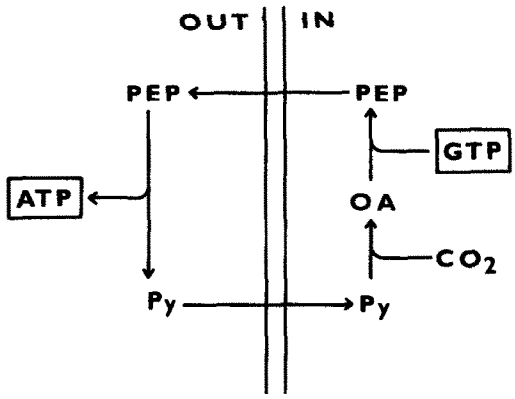

Fig.1. Phosphoenolpyruvate shuttle composed of three enzymes – phosphoenolpyruvate carboxykinase, pyruvate kinase and pyruvate carboxykinase, and of two translocating systems for phosphoenolpyruvate and pyruvate. PEP, phosphoenolpyruvate; Py, pyruvate; OA, oxaloacetate.

We may thus conclude that the phosphoenolpyruvate shuttle proposed for the brown-adipose-tissue mitochondria could be a useful device for exporting intramitochondrially produced GTP also in other tissues where phosphoenolpyruvate carboxykinase is localized in mitochondria. This cycle could help to regulate activity of the Krebs cycle and also to link intramitochondrial substrate-level phosphorylation with cytoplasmic energy-requiring processes.

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