COMPARATIVE AND ONTOGENIC BIOCHEMISTRY

Properties of Some Enzymes of Gluconeogenesis in Platyhelminths

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Abstract—Activities and properties of some enzymes of gluconeogenesis, the “malic” enzyme, phosphoenolpyruvate carboxykinase, glucoso-6-phosphatase, alanine aminotransferase, and aspartate aminotransferase, were studied in turbellarians Phagocata sibirica and trematodes Eurytrema pancreaticum. It was shown that adaptation of trematodes to the parasitic mode of life affected essentially the biosynthetic processes and led to formation of different metabolic pathways.

INTRODUCTION

Whereas transformation of glucose to pyruvate is the central pathway in carbohydrate metabolism, transformation of pyruvate to glucose is the central pathway of gluconeogenesis. These pathways are not identical, although they have several common stages. Gluconeogenesis is usually carried out by transformation of intermediate products of di- and tricarboxylic acid cycle that are metabolized to phosphoenolpyruvate (PEP) and pyruvate and thus provide pathway to substrates. Most stages of gluconeogenesis are catalyzed by reversion of glycolytic enzyme activities. It was established that phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) plays an important role in gluconeogenesis (i.e., synthesis of glucose from non-carbohydrate precursors) by converting, in the presence of GTP and ITP, oxaloacetate (OA) to PEP, the main substrate used subsequently by the cell for synthesis of glucose.

There is only a fragmentary information about gluconeogenesis in parasitic and free-living worms [1, 2]. It is to be taken into account that only in some helminths, glycolysis reaches pyruvate and then lactate. In most parasites there is mixed fermentation, and the main substrate is PEP, therefore, the absence of pyruvate kinase does not yet indicate inability for gluconeogenesis. Other enzyme systems required for gluconeogenesis from intermediates of glycolysis and Krebs cycle were found in helminths.

For pyruvate carboxylation, the “malic” enzyme (ME) revealed in helminths may be an alternative to pyruvate carboxylase, and the high CO2 content in the environment promotes carboxylation of pyruvate, with formation of malate. Then malate is converted to glucose. As a source of glucose, amino acids that are converted by aminotransferases to Krebs cycle metabolites can also be used. The transamination processes in helminths are so far poorly studied.

The goal of the present work was to study the properties and intracellular distribution of some enzymes of gluconeogenesis in the trematode Eurytrema pancreaticum (Trematoda, Dicrocoeliidae) parasitizing in cattle pancreas ducts and in the turbellarian Phagocata sibirica (Turbellaria, Planorriidae) living in mountain brooks of the Primor’e raion and having common ancestors with trematodes. All parasitic flat worms living nowadays take their origin from the free-living forms that are common with ancestors of rectintestinal turbellarians and morphologically similar to them.
MATERIALS AND METHODS

Trematodes were collected at the slaughterhouse and delivered to the laboratory in the Gedon-Fleig medium in thermoses, while turbellarians were collected in brooks and transported in jars with water. For preparation of enzyme extracts, eurytremes and turbellarians were homogenized in 10 volumes of extraction medium by the method described earlier [3]. To isolate mitochondria, the worms were homogenized in a glass homogenizer in the ice-cold medium containing (mM): 0.25 sucrose, 0.05 Tris-buffer, pH 7.5, 0.05 EDTA, and 0.15% albumin. The obtained homogenate was centrifuged for 15 min at 1000 g at 1°C. Supernatant was used as a source of cytosolic enzymes. Mitochondria were washed twice with the isolation medium lacking albumin, suspended in the same medium, and used as a source of mitochondrial enzymes. The purity of the mitochondrial fractions was controlled under electron microscope.

To obtain membrane fraction, mitochondria were sonicated in an ultrasound MSE-disintegrator at 20 kHz twice for 1 min, with a 30 s interval at constant cooling of the mixture on ice. The disrupted mitochondria were centrifuged for 1 h at 105 000 g at 2°C. Supernatant was used as a source of cytosolic enzymes. Mitochondria were washed twice with the isolation medium lacking albumin, suspended in the same medium, and used as a source of mitochondrial enzymes. The purity of the mitochondrial fractions was controlled under electron microscope.

The concentrations of ions, substrates, cofactors, enzyme extracts, buffer, and pH were chosen to provide the maximal rate of reaction. The determination of the PEPCK activity in OA reaction was performed according to Cornish and co-authors [4]. The changes of optic density were registered every minute for 5 min. The complete reaction mixture contained (nM): 0.5 acetic acid buffer, pH 4.9, 40 KHCO3, 1 MnCl2, 5 IDP, 0.5 GDP, 0.25 NADH, 0.5 PEP, 0.5 units MDH, 0.4–0.6 mg of sample protein. The reaction was initiated by addition of PEP after preincubation for 5 min at 30°C. After addition of KHCO3, pH of the reaction mixture rose from 4.9 to 6.2. This value remains constant until the end of determination. At evaluation of the pH-optimum, pH of the incubation medium was adjusted by 1 M KOH. Other components of the mixture were added to the volume of 3 ml; pH was also measured in the end of determination and found to be unchanged. Control was prepared for each value of pH. Activity of PEPCK in the reaction of OA decarboxylation was determined according to Seubert and Huth [5]. In the case of Mg2+-dependent reaction the incubation mixture contained (mM): 50 Tris-HCl buffer, pH 8.5, 1.5 freshly neutralized OA, 2 ITP, 7 MgCl2, 1 dithiotreitol (DTT), 4 NaF; in the case of Mn2+-dependent reaction: 50 Tris-HCl buffer, pH 7.0, 1.5 OA, 8 ITP, 2 MnCl2, DTT, 4 NaF, and 0.4–0.8 mg of enzyme protein. The reaction was started by OA addition after preincubation for 5 min of all components at 37°C. In 10 min the reaction was stopped by addition of 5 mg of freshly prepared KBrH4 in 0.1 ml of water, then the sample was chilled on ice for 2–5 min and acidified by 0.4 ml of 10% HClO4, after cooling 5 ml of 0.05% methyl orange was added and the neutralized with 30% KOH. An aliquot of centrifugate was taken for PEP determination according to Czok and Eckert [6]. The medium for PEP determination contained (mM): 70 Tris-HCl buffer, pH 7.4, 0.3 NADH, 2 ADP, 4 MgCl2, 33 KCl, 5.5 units of LDH, 4 units of pyruvate kinase.

Activity of NAD-dependent (EC 1.1.1.39) and NADP-dependent (EC 1.1.1.40) ME was determined by the method of Ochoa [7]. The complete reaction mixture with the final volume of 3 ml contained (mM): 200 Tris-HCl buffer, pH 6.35–7.0, 3.3–10 MnCl2, pH 6.35–7.0, 0.66 NAD and NADP, 10 L-malate, 4–13.3 OA.

Activity of glucose-6 phosphatase (G6Pase) (EC 3.1.3.9) was determined by the method of Yeung and co-authors [8]. The analyzed medium contained 0.5 ml of 0.2 M Tris-maleate buffer, pH 5.2–6.4, 0.1 ml of 0.07–0.1 M glucose-6-phosphate (G6P) dissolved in the same buffer. After preincubation at 30°C the reaction was initiated by addition of 0.1 ml of protein, and the samples were incubated for 30 min. Reaction was stopped by addition of 6% TCA. After centrifugation of the samples, inorganic phosphate was determined in the supernatant according to Kochetov [9].

Activities of aspartate aminotransferase (AsAT) (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) were determined by the method of Yeung and Oliver [10]. The reaction mixture in a final volume of 3 ml contained (mM) for AIAT: