Turbellaria Phagocata sibirica: Some Enzymes of Carbohydrate and Energy Metabolisms

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Abstract—Activities and properties of some enzymes of carbohydrate and energy metabolisms in free-living turbellaria Phagocata sibirica are studied. The enzymes are studied in various subcellular fractions. A high activity of hexokinase is accompanied by high activity of glucose-6-phosphate dehydrogenase (G6PDG). The level of pyruvate kinase activity is sufficient to provide dissimilation of phosphoenolpyruvate with formation of pyruvate. P. sibirica has highly-active lactate dehydrogenase (LDH) and malate dehydrogenase (MDH); a predominance of MDH activity over LDH and a low activity of phosphoenolpyruvate carboxykinase is revealed. NADP-dependent isocitrate dehydrogenase is found, which is activated by Mn2+ and Mg2+ and inhibited by salts of heavy metals and p-chloromercuribenzoate. Activities and properties of α-ketoglutarate dehydrogenase, succinate dehydrogenase (SDH), and fumarate reductase are studied, and it is concluded that in P. sibirica there is the system of succinate oxidation, whereas the system of fumarate reduction into succinate is absent. Mitochondrial and microsomal fractions from P. sibirica had Mg2+- and Ca2+-dependent adenosine triphosphatases.

INTRODUCTION

For maintenance of their life, most organisms are known to utilize the energy released during dissimilation of organic compounds, first of all carbohydrates. The main pathway of carbohydrate catabolism in invertebrates is glycolysis resulted in transformation of glucose into pyruvic acid. In the cells, in which aerobic processes take place, this acid latter is dissimilated further to CO2 and H2O, while under anaerobic conditions in the process of fermentation, to various semi-oxidized products. Dissimilation of carbohydrates with formation of succinate or lactate is controlled by activity of two enzymes competing with each other: pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK), transforming the same substrate, phosphoenolpyruvate (PEP), either into pyruvate and further into lactate, or into oxalacetate (OA) and further into succinate and volatile fatty acids. Both these enzymes are located in cytosol. Their activity differs in various invertebrates, which indicates adaptation of metabolic pathways to environmental conditions. The ratio of the PK and PEPCK activities can be an indicator of direction of the carbohydrate metabolism. These two enzymes are not able to simultaneously function sufficiently actively; they are rather alternative to each other due condition of their catalytic action and regulatory connections [1]. Pyruvate, the final product of glycolysis under aerobic conditions, is oxidized by enzymes of pyruvate dehydrogenase complex with formation of CO2 and acetyl-CoA that is involved in the citric acid cycle (Krebs’ cycle). All reactions of the citric acid cycle are located in mitochondria, the cell “energy plants.” The key point of the energy metabolism is formation and hydrolysis of ATP molecules in mitochondria by adenosine triphos-
phatases (ATPases). Ginetsinskaya [2] believes the “phylogenetic proximity of trematodes and turbellariae is confirmed not only by similarity of their morphology, but also by biological peculiarities. The tendency for parasitism should be considered to be the most important features of the Rhabdocoela biology as the starting point for evolution of their ancestors towards trematodes. This allows us to share the current concept about the origin of trematodes from the common ancestor with rectal turbellariae.” Therefore, the goal of the present work was to study activity and properties of some enzymes of carbohydrate and energy metabolisms in the free-living turbellaria Phagocata sibirica (Turbellaria, Planariidae) inhabiting fast flowing mountain brooks near Vladivostok, in comparison with similar enzymes of parasitic trematodes.

MATERIALS AND METHODS

Turbellariae P. sibirica were delivered to laboratory in jars with brook water. To prepare enzyme extracts, the turbellariae were homogenized with 10 volumes of isolation medium by the method of Prichard and Schofield [3]. To obtain membrane fraction, mitochondria were sonificated in an ultrasonic MSE-disintegrator at 20 kHz twice for 1 min with 30 s interval at constant cooling of the mixture on ice. The mitochondrial membrane and mitochondrial soluble fractions were obtained by subsequent centrifugation of the suspension at 105 000 g for 60 min.

Concentrations of ions, substrates, cofactors, enzyme extract, buffer, and pH were chosen to provide the maximal reaction rate. Activity of hexokinase (HK) (EC 2.7.1.1) was determined by Govorova’s method [4]. The complete reaction mixture contained (mM): 33 Tris–HCl buffer (pH 8), 3.3 MgCl₂, 1.7 NaF, 0.1 NADP, 1.2 ATP, 4.0 glucose, excess of hexokinase-free G6PDG, the enzyme protein—not higher than 1 mg. The reaction was initiated by addition of glucose or ATP after preincubation of all components (except for glucose and ATP) for 5 min and terminated in 30 min. The protein content was determined by the method of Lowry et al. [5]. Activity of PK (EC 2.7.1.40) was determined by the method of Büchner and Pfeiderer [6] from changes of the reaction mixture optical density at 340 nm for 3 min, using an SF-16 spectrophotometer. The reaction mixture contained (mM): 50 Tris–HCl buffer (pH 7.5), 75 KCl, 2 ADP, 3 MnCl₂, 4 MgCl₂, 3 PEP, 0.2 NADH, 0.4 fructose bis-phosphate, 3.6 lactate dehydrogenase (LDH), the PE enzyme protein—0.4—0.6 mg. The reaction was initiated by adding the substrate. Michaelis constants (Kₘ) were determined graphically [7]. Activity of LDH (EC 1.1.1.27) was determined by the method of Kornberg [8]. The complete reaction mixture contained (mM): 50 imidazole buffer (pH 6.0), 5 sodium pyruvate, 0.24 NADH. The reaction was initiated by adding pyruvate after 5 min preincubation of all components at 30°C. Activity of malate dehydrogenase (MDH) (EC 1.1.1.37) in direct and reverse reactions was determined by the method of Prichard and Schofield [9]. The complete reaction mixture (final volume 3 ml) for direct reaction (malate → oxaloacetate (OA)) contained (mM): 54 triethanolamine buffer (pH 9.0), 0.2 NAD, 10 malate, 0.4—0.5 mg of protein; for reverse reaction (OA → malate)—(mM): 54 triethanolamine buffer (pH 9.0), 5 EDTA, 0.17 NADH₂, 40 OA, 0.4—0.5 mg of enzyme protein. Activity of NADP-dependent isocitrate dehydrogenase (IDH, EC 1.1.1.42) was determined by the method of Ochoa [10] from changes of optical density at 340 nm for 3 min using an SF-16 spectrophotometer in the medium containing (mM): 100 Tris, 1.5 MgCl₂, 1.5 MnCl₂, 0.2 NADP, 0.5 isocitrate, 0.3—0.5 mg enzyme protein. Activity of NAD-dependent IDH (EC 1.1.1.41) was determined by the same method, but 0.2 mM NAD was added into the sample instead of NADP, and ADP at concentrations from 0.2 up to 2 mM; other components of the medium were the same as at determination of NADP-dependent IDH. The reaction was initiated by adding the substrate. Activity of α-ketoglutarate dehydrogenase was determined as reported in [11] with use of potassium ferricyanide as synthetic electron acceptor in the medium containing (mM): 50 phosphate buffer (pH 7.5), 3 α-ketoglutarate, 2 KCN, 1 K₃Fe(CN)₆, enzyme protein. Activity of succinate dehydrogenase (SDH) (EC 1.3.99.1) was determined by the method of Slater and Banner [12] with potassium ferricyanide in the medium containing (mM): 50 sucrose-Tris–HCl buffer (pH 8.0), 1 K₃Fe(CN)₆,