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Localization of pyruvate carboxylase in the cells of *Neurospora crassa*

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Summary. The cell wall of *Neurospora crassa* was digested enzymatically and the cytosolic and the mitochondrial fractions were separated. The activity of pyruvate carboxylase (EC 6.4.1.1) was detected entirely in the cytosolic fraction. This indicates that the location of pyruvate carboxylase of *N. crassa* is in the cytosol, but is not in the mitochondria; this is different from the situation in animal tissues.

Pyruvate carboxylase is known to exist in a wide variety of organisms, including mammals[1,2], birds[1,3,4], and bacteria[5-9]. Fungal pyruvate carboxylase has been reported in *Aspergillus niger*[10], *Neurospora crassa*[11,12], *Penicillium camemberti*[13], *Rhizopus nigricans*[14] and yeasts[15-17]. In some cases, the enzyme was purified and molecular architecture was studied[4,6,8,9,15,17]. Pyruvate carboxylase in most animal tissues is chiefly a mitochondrial enzyme[1,18]. In yeast, on the other hand, this enzyme is reported to be localized in the soluble cytosol[16]. In the course of the study on the relationships between the *suc* gene and pyruvate carboxylase of *Neurospora crassa*, we noticed that the location of the enzyme in *Neurospora* is not in the mitochondria but in the cytosol.

Materials and methods. Mycelium of the wild type strain KG1967a of *Neurospora crassa* was grown in a 1.5 l Roux bottle containing 1 l of Vogel's minimal medium[19] supplemented with 2% sucrose and 0.3% sodium acetate under aeration at 34 °C for 1 day. About 8–9 g of wet mycelium were obtained from a bottle. Mitochondria were obtained by essentially the same method as that used by Greenawalt et al.[20]. Cell wall was digested by β-glucuronidase (crude solution from *Helix pomatia*) from Sigma. Wet mycelium weighing 8–9 g was treated in 33 ml of reaction medium containing sorbitol, 0.63 M; citric acid – K_2HPO_4 buffer (pH 5.8), 0.1 M with respect to citric acid; 2-mercaptoethylamine·HCl, 0.03 M; EDTA, 0.4 mM; and 300,000 units of β-glucuronidase for 1 h at 30 °C. Treated cells were washed twice with 60 ml cold, 0.9 M sorbitol, and collected by centrifugation.

The pellet was resuspended in 60 ml of 0.02 M potassium phosphate buffer, pH 7.5, containing 0.25 M sucrose, 5 mM EDTA and 0.15% bovine serum albumin (BSA buffer). The suspension was homogenized using a glass homogenizer with a Teflon pestle. The crude homogenate was diluted with BSA buffer to 150 ml and centrifuged at 1000 × g for 15 min to remove unbroken cells. The supernatant was centrifuged at 10,000 × g for 20 min, and its supernatant fraction (C1S) was recentrifuged at 180,000 × g for 25 min and the final supernatant was obtained (fraction C18S). The pellet obtained between 1000 and 10,000 × g, which contained essentially all mitochondria, was washed with BSA buffer and suspended in 12 ml of 0.02 M potassium phosphate buffer, pH 7.5 (fraction M1P). The suspension was treated with a Tomy model UR-150P sonicator for 1 min at the power setting of 5. The treated suspension (fraction M1P-S) was again centrifuged at 180,000 × g for 25 min and the supernatant was obtained (fraction M18S).

Pyruvate carboxylase activity was determined spectrophotometrically by the citrate synthase-coupled method described by Payne and Morris[5]. Citrate synthase activity was determined by the method of Parvin[21]. Fumarate hydratase activity was determined by the method of Hill and Bradshaw[22], measuring

Activities of the 3 enzymes in various preparations from disrupted *Neurospora* cell. The naming for each preparation is described in the text

	Preparation	Specific activity (μmol mg^{-1} h^{-1})	Total protein (mg)	Total activity (μmol h^{-1})
Pyruvate carboxylase from 9.1 g wet mycelium	C1S	1.80	221	398
	C18S	2.23	161	359
	M1P	0	54	0
	M1P-S	0	49	0
	M18S	0	26	0
Citrate synthase from 8.1 g wet mycelium	C1S	37.6	184	6920
	C18S	43.0	148	6360
	M1P	37.1	63	2340
	M18S	221.4	31	6860
Fumarate hydratase from 8.1 g wet mycelium	C1S	59.0	184	10860
	C18S	70.8	148	10480
	M1P	57.7	63	3640
	M18S	268.8	31	8330

the absorbance at 240 nm. Specific activity of each enzyme was expressed as µmoles of product per mg protein per h. Protein was determined by the method of Lowry et al.[23].

Results and discussion. Activities of pyruvate carboxylase and the two mitochondrial enzymes, citrate synthase and fumarate hydratase, in various preparations from the *Neurospora* cell are shown in the table. Most of the pyruvate carboxylase activity was found in the cytosolic fractions, C1S and C18S, whereas no activity was detected in the mitochondrial fractions, M1P, M1P-S and M18S. Pyruvate carboxylase of animals and some bacteria requires acetyl-CoA[3,5,6]. Pyruvate carboxylase of *Neurospora* shows its activity in the absence of acetyl-CoA[11,12]. Although the present assay system contains acetyl-CoA as a component of the reaction mixture, the mitochondrial fractions did not show the activity of pyruvate carboxylase. On the other hand, citrate synthase and fumarate hydratase activities were found in both the mitochondrial and the cytosolic fractions. Although some of this activity could have been due to disruption of mitochondria during the preparation, it is obvious that mitochondria contain citrate synthase and fumarate hydratase. Mitochondrial inner membrane is permeable to pyruvate, malate and aspartate, but not to oxalacetate. In animal cells, a shuttle mechanism works for the transmission of oxalacetate out of the mitochondria for gluconeogenesis[24], because pyruvate carboxylase is localized only in the mitochondrial matrix[18]. While predominance of the gluconeogenic role of the enzyme in vertebrates is indicated[3,25], the primary role of the enzyme in microorganisms is believed to be anaplerotic, that is the production of oxalacetate from pyruvate for both the operation of the TCA cycle and the biosyntheses of carbon compounds[15,26]. The present study shows that the pyruvate carboxylase of *Neurospora crassa* is localized outside the mitochondria. Therefore oxalacetate is formed outside the mitochondria and a shuttle mechanism is not necessary for the gluconeogenesis. Rather, oxalacetate must be transferred from the cytosol into mitochondria for supplementation of the substrate of the TCA cycle. The fact that pyruvate carboxylase of *Neurospora* and yeast exists outside the mitochondria indicates that the regulation of organic acid metabolism in fungi is different from that in animal cells.

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Purification and properties of ornithine aminotransferase from rat brain

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Summary. Ornithine aminotransferase (E.C. 2.6.1.13) from rat brain was purified 100-fold by ammonium sulphate fractionation, DEAE cellulose chromatography, calcium phosphate gel and alumina C γ gel. Pyridoxal phosphate was essential for maximum activity of the enzyme. The brain enzyme did not differ from liver and kidney enzymes in properties such as pH optimum, K_m, substrate specificity and the inhibition by branched chain amino acids. Unlike rat liver enzyme, brain ornithine aminotransferase was able to catalyze the reaction between L-lysine and 2-oxoglutarate. Spermidine and spermine inhibited brain ornithine aminotransferase activity.

Ornithine aminotransferase (OAT; E.C. 2.6.1.13) is a mitochondrial enzyme present in many tissues including liver, kidney and brain[2]. It is the only enzyme known to catalyze the reversible step in the metabolic pathway interconverting arginine, proline and glutamate[3]. In the rat liver, OAT is mainly involved in ornithine catabolism[4]. The metabolic role of this enzyme in other tissues has not been completely identified. Comparative data obtained from the purified enzymes from rat liver and kidney indicated that the two proteins are identical[5,6]. Although OAT from liver and kidney has been characterized in detail, very little is known about the properties of OAT from brain. In the present