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Localization of Neurospora ornithine aminotransferase in mitochondria

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Abstract

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Fed. Proc. 30: 1238; Tsai and Tsai 1972 Z. Physiol. Chim. 353: 1573).

Ornithine aminotransferase (OAT) (EC 2.6. 1. 13) occurs widely. In mammalian tissues this enzyme is exclusively localized in the mitochondrial matrix (Peraino and Pitot 1963 Biochim. Biophys. Acta 73: 222; Gamble and Lehninger 1971 The subcellular location of this enzyme in Neurospora is not known. Recently there were some indications in the literature (e.g., 1972 Science 178: 840; 1972 Neurospora Newsl. 19:

12), suggesting that the Neurospora OAT is "non-mitochondrial". The results reported in this communication, however, show that the enzyme is present in the freshly prepared mitochondria of Neurospora.

Table 1. Localization of ornithine aminotransferase in mitochondria.

Ept.	No.	Preparation	OAT activity (mg/ml)
I		Purified mitochondria suspension (32 mg/ml AMT-sucrose)	232
II		Post-mitochondrial supernatant *	69
III		Mitochondrial pellet, resuspended • *	212

• Same as Exp. I, but mitochondria removed by centrifugation at 1200 x g for 10 min. ** Mitochondriol pellet from Exp. II was resuspended in the same volume of AMT-sucrose.

The wild-type N. crassa (strain Em 5256) was used in there experiments. The mitochondria were isolated and purified by the procedure described elsewhere (Küntzel and Schäfer 1971 Nature New Biol. 231: 265). The purified mitochondria were suspended in AMT-sucrose, which is composed of 0.44 M sucrose containing 100 mM NH_4Cl, 10 mM $MgCl_2$ and 10 mhl Tris-HCl (pH 7.5). The assay of OAT was &formed according to Jenkins and Tsai (1970 Methods in Enzymology 17A: 281), except that 10 μg of Lubrol WX were included in the assay mixture (final volume = 1.0 ml).

As shown in Table 1, the OAT activity co-sedimented with mitochondria when it was centrifuaed in AMT-sucrose at *12,000* x g for 10 min. In these sties, we have also observed that OAT in the Neurospora mitochondria is very unstable, in the sense that it lores more than 50% of the initial activity during overnight storage at 5°C. Whereas the present results ore consistent with those obtained from mammalian systems, the question whether the OAT activity observed in the cytoplasmic fraction of Neurospora is authentic or is on artifact due to the leakage from mitochondria has not been examined.

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