Rat Liver Mitochondrial Monoamine Oxidase, a Flavin-Containing Enzyme

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Mitochondrial monoamine oxidase [monoamine-oxygen, oxidoreductase (deaminating), EC 1.4.3.4] of rat liver was purified (Youdim & Sourkes, 1966). The enzyme was pale yellow in colour. When the difference absorption spectrum (oxidized minus reduced) was determined, peaks at 405 and 460 nm appeared. The absorption at 460 nm suggests a flavin moiety. Erwin & Hellerman (1967) reported a similar finding with bovine kidney enzyme. The fluorescent emission and activation spectra of the purified enzyme resembles that of authentic FAD. However, some preparations of the enzyme did not fluoresce, probably owing to the impurity of the enzyme. Treatment of the enzyme with 10% (w/v) trichloroacetic acid resulted in the release of a small amount of flavin-like material. If the purified preparation (1.23 mg/ml) is treated with pronase (1.5 mg/ml) in 0.05 M-phosphate buffer, pH 7.4, at 37°C, then a material fluorescing at 520 nm (activation at 460 nm) is found. The release of this fluorescent material is progressive in the course of digestion with pronase over 4 h. There are losses of fluorescence after 8 h (H. Birnbaum, M. B. H. Youdim & T. L. Sourkes, unpublished work; cf. Sourkes, 1968). This fluorescent material is identical with FAD as regards fluorescent emission and activation. One mol of flavin is associated with 150 000 g of the enzyme. Similar results, starting with enzyme purified from organs of other species, have been reported by Erwin & Hellerman (1967), Tipton (1968) and Gomes, Igaue, Kloeppfer & Yasunobu (1969). The flavin obtained from pronase-treated enzyme and standard solutions of authentic FAD and FMN were subjected to silica-gel t.l.c. in a dark room. After development in butanol-acetic acid-water (3:2:1, by vol.) the chromatogram was dried and examined under u.v. light. The flavin extracted from enzyme showed a major fluorescent spot, with some trailing, behind FAD. No fluorescent material running with FMN could be detected. When the plate was treated with ninhydrin, the enzyme-extracted flavin spot gave a positive reaction, whereas FAD and FMN did not.

The treatment of rats with galactoflavin (2 mg/kg of diet), an anti-metabolite of riboflavin, up to 42 days resulted in the loss of monoamine oxidase

activity in the liver and brain of about 81 and 33% respectively.

From these observations it is concluded that rat liver monoamine oxidase is associated with FAD.

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The Kinetic Properties of Human Placental Choline Acetyltransferase

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Previous studies on the mechanism of choline acetyltransferase (EC 2.3.1.6) have yielded conflicting results. Schuberth (1966) obtained kinetics with human placental enzyme characteristic of a 'Ping Pong' mechanism in which a central acetyl-enzyme complex is formed from acetyl-CoA (in the forward direction) or from acetylcholine (in the reverse direction) independently of the presence of the second substrate (choline or CoA). Potter, Glover & Saelens (1968), on the other hand, obtained evidence of a sequential mechanism for the rat brain enzyme in which both substrates must interact with the enzyme before any products are released.

A study of isotopic exchange between acetylcholine and [*Me*-¹⁴C]choline catalysed by the placental enzyme (Morris & Grewaal, 1969) showed that the 'Ping Pong' mechanism could not be correct since the exchange occurred only in the presence of CoA, a result consistent with a 'sequential' mechanism. The present work is a reinvestigation of the kinetic behaviour of the placental enzyme, which has been extended to include product-inhibition studies.

Choline acetyltransferase activity was assayed by using [*acetyl*-¹⁴C]CoA, by a procedure (D. Morris, A. Maneckjee & C. O. Hebb, unpublished work) modified from that described by McCaman & Hunt (1965) and Schrier & Shuster (1967). Double-reciprocal plots of the initial velocity of acetylcholine synthesis versus the concentration of one

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