acid oxidation in the case of the ethionine experiment, to form ethionine sulphone) on the amino acid analyser showed no trace of the analogues. It is possible that the analogues were metabolysed in other ways[12]. Incorporation of ethionine into peptidase A, making it biologically inactive, is ruled out since the enzyme does not contain sulphur amino acids[4].

Résumé. La DL-éthionine et la *p*-fluorophénylalanine inhibent la formation de la peptidase A extracellulaire chez le *Penicillium janthinellum* sans affecter la synthèse de la protéine cellulaire.

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Identification of Flavin in the Purified Beef Brain Mitochondrial Monoamine Oxidase

Several reports have appeared concerning the physicochemical properties of mitochondrial monoamine oxidase [EC 1.4.3.4. monoamine : oxygen oxidoreductase (deaminating)]. NARA, GOMES and YASUNOBU[1] first reported that beef liver mitochondrial monoamine oxidase is flavo-enzyme. IGAUE, GOMES and YASUNOBU[2] further reported that the flavin in beef liver mitochondrial monoamine oxidase is covalently attached to the enzyme and catalytically involved in the enzyme reaction. ERWIN and HELLERMAN[3] also reported that beef kidney mitochondrial monoamine oxidase contains flavin which is tightly associated with the protein. However, there has been little knowledge about the prosthetic group of brain mitochondrial monoamine oxidase because of the difficulty in its solubilization and purification. TIPTON[4] has reported the extraction of the enzyme from pig brain mitochondria by the combined effects of sonication and freezing-thawing and the extensive purification. TIPTON[5] has also shown that the prosthetic group of pig brain mitochondrial monoamine oxidase is FAD which is dissociable from the protein.

One of the authors showed that the enzyme can be extracted from beef brain mitochondria by sonication in the presence of a detergent[6]. We also found that the enzyme can be extracted by the use of a detergent after the mild heat treatment of beef brain mitochondria[7]. Present communication describes purification of monoamine oxidase from beef brain mitochondria and the identification of the prosthetic group as FAD.

Monoamine oxidase activity was measured by the disappearance of kynuramine[8]. Protein was determined by the method of LOWRY et al.[9]. Beef brain mitochondria were prepared according to the method of BRODY and BAIN[10]. The mitochondrial suspension in 0.01 *M* potassium phosphate buffer, pH 7.4 (10 mg protein/ml) was incubated at 40 °C with continued stirring for 10 min. After cooling the suspension in an ice bath, a 5% (w/v) aqueous solution of Nonion NS-210 (polyoxyethylene-nonyl phenol ether-containing detergent, Nippon Oils & Fats Co. Ltd., Tokyo) was added dropwise to 1% final concentration with gentle stirring in an ice bath. 30 min of equilibration with stirring was allowed. The suspension was centrifuged at 106,000 × g for 1 h and the clear supernatant layer was pipetted out carefully. To the supernatant was added saturated ammonium sulphate solution (pH 7) to 30% saturation. The suspension was then centrifuged at 9,500 × g for 15 min. The precipitate rose to the surface. The solution beneath precipitate was aspirated and discarded. The precipitate was redissolved in 0.01 *M* phosphate buffer (pH 7.4) and extensively dialyzed against the same buffer. The buffer was changed 3 times during the 15 h dialysis. The dialyzed solution was centrifuged, and the supernatant was applied to a DEAE-cellulose column equilibrated with 0.01 *M* phosphate buffer (pH 7.4). Stepwise elution was carried out with 0.01 *M* and 0.1 *M* phosphate buffers and then 0.1 *M* phosphate buffer containing 0.4% Nonion NS-210. The monoamine oxidase activity was eluted with 0.1 *M* phosphate buffer containing the detergent. The active fraction was concentrated by the addition of saturated ammonium sulphate solution (pH 7) to 30% saturation. The precipitate obtained by centrifugation was dissolved in 0.01 *M* phosphate buffer (pH 7.4) and then dialyzed against the same buffer. The dialyzed enzyme solution was subjected to calcium phosphate gel[11]-cellulose powder (gel/powder, 1:5, w/w) column chromatography. The column was equilibrated with 0.01 *M* phosphate buffer (pH 7.4). The elution procedure was the same as in the DEAE-cellulose chromatography. The enzyme was also eluted with 0.1 *M* phosphate buffer containing 0.4% Nonion NS-210 (pH 7.4). The enzyme solution was faintly yellow.

A typical example of the purification is summarized in the Table. Analysis of the purified enzyme preparation by disc electrophoresis showed that it was nearly homogeneous. However, the purification was only 28-fold as shown in the Table. This low value of the increase in the specific activity may be due to inactivation of the enzyme during the purification procedure, especially at the stage of ammonium sulphate fractionation. Freezing of the enzyme preparations after the extraction from beef brain mitochondria caused rapid loss of the enzyme activity.

The absorption spectrum of the purified enzyme exhibited a maximum at 410 nm. The spectrum was nearly identical with that of purified rat liver mitochondrial enzyme reported by YOUDIM and SOURKES[12]. Fluorescence activation spectrum of the native enzyme measured at 530 nm with Aminco-Bowman spectrophotofluorometer

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showed maxima at 280 nm, 370 nm, and 460 nm. Fluorescence emission spectrum of the purified enzyme measured by the excitation at 450 nm showed a shoulder at the vicinity of 520 nm (Figure 1). These fluorescence activation and emission spectra were similar to those of FAD. The shoulder at the vicinity of 520 nm of the fluorescence emission spectrum was decreased by the addition of a substrate, benzylamine (Figure 1). Fluorescence emission spectrum of the supernatant of the enzyme solution boiled with zinc sulphate at pH 7 showed a distinct peak at 520 nm, indicating the liberation of a fluorescent prosthetic group from the holoenzyme. The fluorescent material in the supernatant was extracted by the method of YAGI[13], and analyzed by thin-layer chromatography. The supernatant yielded a single yellowish green fluorescent spot, which Rf value coincided with that of authentic FAD (Figure 2).

These results indicated that beef brain mitochondrial monoamine oxidase has FAD as its prosthetic group which is involved in the enzyme reaction, and that the FAD can be fairly easily separated from the enzyme protein. This finding agrees with the report by TIPTON[4,5] that FAD is the prosthetic group of purified pig brain mitochondrial monoamine oxidase[14].

Purification of monoamine oxidase of beef brain mitochondria

Preparation	Total activity nm*M*/min	Protein mg	Specific activity nm*M*/min per mg protein
Homogenate	9600	9600	1.0
Mitochondria	7060	4600	1.5
Extracted supernatant	2800	1430	2.0
Ammonium sulphate fractionation (0–30%)	840	290	2.9
DEAE-cellulose chromatography	510	36.1	14.1
Calcium phosphate gel-cellulose powder chromatography	300	10.6	28.3

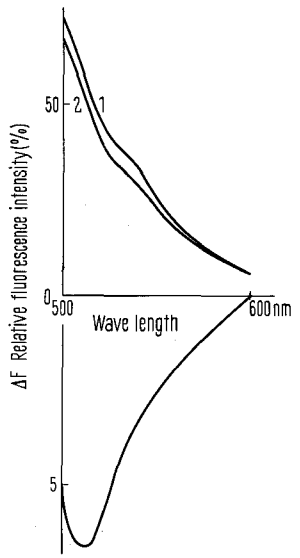

Fig. 1. Fluorescence spectrum change of beef brain mitochondrial monoamine oxidase by the addition of substrate. (1) Native enzyme; (2) native enzyme in the presence of 1.7 m*M* benzylamine.

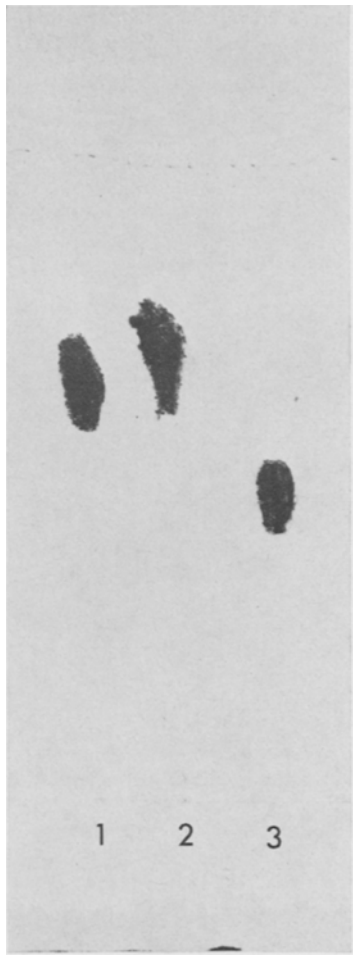

Fig. 2. Thin-layer chromatogram of the flavin in the supernatant after boiling the solution of the purified beef brain mitochondrial monoamine oxidase. Solvent used was acetic acid/pyridine/H_2O (1:10:40, v/v/v). Yellowish green fluorescent spots of flavins were examined under ultraviolet light. (1) Authentic FAD; (2) sample; (3) authentic FMN.

Zusammenfassung. Die Monoaminoxydase in den Mitochondrien des Rinderhirns wurde gereinigt. FAD wurde durch Fluoreszenzspektrum und Dünnschichtchromatographie als prosthetische Gruppe in den gereinigten Präparaten identifiziert.

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