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A simple micro-determination of type B monoamine oxidase

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The existence of type A and B monoamine oxidase [monoamine: oxygen oxidoreductase (deaminating), EC 1.4.3.4] (MAO) is demonstrable both *in vivo* and *in vitro* by their substrate specificity and their selective inhibition [1–3]. Type A MAO preferentially deaminates 5-hydroxytryptamine and norepinephrine, while type B MAO deaminates β-phenylethylamine (PEA) and benzylamine. For the determination of type A MAO, simple and sensitive methods are available [4, 5], and for that of type B MAO, spectrophotometric method using benzylamine as substrate [6] and fluorometric method using PEA as substrate [7] are available. However, the method for the determination of type B MAO using benzylamine is not highly sensitive and that using PEA involves an extraction procedure with organic solvent. Therefore, we have intended to obtain a more simple and sensitive method. The present paper deals with the details of a new, simple and sensitive method for the determination of MAO activity towards PEA by measuring phenylacetaldehyde, the reaction product from PEA catalyzed by MAO.

It has been shown by Samejima *et al.* [8, 9] that phenylacetaldehyde reacts with ninhydrin and a primary amine to yield a highly fluorescent compound, and this can be adopted for a sensitive measurement of phenylacetaldehyde. However, the substrate, PEA, can also react with ninhydrin to yield the same compound [10]. Accordingly, the reaction of PEA with ninhydrin should be suppressed. For this purpose, our previous finding that PEA does not react with ninhydrin below pH 6 [11] can be successfully adopted.

In the case of crude enzyme preparation such as tissue homogenate, the enzymes which destroy phenylacetaldehyde should be inhibited. The responsible enzymes are aldehyde dehydrogenase [12], aldehyde oxidase [13], aldehyde reductase and alcohol dehydrogenase [14]. We tested the inhibitors of each enzyme, *viz.* chloral hydrate, sodium azide, sodium phenobarbital and pyrazole, by adding them to rat brain and liver homogenates. However, only the addition of chloral hydrate to liver homogenate was effective, and the other inhibitors were not effective. It was confirmed that chloral hydrate has no effect on MAO activity.

When tissue homogenate was used as an enzyme source, the recovery of phenylacetaldehyde was found to be rather low. Taking into account the fact that aldehyde derivatives are adsorbed onto brain macromolecules [15], the low recovery was considered to be ascribed to similar adsorption phenomenon in the tissue homogenate, and we tried to avoid such adsorption by adding organic solvents. Among the organic solvents tested, *viz.* acetone, methanol and ethanol, methanol was found to be adoptable, since acetone inhibits ninhydrin reaction and ethanol causes turbidity, even though they seem to be effective.

The fluorescence excitation and emission spectra of the fluorescent compound obtained from the product formed from PEA by liver homogenate were found to be identical with those of the compound obtained from authentic phenylacetaldehyde, having the excitation and emission maxima at 390 nm and 495 nm, respectively.

On the basis of the above data, we propose the following procedure as a standard method for the determination of type B MAO in animal tissues.

Tissue was homogenized with 0.9% NaCl solution in a glass homogenizer fitted with a Teflon pestle. The incubation was carried out in a small centrifuge tube. To 0.5 ml of the homogenate containing 1–10 mg of the tissue, were added 0.15 ml of 0.5 M sodium phosphate buffer (pH 7.4), 0.1 ml of 0.05 M chloral hydrate solution, and 0.5 ml of PEA solution containing 50 μg (final concentration, 0.25 mM) of PEA–HCl. After incubation at 37° for 30 min, the enzyme reaction was stopped by adding 0.1 ml of 30% trichloroacetic acid, then mixed with 2 ml of methanol, shaken vigorously for 2 min and centrifuged (3,000 rpm, 5 min). A 0.5-ml aliquot of the supernatant was incubated with 1.5 ml of 0.5 M sodium phosphate buffer (pH 5.7), 0.5 ml of 0.05 M ninhydrin solution and 0.5 ml of 3 mM L-leucyl-L-alanine solution at 60° for 60 min in an oil bath, and left at room temperature for 15 min. The fluorescence intensity of the reaction product was measured with excitation at 390 nm and emission at 495 nm. As a blank test, the reaction mixture without substrate was incubated, and mixed with trichloroacetic acid and the same amount of PEA solution. Internal standard was taken by adding an appropriate amount of phenylacetaldehyde (Aldrich Chemical Co., Milwaukee, Wis.) to the assay mixture before incubation to permit direct calculation of results. The recovery of phenylacetaldehyde was over 80 per cent.

Figure 1 shows the relationship between MAO activity and the enzyme concentration with liver and brain homogenates as enzyme sources. The linear relationship was observed between MAO activity and the amount of enzyme in the range from 2.5 mg to 10 mg of the tissues in the assay mixture.

Figure 2 shows that there is a linear relationship

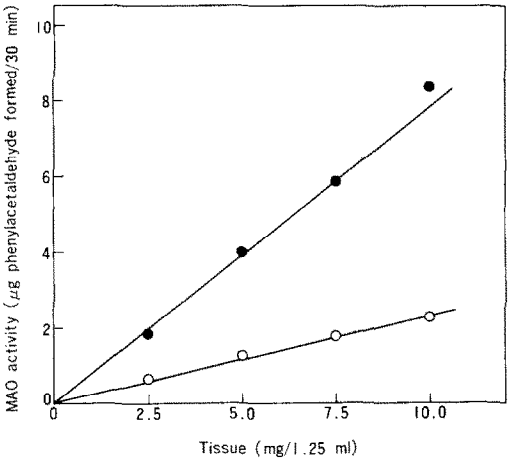

Fig. 1. Relationship between MAO activity and enzyme concentration. Solid circles: rat liver homogenate; open circles: rat brain homogenate. Assay method is described in the text.

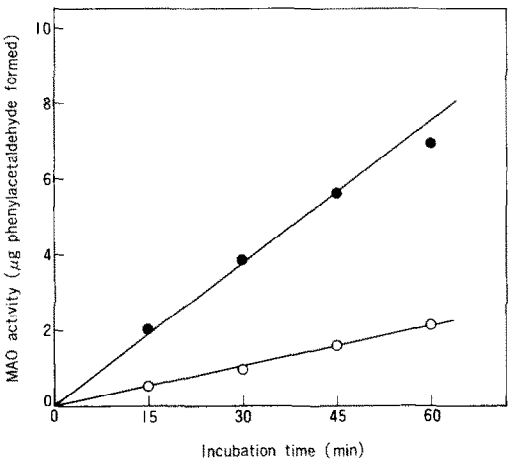

Fig. 2. Relationship between MAO activity and incubation time. The reaction mixture (1.25 ml) contained 5 mg of fresh tissue. Solid circles: rat liver homogenate; open circles: rat brain homogenate. Assay method is described in the text.

Table 1. MAO activity of selected tissues

Tissue	MAO activity (μg phenylacetaldehyde formed/g wet wt/30 min)	
Rat adrenal gland	404 ± 24	(2.4–3.4)
Rat pineal body	768 ± 106	(1.7–2.2)
Chick retina	79 ± 24	(5.1–8.4)

Values were obtained from 3 experiments. Means ± S.D. are given. Values in parentheses indicate the amount of tissue (mg wet wt) in the assay mixture (1.25 ml).

between MAO activity and incubation time for at least 45 min.

Using this method, MAO activities in small tissues were determined. The results are shown in Table 1.

As a criterion of the enzyme specificity, pargyline (4×10^{-4} M), a potent inhibitor of MAO, was added to the assay mixture and the complete inhibition of the enzyme reaction was observed.

The present method can be applied to a wide variety of animal tissues. However, if particular tissue contains strong activities of the above-mentioned phenylacetaldehyde-destroying enzymes, the recovery of the internal standard becomes low. In this case, appropriate inhibitors should be added. When some drugs such as EDTA and potassium cyanide are added into the reaction mixture, they disturb the ninhydrin reaction. In such cases, our previous method for the determination of MAO by measuring PEA disappearance [7] should be used.

The present method made it possible to determine type B MAO activity using PEA as substrate even with a small amount of samples (1–10 mg of wet tissue). As little as 0.2 μg of phenylacetaldehyde formed in the assay mixture could be measured accurately.

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