STUDIES ON MONOAMINE OXIDASE (REPORT LXXVII)
EFFECTS OF pH ON MAO IN RABBIT SERUM

Yuichiro ARAI, Tadamasa UESATO, Sadayuki SHO and Kazuya KAMIJO
Department of Pharmacology, School of Medicine, Showa University,
1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan

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There are numerous reports on the multiplicity of mitochondrial monoamine oxidase (MAO) in various tissues and the existence of at least two types of MAO, type A and type B, in mitochondria is now supported by results using specific inhibitors such as clorgyline and deprenyl (1, 2). In 1962, Yamada and Yasunobu purified the soluble MAO from bovine serum, separated three different MAO activities by column chromatography (3) and suggested the existence of isoenzymes of MAO. In addition, four other MAO activities were separated by chromatography of serum from patients with hepatic diseases (4, 5).

In this work, we studied the effects of pH, inhibitors and heat treatment on rabbit serum MAO.

Blood obtained from the rabbit carotid artery was centrifuged at 3,000 rpm for 10 min. The serum thus obtained was stored frozen at -20°C until use. MAO activity was measured by three methods: (i) by photometric assay of production of benzaldehyde from benzylamine following the method of Nakagawa et al. (6), which is a slight modification of the method of McEwen and Cohen (7); (ii) by measuring the radioactivity of 14C-benzaldehyde produced from 14C-benzylamine as described by Wurtman and Axelrod (8), and (iii) by manometric measurement of O2 consumption during oxidation of benzylamine for one hour at 38°C as described by Kamijo et al. (9). 0.1 M phosphate buffer was used. The reaction mixtures containing 0.1 M phosphate buffer were acidified with 0.1 M HCl at pH 5 or less and alkalized with 0.1 M NaOH at pH 9 or more.

Measurement of MAO activity by the photometric assay procedure (Fig. 1), showed that the pH-activity curve of rabbit serum MAO had two distinct peaks at pH 6 and pH 8. Similar biphasic pH-activity curves were obtained on radiometric and manometric determination of MAO activity. These results suggest that rabbit serum MAO has two distinct pH optima as pH 6 and pH 8, or that the serum contains two MAO enzymes with optima at pH 6 and pH 8, respectively.

Next we examined the effects of MAO inhibitors on the pH-activity curve of rabbit serum MAO. As shown in Fig. 2, the biphasic pH-activity curve of rabbit serum MAO was not influenced by clorgyline (10^-4 M), a specific inhibitor of type A MAO, or deprenyl (10^-4 M), a specific inhibitor of type B MAO. These results suggest that rabbit serum MAO does not contain any type A or type B mitochondrial MAO. Semicarbazide (10^-4 M) inhibited rabbit serum MAO activity by 93%, 83% and 73% at pH 6, 7 and 8, respectively. Cuprizone (10^-4 M) also inhibited rabbit serum MAO activity by 86%, 44% and 51% at
pH 6, 7 and 8, respectively. Thus, both inhibitors inhibited the activity more strongly at pH 6 than at pH 7 or 8. However, almost the same inhibitions at any pH were obtained with pargyline, KCN, phenylhydrazine, harmine, tranylcypromine, diethyldithiocarbamate, o-phenanthroline, 8-hydroxyquinoline, EDTA, 2,2'-dipyridyl imidinoacetic acid and acetylacetone.

Next the effects of heat treatment of rabbit serum MAO at 65°C and 70°C were examined. It was found that the decrease of activity was proportional to the preincubation time at both temperatures and that the inactivation by heat treatment was greater at pH 6 than at pH 7 or 8. For example, about 50% inactivation was found at pH 6 while only 25% inactivation was found at pH 7 or 8 after 4 min preincubation at 70°C.

Three forms of MAOs from human and bovine serum (10, 15) and four forms of MAOs from pig serum (11) have been separated by electrophoresis or chromatography, but there are no reports on the multiplicity of rabbit serum MAO. The optimum pH or purified rabbit serum MAO was reported to be pH 8 (12), but in this work we found that rabbit serum MAO had a biphasic pH-activity curve with maxima at pH 6 and 8. It has been reported
that cuprizone and o-phenanthroline strongly inhibited bovine serum MAO (13), and that cuprizone and sodium azide inhibited human serum MAO (10). In the present work, we found that cuprizone and semicarbazide inhibited rabbit serum MAO more strongly at pH 6 than at pH 7 or 8. On the other hand, it has been reported that the pH-activity curve of human serum MAO has a sharp peak at pH 7.2, which changed to a broad peak at pH 7-8 on heat treatment (14). It has also been reported that three MAOs isolated from human serum have different heat stabilities, and thus that there may be multiple forms of MAO in human serum (15). We found that MAO in rabbit serum was also influenced by heat treatment and had different heat stabilities at pH 6 and 8.

The biphasic pH-activity curve, the differences in inhibition at pH 6 and 8 by various MAO inhibitors and the different effects of heat treatment at pH 6 and 8 observed in this work suggest that there are at least two distinct forms of MAOs in rabbit serum.

REFERENCES

13) YAMADA, H. AND YASUNOBU, K.T.: Monoamine oxidase II. Copper, one of the prosthetic groups of plasma monoamine oxidase. J. biol. Chem. 237, 3077–3082 (1962)