EFFECTS OF METAL SUBSTITUTION ON PIG LIVER MONOAMINE OXIDASE

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SUMMARY. Monoamine oxidase (MAO) from pig liver has been isolated, purified and its native copper(II) removed and replaced by iron(II), nickel(II) or cobalt(II). The metals are seen to effect various kinetic properties. The apparent K_m's at 30°C and pH 9.0 are 1.25 μM, 1.77 μM, 2.17 μM and 3.03 μM for the Co(II), Cu(II), Ni(II) and Fe(II) versions of MAO. The Co(II) monoamine oxidase is approximately one-third more active than the Cu(II) MAO at 30°C in .05 M glycine buffer, pH 9.0. Conversely, the Ni(II) and Fe(II) MAO's possess approximately one-half the activity of normal Cu(II) MAO under the same conditions.

INTRODUCTION. Pig liver mitochondrial monoamine oxidase (MAO) has been isolated and characterized (1,2). This enzyme contains copper, and its removal (2) results in complete loss of enzymatic activity with respect to benzylamine. In our previous work, we were able to restore varying degrees of activity by the removal of copper(II) and its replacement with various divalent metals. In this communication we report the effects of metal substitution on certain enzymatic properties using atomic absorption to determine metal ion content.

MATERIALS AND METHODS. The enzyme assay with benzylamine as substrate was carried out at 30°C as described by Tabor, et al. (3) at 250 nm. The buffer system was .05 M glycine (pH 9.0) containing 1 mm EDTA. Enzyme was prepared from freshly slaughtered pigs as previously described (1,2). Having obtained MAO of specific activity 3,000 (2), we proceeded with the metal substitution at 4°C. First, about 5 ml. of highly purified MAO was dialyzed

overnight against 1 mM sodium diethyldithiocarbamate in .05 mM glycine buffer for six hours, to remove Cu(II). The copper free MAO was then dialyzed against .05 M glycine buffer for six hours to remove excess chelating agent. After this, the enzyme was dialyzed against the divalent metal (Fe, Ni or Co) at a metal concentration of 1 mM in .05 M glycine buffer (pH 9) with the exception of Fe(II). In order to replace Cu(II) with Fe(II), we dialyzed the enzyme against the Fe(II) solution at .05 M glycine buffer solution, pH 7. The metal substituted enzymes were then dialyzed against pH 9 glycine buffer solution containing 1 mM EDTA for two to three hours to remove any excess metal.

A Perkin-Elmer Model 403 atomic absorption spectrophotometer equipped with a deuterium background corrector, a rapid-response strip chart recorder, and Westinghouse single hollow cathode lamps were used for this study. The burner assembly was replaced by a Perkin-Elmer HGA-2000 graphite furnace without further modification. Eppendorf microliter pipets having disposable plastic tips were used for sample introduction.

All reagents were of analytical grade. Deionized water was used for all sample preparations. The stock solutions were a 1000 ppm (1000 μg/ml) certified atomic absorption standard obtained from Fisher Scientific. Intermediate standards were prepared by pipetting the required amount of the stock solution into the appropriate container which was then accurately brought to volume with deionized water.

An aliquot of the test sample was introduced into the graphite tube furnace. The sample was then dried for 30 seconds, charred for 30 seconds and atomized for 5 seconds at the temperatures listed in Table I. The graphite furnace was operated with a nitrogen purge and a water flow of approximately 1.0 and 3 liters/minute, respectively. Standard graphite tubes were employed.

RESULTS AND DISCUSSION. The results of two typical metal analyses are given in Table I. The variation in results from sample to sample is as ex-

TABLE I: RESULTS OF METAL ANALYSIS

Metal	Enzyme Concentration (mg/ml)	Metal Concentration (μg/ml)	Number of Atoms per mole of Enzyme
Co(II)	3.30	1.10	6.72
	4.30	1.59	8.70
Ni(II)	2.91	1.00	6.92
	5.42	1.80	6.77
Fe(II)	1.73	.50	5.45
	4.02	1.59	7.49
Cu(II)	1.79	.22	2.32
	3.50	.34	1.83

The above data indicate that there are between six and eight atoms of metal in each molecule of the modified enzyme, while there are only two atoms of Cu(II) per molecule of MAO. There results suggest a maximum of eight metal sites per mole of enzyme and are consistent with MAO being an octamer (1,2).

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pected, particular, the copper content remains in the vicinity of two atoms per mole of MAO regardless of whether or not native MAO or Cu(II) re-constituted MAO is used. This latter result is in conflict with our earlier value of eight copper atoms per mole (1,2) determined by less sensitive chemical methods, however, it is similar to the value obtained for beef liver mitochondrial MAO (4).

The atomic absorption analysis further indicated only trace amounts of copper, manganese and iron present in samples where these metals were not the major element. Furthermore, no lead or cadmium was detected in any of these samples.

In view of the apparent success of the metal substitution, a pH vs. activity study was completed with the results in Figure 1. The results were obtained using identical protein concentrations in every assay

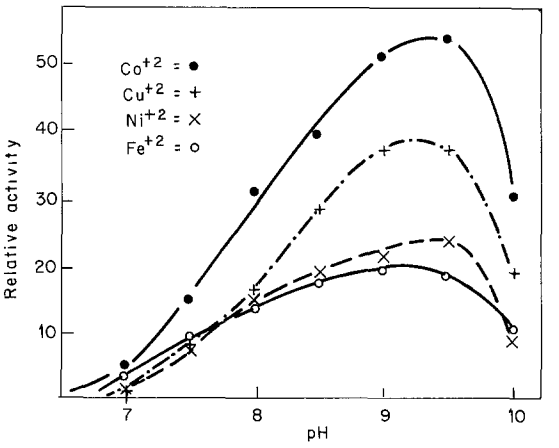

Fig. 1. Relative activity vs. pH for MAO reaction with benzylamine at 30°C in .05 M phosphate buffer.

(E_o = .083 μM). The results are an average of those obtained from the samples analyzed in Table I. There is only a slight shift in the pH maximum, however, the variation in relative activity is considerable.

We determined apparent K_m values for the various metal substituted enzymes at 30° using benzylamine as substrate with .05 mM glycine buffer solution (pH 9). The results are shown in Figure 2. The native enzyme in which Cu(II) metal is necessary for enzymatic activity has a value of 1.77×10^{-6} M. A value of 1.28×10^{-6} M (pH 9.0, 25°C) was reported previously (1,2). Other workers have reported K_m values of 5×10^{-6} M (pH 7.2) for human plasma, (5) 8.4×10^{-5} M (pH 7.8) for rabbit serum, (6) and 2.2×10^{-4} M (pH 7.4) for beef liver monoamine oxidase. (7)

The K_m values for the various metal substituted enzymes range from 1.25×10^{-6} M, 2.17×10^{-6} M and 3.03×10^{-6} M for Co(II), Ni(II) and Fe(II) substituted enzyme respectively. The result shows that the Co(II) substituted MAO has the lowest K_m value with respect to the native enzyme as well as with the other metal substituted enzymes. This result is consistent with its relative activity in Figure 1. Of additional interest

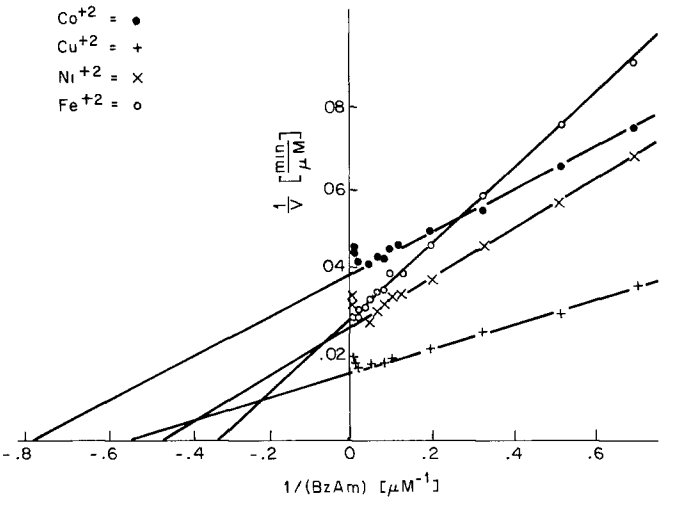

Fig. 2. Lineweaver-Burk plots for various MAO's in .05 M glycine buffer, pH 9.0, 30°C.

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is the disappearance of substrate inhibition for the Fe(II) enzyme, whereas the parent Cu(II) enzyme and its Co(II) and Ni(II) analogues still exhibit this effect. It appears that Fe(II) must in some way affect the unusual polymerization properties of this enzyme (2), which may in part explain the decreased activity of this modified form of MAO.

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