THE NATURE OF THE PROSTHETIC GROUPS OF PLASMA AMINE OXIDASE[1]

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Due to difficulties in obtaining sufficient quantities of highly purified preparations of amine oxidases, the natures of the prosthetic groups have not been established (Zeller, 1951; Davison, 1958; Mann, 1961) qualitatively and quantitatively. Plasma amine oxidase can be obtained in a crystalline form from beef plasma (Yamada and Yasunobu, 1962) and the availability of this preparation has opened the way for a critical examination of the prosthetic groups.

That copper was one of the prosthetic groups of plasma amine oxidase was shown by the fact that there was a direct proportionality between specific activity and copper content during the isolation of the crystalline enzyme. Furthermore, dialysis of the enzyme against sodium diethyldithiocarbamate at pH 7.0 removed the copper from the enzyme with a concomitant loss of enzyme activity. The dialyzed enzyme was specifically reactivated by cupric ions. The four times recrystallized enzyme which was shown to be homogeneous by a number of physicochemical criteria of purity was analyzed for copper content and the results are summarized in Table I. The copper did not undergo a valency change when substrate was added under anaerobic conditions although the absorption spectrum of the enzyme was altered (Fig. 1, insert).

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Table I

Copper, pyridoxal phosphate, and phosphorus content of plasma amine oxidase*

	Method	mμmoles/mg protein	moles/mole enzyme
Cupric copper	Chemical analysis**	14.23	3.7
Cuprous copper	Chemical analysis***	0	0
Pyridoxal phosphate	Spectrophotometry	6.32	1.6
Phosphorus	Chemical analysis	7.22	1.8

*Four times recrystallized enzyme with a specific activity of 500 (Yamada and Yasunobu, 1962) was used.
**Cupric copper was determined by the method of Peterson and Bollier (Peterson and Bollier, 1955).
***Cuprous copper was determined by the method of Griffiths and Wharton (Griffiths and Wharton, 1961).

No other metals such as iron, zinc, molybdenum of manganese were present in the crystalline enzyme in other than trace quantities.

Pyridoxal phosphate was shown to be another prosthetic group of plasma amine oxidase by treatment of the enzyme with hydroxylamine which resulted in a considerable loss of enzyme activity. The activity was partially restored by the addition of both copper and pyridoxal phosphate. Upon the removal of copper by the sodium diethyldithiocarbamate method, the enzyme exhibited an absorption maximum at 380 mu (Fig. 1) which has been reported as the maximum for pyridoxal phosphate (Peterson and Sober, 1954). Since the pyridoxal phosphate in plasma amine oxidase is so strongly bound, neither heat denaturation nor cold acid liberated it quantitatively. The quantity of pyridoxal phosphate present was determined spectrophotometrically on the copper-free enzyme, assuming a molar absorbancy index of 4,900 (Peterson and Sober, 1954) at 380 mμ and pH 7.0. The phosphorus content was determined by the method of Fiske and Subbarow (Fiske and Subbarow, 1925) after wet ashing of the enzyme with perchloric acid. These results are summarized

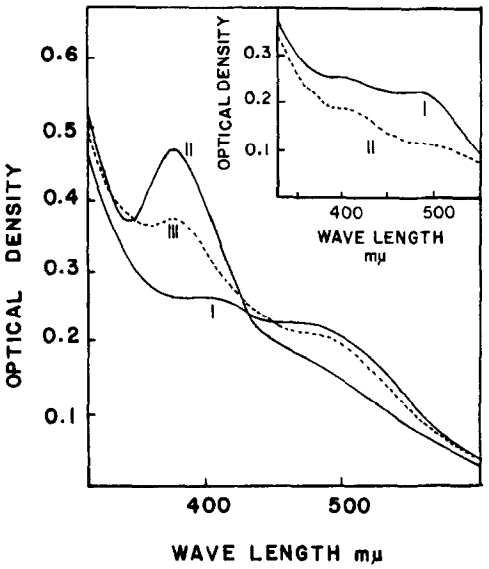

Fig. 1. Absorption spectra of plasma amine oxidase in 0.06 M phosphate buffer, pH 7.0. One ml of the enzyme solution containing 14.9 mg of protein was used in each experiment. (I), (II), and (III) represent recrystallized enzyme, sodium diethyldithiocarbamate treated enzyme and cupric copper-reconstituted enzyme, respectively. The insert represents the recrystallized enzyme (I) and I after the addition of 6 umoles of benzylamine (II).

in Table I. Finally, the digestion of the crystalline enzyme with pronase resulted in the liberation of a pyridoxal phosphate derivative in the deproteinized filtrate. The compound showed absorption and fluorescence characteristics which were similar to pyridoxal phosphate itself but did not respond to the apotryptophanase test[3] (McCormick, et al, 1961). The chemical structure of this compound is under investigation.

No flavins were detected in the crystalline plasma amine oxidase after heat denaturation, acid treatment or after digestion with proteases (Kearney, 1960).

Therefore, the plasma amine oxidase can be represented as protein-$(Cu^{++})_4$-(pyridoxal phosphate)$_2$. The pyridoxal phosphate and copper

[3]We are indebted to Dr. E. L. Oginsky, Department of Bacteriology, University of Oregon Medical School, for a gift of E. coli (Crookes strain).

appears to be present as a chelate since the native enzyme which is pink in color exhibits an absorption maximum at 480 mμ while the copper-free enzyme exhibits a maximum at 380 mμ and the maximum at 380 mu is reconverted to 480 mu upon the addition of cupric copper to the copper-free enzyme (Fig. 1). The maximum at 480 mu is neither characteristic of a pyridoxal enzyme nor a copper enzyme reported to date.

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