THE PROSTHETIC GROUPS OF ANIMAL AMINE OXIDASES*

Sakari Nara, Ikuo Igaue, Benedict Gomes, and Kerry T. Yasunobu

Dept. of Biochemistry and Biophysics, University of Hawaii
Honolulu, Hawaii

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Amine oxidases are widely distributed in various animal tissues and carry out the following reaction.

$$RCH_2NH_2 + O_2 + H_2O = RCHO + NH_3 + H_2O_2$$

The early investigators have classified the animal amine oxidase as either monoamine or diamine oxidases (Zeller, 1951; Blaschko, 1963). As far as the enzymes of the diamine type are concerned, it has been demonstrated that beef and pig plasma amine oxidases are copper-pyridoxal phosphate dependent enzymes (Yamada and Yasunobu, 1962, 1963; Blaschko and Franconi, 1965). Although Kapeller-Adler and Macfarlane (1963) claim that hog kidney diamine oxidase requires FAD, pyridoxal-phosphate and copper as prosthetic groups, Goryachenkova and Ershova (1963) and Mondovi (1965) have been unable to detect flavins in highly purified preparations of hog kidney diamine oxidase. The only studies of the prosthetic group of beef liver mitochondrial monoamine oxidase demonstrate that it is a copper-protein (Nara, et al. 1966). In the present communication, evidence is presented which demonstrates that this enzyme is a flavo-enzyme.

Highly purified preparations of beef mitochondrial amine oxidase were prepared as previously described (Nara, et al. 1966). The prepara-

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tions were homogeneous in the ultracentrifuge and also when examined by disc electrophoresis.

The evidence which demonstrates that the beef liver mitochondrial monoamine oxidase is a flavo-enzyme is summarized below. (1) Microbiological assays of both crystalline beef plasma and beef liver mitochondrial amine oxidase were carried out using L. casei strain e. The results are summarized in Table I. The flavin content was estimated to be one

TABLE I

FLAVIN CONTENT OF SOME ANIMAL AMINE OXIDASES*

	Enzyme	mg Protein	Specific** Activity	µg Flavin/ mg protein
1.	Beef plasma amine oxidase	4.2	400	0
2.	Beef mitochondrial monoamine Oxidase	4.1	4,000	1.2

* Determined by microbiological assay using L. casei as described by Snell and Strong (1939).
**The specific activity of the enzyme was determined using the spectrophotometric assay in which benzylamine is used as the substrate (Tabor et al., 1953).

mole of flavin per 200,000 gm of protein. (2) The difference spectrum of the native enzyme (oxidized) and the reduced (sodium hydrosulfite) showed maxima at about 400 and 460 mu. (Fig. 1a). The spectrum of the flavopeptide obtained by tryptic digestion of the enzyme showed maxima at about 375 and 460 mu. These maxima are similar to that reported for other flavoenzymes (Fig. 1b). (3) Although the native enzyme does not fluoresce at the wavelengths expected for flavins, the flavopeptide showed the characteristic fluorescence properties (Fig. 1d) of flavin.

The following data can be cited to indicate that the flavin plays a catalytic role in the reaction. (1) The addition of the substrate tyramine to the enzyme under anaerobic condition resulted in the bleaching

of the maximum at 450 mu. The difference spectrum of the native enzyme versus the substrate reduced enzyme showed maxima at 400 and 460 mu.

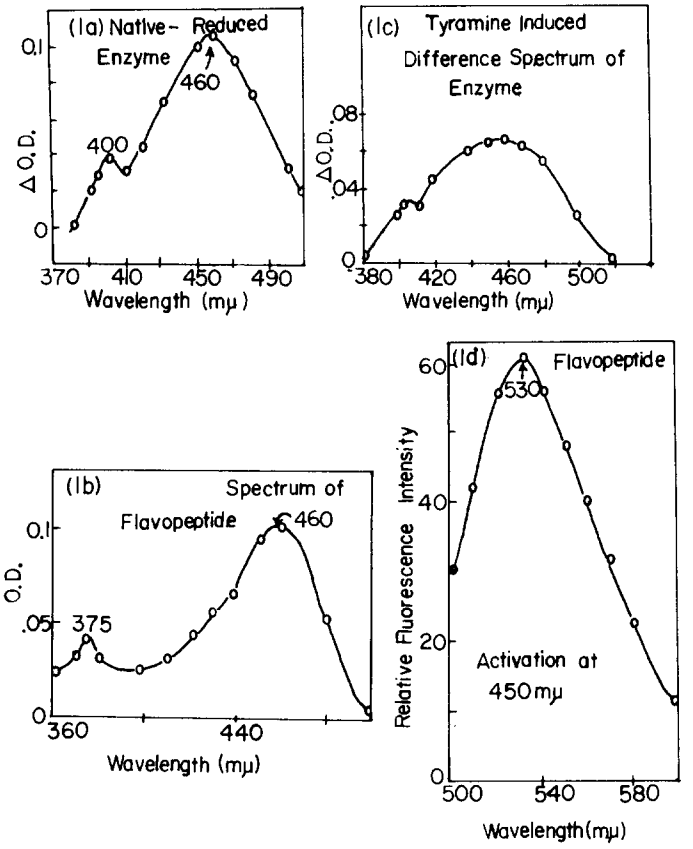

Figure 1a- The enzyme (1.75 mg/ml, specific activity 3,400) was dissolved in 0.1 M phosphate buffer, pH 7.4. The reduced form of the enzyme was obtained by addition of 1 mg of sodium hydrosulfite and the difference spectrum is shown.

Figure 1b- The enzyme (3.5 mg/ml) was dissolved in 0.1 M phosphate buffer, pH 7.4. The spectrum was recorded and after removal of oxygen, 1 mg of solid tyramine was added from the side arm and the spectrum recorded. The difference spectrum is shown.

Figure 1c- For the experiment, 2 ml of enzyme (specific activity 2,650 in 0.1 M phosphate buffer, pH 7.4) was denatured by the addition of 1.0 ml of 20% trichloroacetic acid. The solution was centrifuged and 3 ml of 0.1 M phosphate buffer, pH 7.4 added. The pH was adjusted to 8.0 and about 0.5 mg of trypsin added. The reaction was allowed to proceed for 4 hrs. at 40°. The reaction was terminated by the addition of 1.0 ml of 20% trichloroacetic acid and centrifuged. The supernatant was extracted with 3.5 ml of ether three times and the spectrum recorded.

Figure 1d- The peptide was obtained as reported in Fig. 1c. The excitation wavelength was 450 mu.

(Fig. 1c). (2) During the purification of the enzyme, the absorbency ratio at 410/450 mu decreased to a constant value of about 1.65 suggesting that the flavin is an essential component of the enzyme.

The fact that the mitochondrial monoamine oxidase is a flavoenzyme is in agreement with the finding of Hawkins and Burr (1952) that the liver of riboflavin deficient rats showed a decreased level of monoamine oxidase. The results also explain in part the finding of Gorkin et al. (1964) who found that proflavine is an inhibitor of the rat liver monoamine oxidase.

In summary, the diamine oxidase type of enzymes are copper-pyridoxal-phosphate dependent enzymes. However, the beef liver mitochondrial monoamine oxidase is a copper-flavoenzyme. At the present time we are unable to determine whether the flavin is present as FAD or FMN since the flavin appears to be covalently bonded to the enzyme and is not released from the enzyme even by trichloroacetic acid. However, it is of interest that the monoamine oxidase is the only known example of an enzyme requiring both copper and flavin as prosthetic groups.

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