OXIDATION OF PHENAZINE METHOSULFATE BY HEPATIC ALDEHYDE OXIDASE

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Rabbit liver aldehyde oxidase exhibits a dual catalytic specificity; it catalyzes the oxidation of a wide variety of aldehydes (e.g., formaldehyde, acetaldehyde, salicylaldehyde, pyridoxal) and also catalyzes the hydroxylation of nitrogen-containing aromatic heterocycles such as N^1-methylnicotinamide, quinoline and quinine (Knox, 1946). Oxygen, ferricyanide, methylene blue, 2,6-dichlorophenolindophenol, cytochrome c, nitroblue tetrazolium as well as phenazine methosulfate (PMS) serve as electron acceptors for the highly purified enzyme (Rajagopalan, Fridovich and Handler, 1962). In the course of experiments in which PMS was to serve as electron acceptor it was observed that, in the absence of a conventional substrate, aerobic incubation of this dye with the enzyme resulted in the appearance of a red color. Further examination revealed that PMS can serve not only as electron acceptor but as an oxidizable substrate (electron donor) for aldehyde oxidase. Since this dye has occasionally been observed to serve as a reductant when introduced into biological systems as an oxidant (Kok and Hoch, 1961), it appeared desirable to record the present findings.

Absorption Spectrum of Oxidized PMS

Purified aldehyde oxidase was prepared from rabbit liver by the method previously described (Rajagopalan et al., 1962). Five µg of the enzyme was allowed to react aerobically with 2×10^{-5} M PMS, in 0.05 M phosphate, pH 7.8, containing .005% versene-Fe3 in spectrophotometric cuvettes until reaction was complete. The absorption spectrum of the red product as well as that of PMS was obtained with a Cary Model 14 recording spectrophotometer. The absorption spectra of PMS and its red oxidized derivative are presented in Fig. 1. The molar extinction coefficients of the two compounds at several wavelengths are listed in Table I. The increase in extinction at 520 mμ on oxidation of PMS affords the most sensitive spectrophotometric assay for aldehyde oxidase available.

K_m for PMS was found to be 1.9×10^{-4} M; the values for N^1-methyl nicotinamide and acetaldehyde are 2.8×10^{-4} M and 1.0×10^{-3} M, respectively.

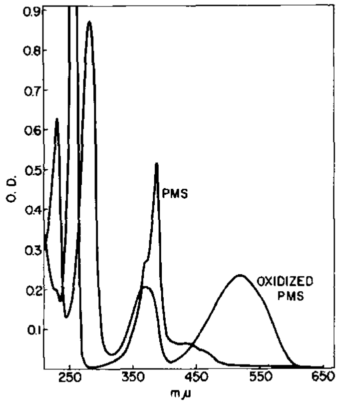

Figure 1. Absorption spectra of PMS and oxidized PMS.

TABLE I

Molar extinction coefficients of PMS and oxidized PMS

Wavelength mμ	Molar extinction coefficient (1 cm. light path)	
	PMS	OXIDIZED PMS
230	9,900	31,500
260	82,000	12,500
284	250	43,500
387	25,700	6,500
520	400	11,700

Probable Identity of the Oxidation Product

The red oxidation product (I) was reduced to a leuco form (II) by each of two procedures: (a) judicious addition of dithionite or (b) anaerobic incubation with aldehyde oxidase in the presence of N^1-methyl nicotinamide as electron donor in 0.05 M phosphate, pH 7.8. In both cases, reintroduction of oxygen resulted in reoxidation to the original red form. Thus, reduction of (I) did not result in reformation of phenazine methosulfate.

Anaerobic incubation of PMS with aldehyde oxidase yielded rather complex data. Initially, PMS was oxidized to its red derivative (I) with other molecules of PMS serving as electron acceptors in the conventional manner, resulting in the formation of leuco PMS (III). Subsequently, as (I) also served as electron acceptor an equilibrium mixture was obtained which contained PMS as well as compounds I, II and III.

The red compound (I) exhibited several properties which resembled those described for pyocyanine (10 methyl phenazin-1-one). Thus, Friedheim and Michaelis (1931) reported that pyocyanine, which is blue in alkaline medium, is converted to a leucoform by a 2-electron reduction. In acid solution, however, pyocyanine is wine red; addition of dithionite results in formation of a stable green free radical which may then be further reduced to a colorless form. In comparison, the oxidation product (I) derived from phenazine methosulfate is red at alkaline pH and yielded an orange-yellow solution at acid pH. Addition of a small amount of dithionite yielded a green solution which was completely bleached by a further addition of dithionite.

In contrast to the behavior of (I), the photoxidation product of PMS, prepared by aerobic illumination of PMS in 0.05 M phosphate buffer, pH 7.8, completely resembled pyocyanine when treated with dithionite in acid or alkaline solution. Kehrmann and Cherpillod (1924) reported the synthesis and properties of oxidized forms of 10-methyl phenazine with oxygen functions in the 2- and 3-positions respectively. Comparison of the properties of the latter compounds with those of (I) indicated that the latter is identical with 10-methyl phenazin-3-one (Fig. 2). This is compatible with the fact that the electron distribution around carbon 3 of phenazine methosulfate resembles that about the 6-carbon of N^1-methyl nicotinamide, a conventional substrate for aldehyde oxidase. It is noteworthy that some tumor tissues

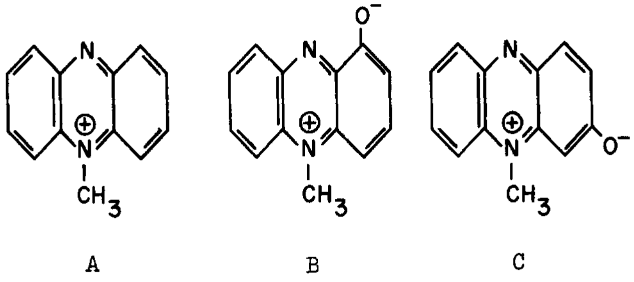

Figure 2. A - 10-methyl phenazine. B - 10-methyl phenazin-1-one (Pyocyanine). C - 10-methyl phenazin-3-one.

have been found to oxidize PMS to the same product as obtained with aldehyde oxidase (Dickens, 1936; Dickens and McIllwain, 1938).

N-methyl acridine and N-methyl (1) acridone have also been observed to serve as substrates for aldehyde oxidase, whereas pyocyanine acts solely as an acceptor. Milk xanthine oxidase, which resembles aldehyde oxidase in many respects, does not oxidize any of these compounds under the same conditions.

Recently Kok and Hoch (1961) reported that PMS, contrary to expectation, acted as a source of "reducing power" in photosynthesizing preparations derived from various algae. Horio and Kamen (personal communication) have observed the reducing capacity of PMS in bacterial preparations capable of conducting photophosphorylation. The present data, demonstrating the oxidation of PMS by an enzyme which can use cytochrome c as electron acceptor, suggest the presence of a similar enzyme in these photosynthesizing systems.

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REFERENCES

Dickens, F., Biochem. J. 30, 1064 (1936).
Dickens F., and McIllwain, Biochem. J., 32, 1615 (1938).
Friedheim, E., and Michaelis, L., J. Biol. Chem. 91, 355 (1931).
Horio, T., and Kamen, M. D., Personal communication.
Kehrmann, F., and Cherpillod, F., Helv. Chim. Acta, 7, 973 (1924).
Kok, B., and Hoch, G., in McElroy, W. D. and Glass, B., Ed., A Symposium on Light and Life, The Johns Hopkins Press, Baltimore, 1961, p. 397.
Rajagopalan, K. V., Fridovich, I., and Handler, P., J. Biol. Chem. 237, 922 (1962).