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SHORT COMMUNICATION

FUNGAL METABOLISM—IV.

THE OXIDATION OF PSILOCIN BY *p*-DIPHENOL OXIDASE (LACCASE)

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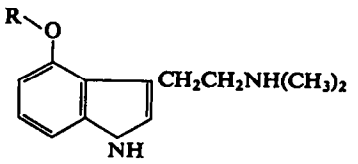
Abstract—The oxidation of psilocin catalysed by *p*-diphenol oxidase has been studied. The corresponding phosphorylated compound psilocybin, was found not to be a substrate of this enzyme. The K_m for psilocin was obtained by a spectrophotometric method. The oxygen uptake occurring during the oxidation of psilocin was studied manometrically. A study of the effect of pH on the oxidation of psilocin showed a broad pH optimum below pH 4·0. The colour of the blue oxidation product was found to be rapidly bleached by ascorbic acid.

INTRODUCTION

PSILOCYBIN (3-2′-dimethylaminoethylindol-4-phosphate) (I) and the corresponding phenol psilocin (II) were isolated in 1958 as the active principles in the psychotropic mushrooms of the *Psilocybe* genus and in *Stropharia cubensis*.[1] Blaschko and Levine[2] have already shown that the copper oxidase, ceruloplasmin, which has a similar substrate specificity to *p*-diphenol oxidase from other sources[3] catalyses the oxidation of hydroxyindoles such as psilocin, and hence it was considered worthwhile to study the action of *p*-diphenol oxidase from a fungal source on these compounds, especially since psilocin and psilocybin are products of fungal origin.



(I) R = PO_3H
(II) R = H

RESULTS AND DISCUSSION

In the studies reported here, psilocin was used as the substrate. The product of the enzymically catalysed reaction is blue in colour and its u.v. spectrum show absorption maxima at 610 nm and 395 nm The product is similar in colour and its u.v. spectrum to

[1] A. HOFMANN, R. HEIM, A. BRACK and H. KOBEL, *Experientia* **14**, 107 (1958).
[2] H. BLASCHKO and W. G. LEVINE, *Brit. J. Pharmacol.* **15**, 625 (1960).
[3] J. PEISACH and W. G. LEVINE, *J. Biol. Chem.* **240**, 2284 (1966).

the product obtained when pscilocin is oxidized with ferric chloride. The blue product was found to be very hydrophilic, and cannot easily be extracted into the usual organic solvents. The oxidation of psilocin was studied spectrophotometrically and the assay was based on the increase in absorbancy at 610 nm, a short induction period was found to occur before a steady state was reached. The enzyme displayed Michaelis–Menton kinetics with psilocin as substrate, and reciprocal plots of initial velocity and substrate concentration gave straight lines. The K_m for psilocin at pH 3·6 was found to be 2×10^{-7} M.

Under identical conditions, psilocybin, the phosphorylated ester of psilocin was not oxidized. Hence it appears that the free hydroxyl group on the aromatic ring is essential for oxidation catalysed by this enzyme. The oxidation of psilocin catalysed by *p*-diphenol oxidase was also studied in experiments using conventional manometry. A study of the effect of pH on the oxidation of psilocin using sodium acetate buffer showed a broad pH optimum on the acid side below pH 4·0 (Fig. 1), this is very similiar to the effect of pH on the oxidation of 2,6-dimethoxyphenol by this enzyme.[4]

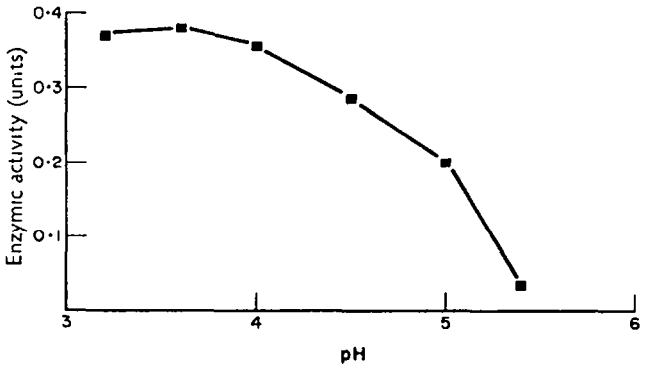


FIG. 1. THE EFFECT OF pH ON THE OXIDATION OF PSILOCIN BY *p*-DIPHENOL OXIDASE (USING SODIUM ACETATE–ACETIC ACID BUFFER).

The blue product formed during the oxidation of psilocin was found to be readily reduced to a colourless (straw coloured in concentrated solution) product by ascorbic acid or sodium dithionite, and is thus probably quinonoid in nature. Studies previously reported of the oxidation of phenols such 2,6-dimethoxyphenol and 2,6-dimenthylphenol have shown that dimeric quinones are obtained as products, these quinones were readily reduced by ascorbic acid or sodium dithionite.[4]

EXPERIMENTAL

The enzyme used in this study was obtained from the fungus *Polyporus versicolor*. The organism was grown as described by Bocks[4] and the enzyme preparation carried out according to the method previously described by Malström *et al.*[5]

Determination of p-diphenol oxidase activity was carried out by a spectrophotometric method using 2,6-dimethoxyphenol as substrate.[4] In kinetic studies of the oxidation of psilocin, a range of substrate concentrations from 0·25 μmoles to 3·0 μmoles were used. The increase in absorbancy was measured at 610 nm and the initial velocity of the reaction was measured at 25° from the linear portion of the curve. All other conditions were similar to those described for kinetic studies of the oxidation of 2,6-dimethoxyphenol by this enzyme.

[4] S. M. BOCKS, *Phytochem.* **6**, 785 (1967).
[5] B. MALSTRÖM, G. FAHRAEUS and R. MOSBACH, *Biochim. Biophys. Acta* **28**, 652 (1958).

The ascorbic acid was obtained from Koch-Light Laboratories, Colnbrook, Bucks., and the chemicals used in the preparation of buffers were of Analar grade.

Oxygen uptake was measured by the conventional manometric technique, using 5·0 μmoles of the substrate (psilocin) per flask. Control experiments (minus enzyme) showed that no measurable oxygen uptake occurred due to autoxidation of the substrate. Each manometer flask also contained sodium acetate buffer, pH 3·6 (2·4 μmoles) and 10 units of enzyme, any carbon dioxide present was absorbed by KOH in the centre well. The experiments were carried out at 30°.

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