STEREOCHEMISTRY OF OXIDATION OF BENZYLAMINE BY THE AMINE OXIDASE FROM BEEF PLASMA

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1. Introduction

Beef-plasma amine oxidase catalyses the oxidative deamination of various amines to give the corresponding aldehydes, hydrogen peroxide and ammonia. The enzyme contains cupric copper and a prosthetic group of, as yet, undetermined chemical structure. This paper describes the determination of the stereochemistry of oxidation of benzylamine by the plasma enzyme.

2. Materials and methods

2.1. *Enzyme and substrates*

The enzyme was purified to homogeneity by a modification of method in [1] and will be detailed in [2]. The enzyme was stored as a suspension in 0.1 M Na_2HPO_4/KH_2PO_4 (pH 7.0) buffer containing ammonium sulphate to 0.55 saturation. Purified enzyme had spec. act. 0.36 units/mg protein; 1 unit catalyses the formation of 1 μmol benzaldehyde ($\epsilon_{250} = 1.28 \times 10^4\ M^{-1}\ cm^{-1}$) per minute at 25°C under the standard assay conditions [3].

(*RS*)-, (*R*)- and (*S*)-[*methylene*-$^3H_1$]benzylamines were synthesised as in [4]. Benzylamine samples were assayed using ninhydrin [5]. Liver alcohol dehydrogenase, catalase and NADH were obtained from Sigma.

2.2. *Benzylamine oxidation by beef-plasma amine oxidase*

The following components were incubated in 4.0 ml 0.1 M sodium phosphate buffer (pH 7.2) at 30°C: stereospecifically tritiated benzylamine sample (~8 mM); NADH (10 mM); plasma amine oxidase (0.15 U); liver alcohol dehydrogenase (4 U) and catalase (10 U). The course of the reaction for the enzyme-catalysed oxidation of tritiated benzylamine was monitored by the removal of aliquots (10 μl) at timed intervals, which were diluted with 1.0 ml water before determining ΔA_{340}. After ~30% of the benzylamine had been oxidised, the reaction was stopped by the addition of 2 N perchloric acid (0.5 ml) and the precipitated protein removed by low speed centrifugation using an MSE Bench centrifuge. To the clear aqueous solution unlabelled benzyl alcohol (100 μmol) was added and the diluted, radioactive benzyl alcohol extracted with a 10% solution (v/v) of toluene in methylene chloride (2 × 4.0 ml). The organic phase was washed successively with small volumes of H_2O, 3 N HCl, H_2O saturated $NaHCO_3$, H_2O, saturated NaCl and dried over anhydrous $MgSO_4$. After removal of the bulk of CH_2Cl_2, using a steady stream of dry nitrogen gas, phenylisocyanate (100 μmol) was added to the residual toluene solution and the mixture was heated at 100°C for 5 min. On cooling, the solution was evaporated to dryness, then extensively dried in vacuo over fresh phosphoric oxide. The crude phenylurethane was purified by preparative thin-layer chromatography on a single 20 × 20 × 0.2 cm silica 60 F_{254} plate developed with ether : *n*-pentane (1:1), and finally was crystallised several times from *n*-heptane/*n*-pentane.

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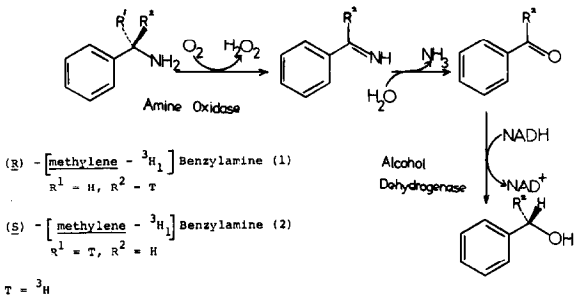
3. Results and discussion

The 3 tritiated benzylamines were each mixed with a small amount of [*methylene*-^{14}C]benzylamine which provides a standard against which the extent of tritium retention can be measured in the subsequent incubation with plasma amine oxidase. The results of this study are shown in table 1 and clearly establish that the oxidative deamination of benzylamine occurs stereospecifically with loss of the hydrogen atom in *Si*-space.

The difficulties of handling the unstable product, benzaldehyde, have been discussed [4] and consequently a coupled reductive enzyme system was included in the incubation mixture to reduce the benzaldehyde to benzyl alcohol (see scheme 1). Derivatization of the benzyl alcohol as the crystalline phenylurethane considerably simplifies both the purification and radioassay of the product. The present study complements our earlier work on the Cu-amine oxidase from pea seedlings [4] which also removes the *Si*-hydrogen from benzylamine. However, the two amine oxidases diverge markedly in their behaviour towards phenethylamines: oxidation is stereospecific with the pea seedling enzyme [6,7], but apparently not with the plasma enzyme [8].

p-Hydroxybenzylamine is also [9] oxidised in the presence of the plasma enzyme with stereospecific removal of the *Si*-hydrogen. The reason for the nonstereospecificity of the oxidation of phenethylamines is under investigation [2].



Scheme 1

Acknowledgements

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References

[1] Yamada, H. and Yasunobu, K. T. (1962) J. Biol. Chem. 237, 1511–1516.
[2] Summers, M. C., Klinman, J. P. and Markovic, R. (1979) Biochemistry, in press.
[3] Tabor, C. W., Tabor, H. and Rosenthal, S. M., (1954) J. Biol. Chem. 208, 645–661.
[4] Battersby, A. R., Staunton, J. and Summers, M. C. (1976) J. Chem. Soc. Perkin Trans. I, 1052–1056.
[5] Troll, W. and Cannon, R. K. (1953) J. Biol. Chem. 200, 803–811.
[6] Battersby, A. R., Southgate, R., Staunton, J. and Summers, M. C. (1979) J. Chem. Soc. Perkin Trans. I, in press.
[7] Staunton, J. and Summers, M. C. (1978) in: Techniques in Protein and Enzyme Biochemistry, part II (Tipton, K. F. ed) vol. B116, pp. 1–39, Elsevier/North-Holland Biomedical Press, Amsterdam, New York.
[8] Klinman, J. P., Markovic, R. and Summers, M. C. (1978) Fed Proc. Fed. Am. Soc. Exp. Biol. 37, 833.
[9] Suva, R. H. and Abeles, R. H. (1978) Biochemistry 17, 3538–3545.

Table 1

Benzylamine	^{3}H/^{14}C	Benzyl alcohol[a] (^{3}H/^{14}C)	^{3}H retention (%)
(*RS*)-[*methylene*-$^3H_1$,^{14}C] (1) + (2)	10.0 ± 0.2	5.7 ± 0.1	57 ± 2%
(*R*)-[*methylene*-$^3H_1$,^{14}C] (1)	8.35 ± 0.3	8.0 ± 0.3	96 ± 3%
(*S*)-[*methylene*-$^3H_1$,^{14}C] (2)	6.5 ± 0.2	0.3 ± 0.1	4.6 ± 2%

[a] Radioassay as phenylurethane derivative