INHIBITION OF BEEF PLASMA AMINE OXIDASE ACTIVITY BY GLYCINE

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1. Introduction

Beef plasma amine oxidase catalyzes the oxidative deamination of various monoamines. It contains copper and a prosthetic group interacting strongly with carbonyl reagents [1]. Histamine, putrescine, spermidine and spermine were observed [2] to oxidize more rapidly in Tris–HCl than in glycine buffer by mouse liver diamine oxidase. We observed that benzylamine oxidation by bovine plasma amine oxidase is lower in Tris–glycine than in phosphate buffers at the same pH and ionic strength. These results suggest that glycine may be an inhibitor of these enzymes. A comprehensive study of this hypothesis is reported here. Both spectral and kinetic evidence suggest that the site of glycine inhibition is the carbonyl cofactor of the enzyme.

2. Materials and methods

Glycine was obtained from Merck, Darmstadt. Ammonia-free glycine hydrochloride from Sigma Co., St Louis. Other reagents used were of highest available purity. Beef plasma amine oxidase was purified as in [3]. Enzyme activity was assayed spectrophotometrically as in [4]. Enzyme kinetics were studied by polarographic determination of oxygen uptake in a GME oxygraph model KM, equipped with a Clark electrode, in a 1.5 ml reaction vessel, thermostated at 38°C with 0.1 M potassium phosphate buffer, pH 8. Enzyme units are expressed according to [5]. Enzyme concentration is expressed in terms of the molecular weight of monomer, i.e., 86 000 [1]. Absorption spectra were recorded on a Cary 14 ratio recording spectrophotometer.

3. Results and discussion

Figure 1 shows a Dixon plot of the enzymic oxidation of benzylamine in solutions containing varied amounts of glycine. The graph indicates a non-competitive inhibition of the enzyme by glycine with a K_i $\sim 10^{-2}$ M. Possible ionic strength effects may be ruled out because 0.4 M alanine does not inhibit at all benzylamine oxidase activity. The optical spectrum of the enzyme in the presence of glycine shows a broad peak at 415 nm (fig.2). Enzyme activity and optical spectrum, typical of the native enzyme, were restored after 48 h dialysis against 0.1 M phosphate buffer pH 8 (fig.2, table 1).

Since a 415 nm band in enzymes containing carbonyl cofactors is generally ascribed to an amino acid Schiff base [6], and its extinction coefficient is similar to that for pyridoxal-5-phosphate bound to

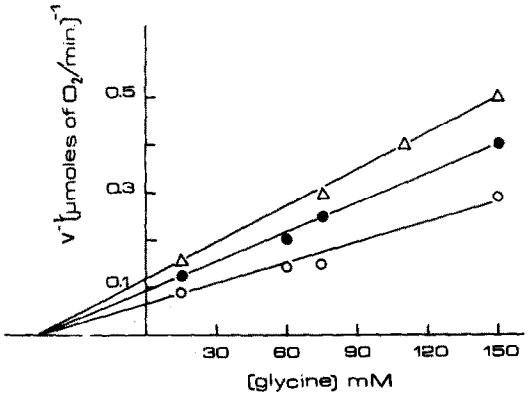

Fig.1. Dixon plot of benzylamine oxidation in presence of glycine as inhibitor. Enzyme concentration 2×10^{-6} M. (o–o) 1.67×10^{-3} M benzylamine. (□–□) 0.83×10^{-3} M benzylamine. (△–△) 0.53×10^{-3} M benzylamine.

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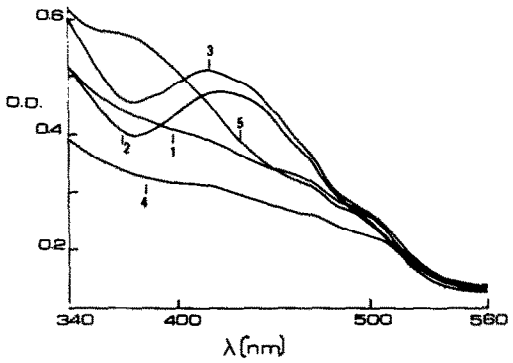

Fig.2. Amine oxidase spectra in the presence of glycine and cycloserine. (1) Enzyme 10^{-4} M. (2) Enzyme + 0.4 M glycine. (3) Enzyme + 0.4 M glycine + 10 mM cycloserine. (4) The sample of curve 3 dialysed 48 h against 0.1 M phosphate buffer pH 8. (5) Enzyme + 10 mM cycloserine + 0.4 M glycine. After dialysis spectrum did not change.

glycine at neutral pH [7], the enzyme was treated with cycloserine and phenylhydrazine, well known irreversible reagents of carbonyl groups: in particular, cycloserine has a marked specificity [8,9]. In the presence of cycloserine, enzyme activity was completely abolished (table 1) and a shoulder at 370 nm appeared (fig.2), which may be imputable to a pyridoxal-5-phosphate–cycloserine Schiff base [8,9].

Table 1
Residual activity of bovine plasma amine oxidase (AO) (2×10^{-6} M) after treatment with glycine (0.4 M) and cycloserine (10 mM)

	% act. in presence of inhibitor	% act. after 48 h dialysis
AO	100	100
AO + glycine	14.3	86
AO + cycloserine	0	0
AO + glycine + cycloserine	0	80
AO + cycloserine + glycine	0	0

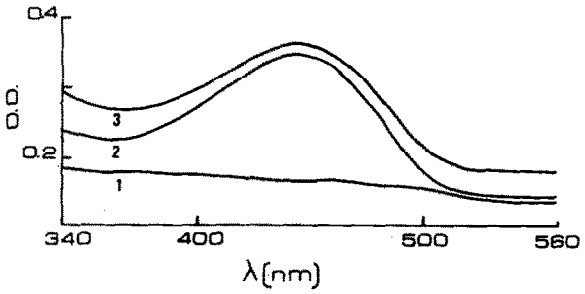

Fig.3. Amine oxidase spectra in the presence of phenylhydrazine and cycloserine. (1) Enzyme 4×10^{-5} M. (2) Enzyme + 4×10^{-5} M phenylhydrazine. (3) Sample of curve 2 plus 4×10^{-5} M cycloserine: also in the presence of glycine spectrum does not change.

These effects were not reversible on exhaustive dialysis (table 1, fig.2).

If both glycine and cycloserine were added to the same enzyme solution, results were found to depend on the addition sequence being the effects typical of glycine and cycloserine not affected by the subsequent addition of the other reagent (fig.2, table 1).

Glycine and cycloserine were unable to give their characteristic spectra when phenylhydrazine was present (fig.3).

In conclusion, the spectrum of the glycine-treated enzyme and the competition of well-known carbonyl reagents with glycine suggest that the enzyme binding site for glycine is a carbonyl group, probably that of pyridoxal-5-phosphate. These results add new evidence for the presence of this prosthetic group in amine oxidases.

Acknowledgements

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References

[1] Yasunobu, K. T., Ishizaki, H. and Minamiura, N. (1976) Mol. Cell. Biochem. 13, 3–29.
[2] Syatkin, S. P. and Galaev, Yu. V. (1977) Biokhimiya 42, 783–786.

[3] Turini, P., Costa, M. T., Sabatini, S. and Mondovì, B. (1978) Ital. J. Biochem. in press.
[4] Tabor, C. W., Tabor, H. and Rosenthal, S. M. (1954) J. Biol. Chem. 208, 645–661.
[5] Yamada, H. and Yasunobu, K. T. (1962) J. Biol. Chem. 237, 1511–1516.
[6] Fasella, P. (1967) Ann. Rev. Biochem. 36, 185–210.
[7] Metzler, D. E. (1957) J. Am. Chem. Soc. 79, 485–490.
[8] Churchich, J. E. (1967) J. Biol. Chem. 242, 4414–4417.
[9] Finazzi-Agrò, A., Guerrieri, P., Costa, M. T. and Mondovì, B. (1977) Eur. J. Biochem. 74, 435–440.