The presence of an inhibitor of benzylamine oxidase in human blood plasma

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The presence of an inhibitor of benzylamine oxidase in human blood plasma was observed. It may be removed by use of either a DEAE-cellulose column or a Sephadex G-200 column.

The benzylamine oxidase of human serum has been previously described by McEwen & Cohen (1963), and it has been partially purified by McEwen (1965*a,b*). This enzyme seems very similar in its substrate-specificity and inhibitor-sensitivity to pig plasma benzylamine oxidase (Buffoni, 1966; Buffoni & Della Corte, 1972). In the present paper the presence in human blood plasma of an inhibitor of benzylamine oxidase is described.

Materials and methods

Benzylamine oxidase activity was assayed by the spectrophotometric method described by Tabor *et al.* (1954) (1 unit is defined as the increase of 0.001 A_{250} unit/min) and by a radioisotopic method with [^{14}C]benzylamine as substrate as described by McCaman *et al.* (1965). The enzymic activity was also determined by measuring the ammonia released, by using the micro-diffusion method (Conway, 1962) and the Nessler spectrophotometric method (Hawk *et al.*, 1954). The formation of products was linear up to 60 min at different protein concentrations (Fig. 1). Protein concentration was determined by the method of Waddell (1956). [^{14}C]Benzylamine hydrochloride was obtained from ICN Pharmaceuticals (Irvine, CA, U.S.A.). General chemicals were all AnalaR grade from BDH Chemicals (Poole, Dorset, U.K.), DEAE-cellulose (DE-32, granular) was from Whatman Biochemicals (Maidstone, Kent, U.K.) and Sephadex G-200 was from Pharmacia (Uppsala, Sweden). The human blood plasma was obtained from the Blood Bank of the S. Maria Nuova Hospital of Florence. When fresh blood was used it was collected with heparin in

a sterilized syringe, and the plasma was obtained by centrifugation at 500 *g* for 20 min at 4°C.

Results and discussion

Fig. 1 shows that the measurement of benzylamine oxidase activity in human blood plasma and of its 35–55%-satn.-(NH_4) $_2$ SO $_4$ precipitate is not possible at high protein concentrations. Similar results were obtained with measurement of either product, ammonia or benzylaldehyde. Seven different samples were analysed and each gave similar results (five were out-dated human plasma, two were fresh human plasma). This behaviour is consistent with the presence of an inhibitor that is still present in the protein obtained by precipitation with 35–55%-satd. (NH_4) $_2$ SO $_4$. Table 1 shows the partial purification of benzylamine oxidase. The precipitation by 0–55%-satd. (NH_4) $_2$ SO $_4$ of the active pooled fractions coming from the DEAE-cellulose column eliminated the inhibitor; the activity was now independent of the protein concentration, which indicates that the inhibitor had been removed by this step. Gel filtration on Sephadex G-200 of the 35–55%-satn.-(NH_4) $_2$ SO $_4$ precipitate from plasma also removed the inhibitor, which was a yellow substance with M_r below 200 000, coming before the enzyme. The active fractions were inhibited by the fractions containing this material, and the inhibition was decreased by dilution (Fig. 2). We may conclude that in human blood plasma benzylamine oxidase activity is very low because there is an inhibitor present. We do not know the physiological significance of this observation, but the presence of this inhibitor in human blood plasma might be

Fig. 1. *Benzylamine oxidase activity of human plasma at different protein concentrations*

When the radioisotopic method was used the reaction mixture contained 0.2 ml of enzyme solution at different concentrations in 60 mM-sodium phosphate buffer, pH 7.4, 0.05 ml of the same buffer, and 0.05 ml of 10 mM- $[^{14}\text{C}]$ benzylamine (0.5 mCi/mmol). When the spectrophotometric method was used the reaction mixture contained 1.0 ml of enzyme solution at different concentrations, 0.2 ml of catalase (21 units/ml), 0.2 ml of 0.1 M-benzylamine and 4.6 ml of 60 mM-sodium phosphate buffer, pH 7.4. In both cases the incubation was at 37°C in air in a Dubnoff water bath. The preincubation was for 10 min, the incubation for 30 min. The activity is always expressed in spectrophotometric units. When the radioisotopic method was used, transformation of nmol/min per mg of protein into spectrophotometric units was obtained with the following expression: (nmol/min per mg) $\times$ 11.3 = 1 unit/mg. An AKTA III spectrophotometer and a Packard liquid-scintillation counter were used. The efficiency in Instagel was $88 \pm 0.1\%$ (mean $\pm$ s.e.m., $n = 50$). (a) ●, Human plasma; O, 35–55%-satn.-(NH_4) $_2$ SO $_4$ precipitate of human plasma fully dialysed against 10 mM-sodium/potassium phosphate buffer, pH 7.0. (b) 35–55%-satn.-(NH_4) $_2$ SO $_4$ precipitate of human plasma at the following concentrations in the reaction mixture: ●, 2.4; O, 0.8; ■, 0.4; ▽, 0.24 mg of protein/6 ml.

Table 1. *Partial purification of the benzylamine oxidase of human plasma*

The DEAE-cellulose column (2.5 cm $\times$ 33 cm) was equilibrated with 10 mM-sodium/potassium phosphate buffer, pH 7.0. Fractions of volume 10 ml were collected. The concentration of the eluting buffer was increased to 30 mM after 150 ml, and to 0.1 M after 510 ml. The enzyme was eluted with the last buffer.

Step	Volume (ml)	Protein (mg/ml)	Activity (units/ml)	Yield (%)
Plasma	360	80	0.44 $\pm$	100
35–55%-satn.-(NH_4) $_2$ SO $_4$ precipitate*	150	22	9.32 $\pm$	243
DEAE-cellulose column eluate pool	170	0.67	88 $\pm$	79
Further 35–55%-satn.-(NH_4) $_2$ SO $_4$ precipitate†	20	1.29	109§	22

* Fully dialysed against 10 mM-sodium/potassium phosphate buffer, pH 7.0.

† Fully dialysed against 6 mM-sodium/potassium phosphate buffer, pH 6.8.

‡ This activity was the maximal obtained at low protein concentration.

§ This activity was independent of the protein concentration within the range 0.01–0.0025 mg/ml.

Fig. 2. Gel filtration of the benzylamine oxidase of human blood plasma on Sephadex G-200

A Sephadex G-200 column (4 cm $\times$ 100 cm) was equilibrated with 10 mM-sodium/potassium phosphate buffer, pH 7.0. The 35–55%-satn.-(NH₄)₂SO₄ precipitate of 5 litres of plasma was put on the column. Two pools of active fractions eluted by 0.1 M-phosphate buffer, pH 7.0, were collected: one (peak 1), coming on the tail of the peak of a yellow material, still contained the yellow colour, and the other (peak 2) was completely colourless. The addition of peak-1 material to peak-2 material inhibited the activity of the peak-2 material. The inhibition was decreased by dilution of the inhibitory fraction. The protein content of the pooled peaks was 12.7 mg/ml. The radioisotopic assay was performed in the following reaction mixture: 0.2 ml of pooled peaks, 0.05 ml of catalase (21 units/ml) and 0.05 ml of 10 mM-[¹⁴C]benzylamine. The incubation was for 30 min at 37°C in air with shaking. The

responsible for the fluctuations in benzylamine oxidase activity observed in different pathological conditions (McEwen, 1972).

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activity was the maximal obtained at low protein concentration within the range 0.05–0.005 mg/ml. (a) Activity at different protein concentrations. (b) ●, Protein; ■, activity.