α-Allenyl putrescine, an enzyme-activated irreversible inhibitor of bacterial and mammalian ornithine decarboxylases

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α-Allenyl putrescine (5,6-heptadiene-1,4-diamine) was designed as a new potential enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC). This compound, and more specifically its (*R*)-enantiomer, produced time-dependent inhibitions of *E. coli* and rat liver ODC. The inhibitions exhibit saturation kinetics and were not relieved by prolonged dialysis of the inactivated enzyme. Selective inactivation of the two types of ODC by the (*R*)-enantiomer is in agreement with the stereochemistry reported for ornithine decarboxylation by the enzyme. Kinetic constants of *E. coli* ODC inactivation by α-(*R*)-allenyl putrescine compare favorably with other irreversible inhibitors of this enzyme.

α-Allenyl putrescine *Enzyme-activated irreversible inhibitor* *Suicide substrate* *Ornithine decarboxylase* *Polyamine* *Stereochemistry*

1. INTRODUCTION

Considerable progress in the knowledge of the functional role of polyamines was achieved thanks to the availability of specific inhibitors of ornithine decarboxylase (EC 4.1.1.17, ODC), the enzyme responsible for the biosynthesis of putrescine [1,2]. Among these inhibitors, enzyme-activated irreversible inhibitors (also referred to as suicide substrates) [3,4] have proven to be particularly valuable. Such ODC inhibitors were α-halomethyl, α-vinyl and α-ethynyl analogues of ornithine and putrescine [5,8]. Mechanistic considerations led to suggest that the α-allenyl analogues were also potential enzyme-activated irreversible inhibitors of ODC [9]. We recently described the synthesis of α-allenyl putrescine (5,6-heptadiene-1,4-diamine) and of its (*R*)- and (*S*)-enantiomers [9]. Here, we report the effects of these compounds on bacterial and mammalian ODC, in vitro.

2. MATERIALS AND METHODS

2.1. Chemicals

DL-[1-^{14}C]Ornithine (58 Ci/mol) was purchased from the Radiochemical Centre, Amersham. All other chemical products were of the purest grade commercially available.

2.2. Enzymes

Biosynthetic *E. coli* ODC was prepared as following: *E. coli* (PR 7 strain), a gift from Dr J.E. Davies, was grown according to [10]. Cells were washed with 30 mM phosphate buffer (pH 7.8) and resuspended in 30 mM Tris–HCl buffer (pH 8.2) containing pyridoxal phosphate (0.1 mM), dithiothreitol (5 mM) and EDTA (0.1 mM). After sonication, the cell debris were removed by centrifugation at 20 000 × *g* for 30 min. The 105 000 × *g* supernatant, dialysed against the buffer used for homogenisation, was used for enzyme activity measurements. Specific activity of this preparation was 16 nmol CO_2/min per mg protein.

Mammalian ODC was prepared from livers of

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rats which had been injected with thioacetamide (150 mg/kg body wt) 18 h before sacrifice, and was purified about 10-fold from the homogenate by acid treatment at pH 4.6 as in [11]. Specific activity of this preparation was 0.2 nmol CO_2/min per mg protein.

2.3. Assays of time-dependent inhibition of ODC

For a typical experiment, 320 μl of stock solution of enzyme were mixed at time 0 with 80 μl of a solution of α-allenyl putrescine in water and incubated at 37°C. At various times, 25-μl aliquots were transferred into a 0.975 ml assay medium in a closed vessel in which a filter paper moistened with 50 μl of hyamine hydroxide (1 M) was fitted. The assay medium for the *E. coli* enzyme contained Tris–HCl (30 mM, pH 8.2), pyridoxal phosphate (0.01 mM), dithiothreitol (5 mM), L-ornithine (5 mM) and DL-[1-^{14}C]ornithine (1.25 μCi). For the rat liver enzyme, the assay medium contained sodium phosphate (30 mM, pH 7.1), pyridoxal phosphate (0.1 mM), dithiothreitol (5 mM), L-ornithine (0.09 mM) and DL-[1-^{14}C]ornithine (1.25 μCi). The reaction was allowed to proceed for 60 min at 37°C and was then terminated by the addition of 0.5 ml of 40% trichloroacetic acid. After an additional 30 min, the $^{14}CO_2$ absorbed on the filter paper was counted in a standard scintillation cocktail.

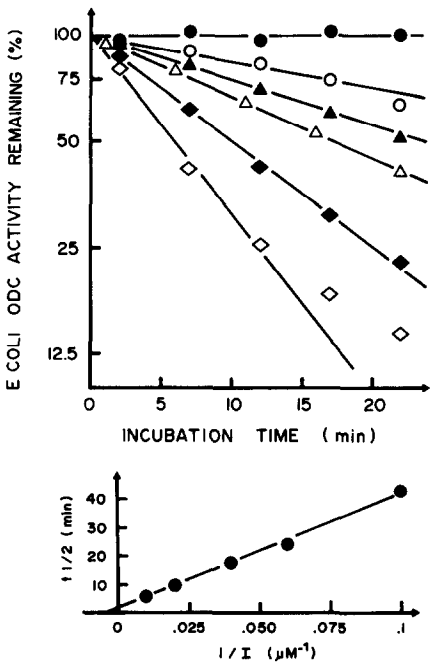

Fig.1. Time- and concentration-dependent inhibition of *E. coli* ODC in vitro. ODC was incubated at 37°C with Tris–HCl buffer (30 mM, pH 8.2), dithiothreitol (5 mM), pyridoxal phosphate (0.1 mM), EDTA (0.1 mM) and various concentrations of α-allenyl putrescine: (●) control; (○) 10 μm; (▲) 17 μM; (△) 25 μM; (◆) 50 μM; (◇) 100 μM). In the lower part of the figure, the times of half inactivation ($t_{1/2}$) are plotted against the reciprocal of the inhibitor concentration.

3. RESULTS

Incubation of the *E. coli* ODC preparation with α-allenyl putrescine resulted in a time-dependent loss of enzyme activity which followed pseudo first-order kinetics for approximately two half-lives. Over longer time periods, the semilogarithmic plot deviated from linearity (fig.1). Loss of activity was related to the concentration of inhibitor. By plotting the time of half inactivation ($t_{1/2}$) as a function of the reciprocal of the inhibitor concentration ($1/I$) according to [12], a straight line was obtained (fig.1). This line does not pass through the origin but intercepts the positive y-axis, demonstrating a saturation effect which involves the enzyme's active site in the inhibitory process. Kinetic constants for the time-dependent inhibition (K_I, the apparent dissociation constant and $\tau_{1/2}$ the time of half inactivation at infinite concentration of inhibitor) can be extrapolated from fig.1 and are given in table 1. The presence of dithiothreitol (5 mM) in the preincubation medium and the absence of a lag-time before the onset of inhibition rule out the possibility of inactivation via affinity labeling mode by a diffusible alkylating species [13]. Incubation with α-allenyl putrescine (0.1 mM) resulted in 95% inactivation of *E. coli* ODC after 30 min. Prolonged (24 h) dialysis of the inactivated ODC against a buffer solution containing Tris–HCl (30 mM, pH 8.2), pyridoxal phosphate (0.1 mM) and dithiothreitol (5 mM) (conditions under which the native enzyme is stable) did not lead to regeneration of enzyme activity, thus demonstrating the irreversibility of the inhibition.

Further experiments with the two optical isomers of α-allenyl putrescine showed that the inhibitory activity resides only in the (*R*)-enantiomer (fig.2). This enantiomer has a K_I of 140 μM, i.e., approximately half that of the racemic mixture.

Table 1
Kinetic constants of ODC inactivation by α-allenyl putrescine and its enantiomers

	E. coli ODC		rat liver ODC	
	K_I (μM)	$\tau_{1/2}$ (min)	K_I (μM)	$\tau_{1/2}$ (min)
α-(*RS*)-allenyl putrescine	300	1.4	160	8
α-(*R*)-allenyl putrescine	140	2	70	10
α-(*S*)-allenyl putrescine	No time-dependent inhibition at 2 mM[a]		3400	25

[a] α-(*S*)-allenyl putrescine was found to be a weak competitive inhibition of *E. coli* ODC (K_I = 1.5 mM)

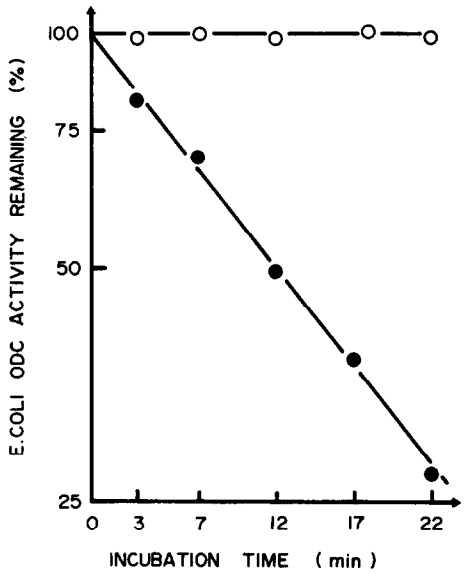

Fig.2. Time-dependent inhibition of *E. coli* ODC α-allenyl putrescine enantiomers. ODC was incubated at 37°C under conditions described in fig.1 with (●) 0.03 mM α-(*R*)-allenyl putrescine, or (○) 3 mM α-(*S*)-allenyl putrescine.

α-Allenyl putrescine and its enantiomers were also tested as time-dependent inhibitors of mammalian ODC. Results were qualitatively similar to those obtained with the bacterial enzyme. The main difference is that the (*S*)-enantiomer, at millimolar concentrations, inactivates rat liver ODC in a time-dependent manner (see table 1).

4. DISCUSSION

Our results represent the first example of an α-allenyl derivative of an amine as being an enzyme-activated irreversible inhibitor of a pyridoxal phosphate-dependent decarboxylase. Other allenyl amines were reported to be irreversible inhibitors of the flavin-enzyme monoamine oxidase (EC 1.4.3.4.) [14,15]. α-Allenyl GABA was claimed to be an irreversible inhibitor of GABA transaminase (EC 2.6.1.19) but results were not shown [16].

Here, we show the (*R*)-enantiomer of α-allenyl putrescine is the active optical isomer in the inhibition of both bacterial and mammalian ODC. This is in agreement with a previous finding that the (*R*)-enantiomer of γ-ethynyl putrescine inactivates ODC [17,18] and with the stereochemistry of bacterial ODC which decarboxylates L-ornithine with retention of configuration [19]. Inactivation of mammalian ODC by high concentrations of α-(*S*)-allenyl putrescine represents a further example of the lack of stringent stereospecificity in the inactivation of various pyridoxal phosphate-dependent enzymes by suicide substrates discussed recently [18].

α-Allenyl putrescine and its (*R*)-enantiomer do not compare favorably with other enzyme-activated irreversible inhibitors of mammalian ODC [8]. However, α-(*R*)-allenyl putrescine inactivates *E. coli* ODC at the same rate as α-monofluoromethyl putrescine which, so far, was the most potent inhibitor of this enzyme [20]. Inhibitors of polyamine biosynthesis have been used successfully to block bacterial growth [2,21,22]. In this respect, α-(*R*)-allenyl putrescine represents a promising alternative.

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