

Inhibition of Cell Proliferation by DL- α -Difluoromethylornithine, a Catalytic Irreversible Inhibitor of Ornithine Decarboxylase*

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The polyamines putrescine, spermidine and spermine have been implicated in a number of cellular processes, such as DNA, RNA and protein synthesis. With the recent development of specific polyamine synthesis inhibitors, it has become possible to directly analyze the cellular function(s) of the polyamines. In an attempt to study the role of polyamines in cell proliferation we have used a substrate analog of ornithine (α -difluoromethylornithine) in order to block the activity of ornithine decarboxylase (ODC; L-ornithine carboxylase; EC 4.1.1.17), the initial and rate-limiting enzyme in polyamine biosynthesis.

The possible mechanism of action of α -difluoromethylornithine^{1,2} is shown in Fig. 1. After the formation of a Schiff's base between the inhibitor and the pyridoxal 5'-phosphate cofactor in the active site of the enzyme (Step 1; Fig. 1), CO₂ and fluorine are eliminated (Step 2; Fig. 1). This results in a highly reactive electrophilic intermediate (a conjugated imine) which can alkylate a nucleophilic residue at or near the active site of the enzyme, thus covalently binding the inhibitor to the enzyme (Step 3; Fig. 1). Only one optical isomer (-)

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Table 1. Effects of α -difluoromethylornithine on polyamine metabolism and growth of Ehrlich ascites tumor cells in culture.

Treatment	Time after seeding/h	ODC activity/ nmol of ¹⁴ CO ₂ h ⁻¹ (10 ⁶ cells) ⁻¹	Putrescine	Spermidine	Spermine	Cell number/ 10 ⁶
			nmol (10 ⁶ cells) ⁻¹			
Control	0	0	0.73	3.21	1.33	10
	22	1.13	3.24	7.32	2.04	21
	48	0.48	2.97	6.86	1.86	95
	72	0.03	2.01	3.29	1.10	163
α -Difluoro- methylorni- thine (5 mM) ^a	22	0.34	0.16	0.42	2.82	19
	48	0.21	0.10	0.06	1.74	45
	72	0.17	0.06	0	1.42	60

^a The ODC inhibitor was added to the cell cultures at the time of seeding.

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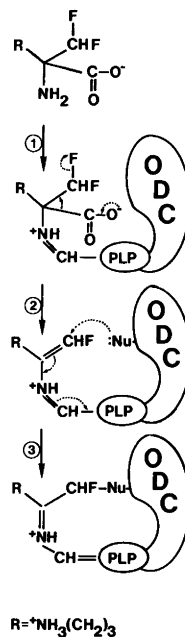


Fig. 1. Hypothetical mechanism of the catalytic irreversible inhibition of ODC by the substrate analog, α -difluoromethylornithine, according to Bey *et al.*¹ PLP, pyridoxal 5'-phosphate; Nu, nucleophilic residue.

possesses inhibitory activity, the other isomer(+) is essentially inactive.¹

Even though α -difluoromethylornithine is an irreversible inhibitor of ODC, there was no complete inhibition of the enzyme activity in the Ehrlich ascites tumor cells (Table 1). This is probably due to the high rate of turnover of this enzyme ($t_3 = 15$ min). The ODC-inhibition caused by α -difluoro-

methylornithine treatment resulted in an excessive depletion of cellular putrescine and spermidine (Table 1). The cellular spermine content, however, was found to be significantly increased, probably as a consequence of the putrescine-deficient state; at normal levels putrescine exerts an inhibitory action on spermine synthase.³

The depletion of putrescine and spermidine resulted in a deceleration of the growth rate of the Ehrlich ascites tumor cells, beginning approximately 22 h after seeding, *i.e.* when the cells had traversed almost two cell cycles. This suggests that the cells possess an excess of polyamines that is sufficient for a limited time of unperturbed growth and division. Once the polyamine content has been significantly reduced, however, the cells grow slowly and synthesize their macromolecules very poorly, suggesting that polyamines (at least putrescine and spermidine) are essential for a maximum rate of cell proliferation.

Experimental. A hyperdiploid subline of the Ehrlich ascites tumor was adapted to suspension growth as previously described.⁴ In the present experiment 1×10^7 plateau phase cells were suspended in 100 ml of fresh growth medium in the presence or absence of 5 mM DL- α -difluoromethylornithine in a 150 cm² Costar flask. At various times after seeding, the cell number was determined in a Coulter counter and samples were taken for ODC assay and polyamine analysis. A radioassay was used for the determination of the ODC activity,⁵ and a thin-layer chromatographic technique was used for the analysis of polyamines.⁶

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