THE EFFECTS OF 1,4-DIAMINOBUTANONE ON POLYAMINE SYNTHESIS IN *ASPERGILLUS NIDULANS*

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1. Introduction

The naturally occurring polyamines, putrescine, spermidine and spermine are synthesized in most eucaryotes from ornithine and methionine [1,2] by the pathway outlined in fig.1. The functions of these amines which often occur in millimolar concentrations [1,2] are unknown. They are synthesized most rapidly in proliferating cells [2]. In order to try to establish their functions much work has recently focussed on the development of specific inhibitors of polyamine synthesis [3–11].

Putrescine occupies a central position in the synthesis of spermidine and spermine:

(i) It has been found to inhibit the production of ornithine decarboxylase [12–17]

(ii) It is a weak competitive inhibitor of ornithine decarboxylase [17–20]

(iii) It is an activator of *S*-adenosyl methionine decarboxylase [17,20–23]

(iv) It is a substrate for spermidine synthase [1,2]

(v) It is a competitive inhibitor of spermine synthase [24].

During a search for a gratuitous repressor of ornithine decarboxylase such as has now been found for this enzyme in regenerating liver [25] we tested a number of compounds having structures similar to putrescine and found that one of these, 1:4 diaminobutanone, stimulated the increased production of ornithine decarboxylase in germinating conidia of *Aspergillus nidulans*. On further examination this compound was found to be a powerful competitive inhibitor of ornithine decarboxylase, it could replace putrescine as an activator of *S*-adenosyl methionine decarboxylase, and it also caused a reduction in the intracellular concentrations of spermidine and spermine.

2. Materials and methods

The amines tested in this paper were purchased either from Aldrich Chemical Co. Ltd., BDH Ltd., or Sigma Chemical Co. *Aspergillus nidulans* BWB 272 was maintained on agar-slopes and grown in submerged culture as described previously [17]. Enzyme extracts

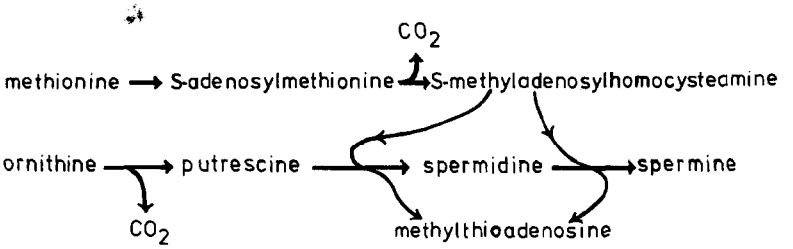

Fig.1. Pathway of polyamine biosynthesis.

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were prepared by grinding conidia with acid washed sand and the extracts were centrifuged at 25 000 × *g* for 15 min [17]. Ammonium sulphate, 410 mg, was added/ml supernatant. The precipitated protein was redissolved in the extraction buffer and dialysed overnight [17]. Ornithine decarboxylase and *S*-adenosyl methionine decarboxylase assays were carried out as described previously [17]. Polyamines were extracted into perchloric acid, dansylated and separated by thin layer chromatography [26,27].

3. Results and discussion

A number of amines were tested as possible inhibitors of ornithine decarboxylase. The following were added to the ornithine decarboxylase incubation mixtures at final concentrations of 0.13 mM and 1.3 mM:

$CH_3 . NH . (CH_2)_3NH_2$, $(CH_3)_2N . (CH_2)_3 . N(CH_3)_2$,

$H_2N . CH_2 . CHOH . CH_2 . NH_2$,

$CH_3NH . (CH_2)_4NH . CH_3$,

$H_2N . CH_2CO . CH_2 . CH_2 . NH_2$,

$H_2N . CH_2 . CH_2 . O . CH_2 . CH_2 . NH_2$,

$H_2N . NH . CO . CO . NH . NH_2$, $H_2N(CH_2)_2NH_2$,

$H_2N(CH_2)_3NH_2$, $H_2N(CH_2)_5NH_2$ and

$H_2N(CH_2)_6NH_2$.

None of these amines, except for 1,4-diaminobutanone, reduced ornithine decarboxylase activity by more than 25% as compared with the controls. Both 0.13 mM and 1.3 mM 1,4-diaminobutanone reduced ornithine decarboxylase activity to undetectable levels. Inhibition by 1,4-diaminobutanone was found to be competitive with respect to ornithine [fig.2] having a $K_i = 0.91$ μM. Thus 1,4-diaminobutanone is a much stronger competitive inhibitor of ornithine decarboxylase than putrescine, the latter having $K_i = 60$ μM [17]. The same amines were tested as possible activators of *S*-adenosyl methionine decarboxylase. All were found to be weak activators except for 1,4-diaminobutanone which causes activation comparable with that of putrescine (table 1).

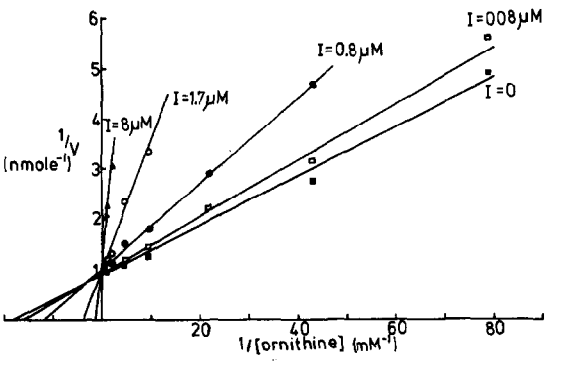

Fig.2. The effect of 1,4 diaminobutanone (= *I*) on ornithine decarboxylase.

The effect of adding 0.01 mM and 0.6 mM concentrations of the above amines to the growth medium was tested in order to see whether they affected the levels of ornithine decarboxylase during germination. Again only 1,4-diaminobutanone was found to affect the ornithine decarboxylase activity. It induced higher levels of ornithine decarboxylase (when measured in dialysed extracts) and it also reduced significantly the intracellular concentrations of spermidine but it raised the levels of putrescine (table 2). Diaminobutanone, 0.6 mM, caused approx. 1 h delay in conidial germination whereas 0.01 mM did not detectably alter the time of germination.

1,4-Diaminobutanone is thus a powerful competitive inhibitor of ornithine decarboxylase having an affinity at least as great, if not greater, than α-methylornithine [5], α-hydrazino-ornithine [4], *N*-(5-phosphopyridoxy)-ornithine [9], 1,4-diamino-*trans*-butene [8] and dehydro-ornithine. It activates *S*-adenosyl methionine decarboxylase but to a lesser extent than

Table 1
Activation of *S*-adenosyl methionine decarboxylase by 1,4-diaminobutanone

Amine added		nmol $^{14}CO_2$/30 min
None		0.087
Putrescine	1.0 mM	1.87
Diaminobutanone	0.5 mM	0.33
Diaminobutanone	1.0 mM	0.60
Diaminobutanone	2.0 mM	0.96
Diaminobutanone	4.0 mM	1.39

Table 2
The effect of 1,4-diaminobutanone on ornithine decarboxylase and intracellular polyamine levels in 8 h germinating conidia of *A. nidulans*

Concentration of diaminobutanone in medium	Ornithine[a] decarboxylase	Putrescine	Spermidine	Spermine
		(μmol/10^{10} conidia)		
None	97	0.27	1.46	0.36
0.01 mM	180	0.6	1.19	0.46
0.6 mM	205	0.88	0.22	0.15

[a]nmol CO^2/30 min/10^9 conidia

putrescine. In all tissues and organisms in which ornithine decarboxylase turnover has been studied it has been found to be rapid.

Diaminobutanone may therefore combine with ornithine decarboxylase and stabilize it against proteolysis thus accounting for the increased level in dialysed conidial extracts. It is possible that the decreased intracellular concentrations of spermidine or spermine observed in the presence of diaminobutanone may be due to the latter inhibiting spermidine and spermine synthase. Since diaminobutanone has such a marked effect on polyamine synthesis it may prove a useful tool for further understanding the control of polyamine biosynthesis and it may throw light on the functions of polyamines. We are investigating its potential further.

Acknowledgement

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