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BETAINE-HOMOCYSTEINE METHYLTRANSFERASE IN THE FUNGUS *Aspergillus nidulans*

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SUMMARY: A betaine:homocysteine methyltransferase activity was demonstrated in the cell-free extracts from the fungus *Aspergillus nidulans*. Among methionine-requiring mutants which do not grow on homocysteine one class responds to betaine indicating that this compound can serve as a methyl donor in methionine synthesis *in vivo*. Mutants of the second class which grow only on methionine were shown to have betaine:homocysteine - and methyltetrahydrofolate:homocysteine methyltransferases simultaneously impaired.

INTRODUCTION

Fungi posses a vitamin B-12 independent methyltetrahydropteroil-tri-glutamate:homocysteine methyltransferase (EC 2.1.1.14) which utilizes the triglutamate derivative of methyltetrahydrofolate as methyl donor (1,2). The exsistance of an alternative reaction of homocysteine methylation was indicated by the fact that the methionine requirement of an *Aspergillus nidulans* mutant could be satisfied by betaine or choline (3). In this paper we describe the presence of an enzyme with betaine:homocysteine methyltransferase (EC 2.1.1.5)

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Abbreviations used: *meth*, *pyro*, *ad*, *bi*, *pro*, *paba* - requirements of methionine, pyridoxine, adenine, biotin, proline and paraaminobenzoic acid, respectively. y - yellow conidia, *aga* - inability to use arginine as a nitrogen source, THF - tetrahydrofolate.

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activity in cell-free extracts from this fungus. Betaine:homocysteine methyltransferase has been observed previously only in animals (4,5,6,7,8,9) and the bacterium Pseudomonas denitrificans (10). This is the first demonstration of this enzyme in a fungus. Both genetic and biochemical findings suggest that in the studied organism the betaine:homocysteine- and methyltetrahydrofolate:homocysteine methyltransferases may have a common component or are the same enzyme protein.

MATERIAL AND METHODS

Strains, culture conditions and extract preparation: Two methionine-requiring mutants of A.nidulans, methH2/pyroA4, yA1/ and methD10/pyroA4,yA1/ used in this work were described previously (3,11). Strain methH3/adC1,proA6,pabaA1,biA1,aga90/ carrying a methionine mutation allelic to methH2 was obtained from the Department of Genetics, University of Warsaw. The mutants methH2 and methH3 are slightly leaky. They grow at a normal rate on methionine only, whereas methD10 grows as well on choline and betaine. Neither strain grows on homocysteine. Strain pyroA4,yA1 referred to hereafter as the wild type, was used as the reference in the experiments.

The cells were grown in liquid minimal medium (12) with L-methionine (0.1mM) or betaine (4mM) and all necessary supplements in a rotary shaker at 30°C for 16-18 hours. The cells were harvested and enzyme extracts prepared as described previously (13). Crude extracts were used for enzyme determinations. Protein was estimated by the method of Lowry et al. (14).

Enzyme assays: Methyltetrahydrofolate:homocysteine methyltransferase was assayed as described previously (13). Betaine: homocysteine methyltransferase was determined by the method of Finkelstein and Mudd (8), serine hydroxymethyltransferase (EC 2.1.2.1) and methylenetetrahydrofolate oxydoreductase (EC 1.5.1.5) according to Scrimgeour and Huennekens (15). Methylenetetrahydrofolate reductase (EC 1.1.1.68) was determined as described by Mangum and North (16).

RESULTS

The data given in Table 1 indicate that the mutants methH2 and methH3 exhibit a lower specific activity of methyltetrahydrofolate:homocysteine- and betaine:homocysteine methyltransferases as compared with the wild type, while both activities are evidently elevated in methD10. On the other hand, the latter strain shows lower activities of the two enzymes involved in

Table 1. Activity of CH_3THF:homocysteine methyltransferase and betaine:homocysteine methyltransferase in the wild type and methH2, methH3 and methD10 strains of A.nidulans

Strain	Specific activity nmole/h/mg protein	
	CH_3-THF: homocysteine	Betaine: homocysteine
wild type	6.0	18.5
methH3	1.4	4.2
methH2	4.6	12.9
methD10	11.2	32.7

The numbers are based on 4-6 experiments

the synthesis of methyltetrahydrofolate, i.e. serine hydroxymethyltransferase and methylenetetrahydrofolate reductase (Table 2). This suggests that the methionine requirement of this mutant results from impairement in the synthesis of a methyl donor, which, however, can be substituted by betaine.

Interestingly, both methyltransferases are more thermolabile in the methH2 strain than in the wild type (Fig. 1).

Table 2. Activity of methylenetetrahydrofolate reductase, serine hydroxymethyltransferase and methylenetetrahydrofolate oxydoreductase in the wild type, and methH2 and methD10 strains of A.nidulans

Strain	Methylenetetrahydrofolate reductase[a]	serine hydroxymethyl transferase[b]	methylenetetrahydrofolate oxydoreductase
	nmole/h/mg protein		
wild type	0.60	4353	-
methH2	0.77	3394	315
methD10	0.31	1737	306

a - nmoles of formaldehyde liberated
b - nmoles of formaldehyde used

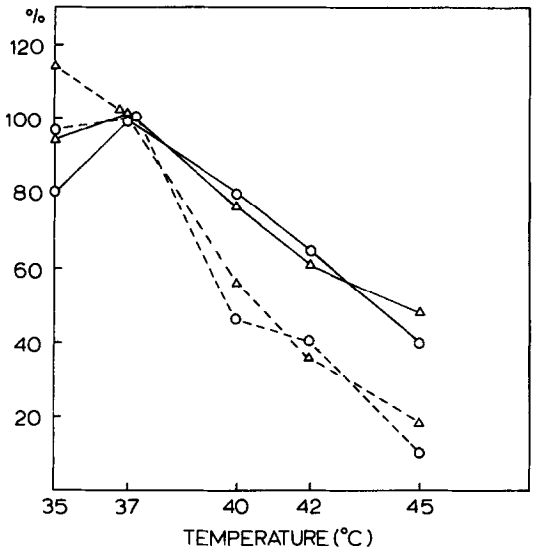

Fig. 1: Dependence of methyltetrahydrofolate: homocysteine-(Δ) and betaine: homocysteine (o) methyltransferases on temperature: ——— wild type, ------ methH2.

The results shown in Table 3 indicate that addition of betaine to the growth medium results in an elevation of the levels of both transmethylases.

Table 3. Effect of betaine in the growth medium on the levels of CH_3THF:homocysteine- and betaine:homocysteine methyltransferases in A.nidulans

Exp.	Medium	Specific activity nmole/h/mg protein	
		CH_3THF: homocysteine[a]	Betaine: homocysteine
1.	minimal	3.4	12.2
	minimal + betaine (4mM)	6.9	31.4
2.	minimal	3.5	15.0
	minimal + betaine (4mM)	5.8	42.5

a - non-induced levels of CH_3THF:homocysteine methyltransferase in these experiments were much lower than that observed previously (Table 1) due to a different preparation of methyltetrahydrofolate.

DISCUSSION

Aspergillus nidulans posseses an enzyme which can utilize betaine as a methyl donor in methionine synthesis. The existance of mutants in which methionine requirement can be satisfied by betaine indicates that it can serve as a methyl donor for methionine synthesis *in vivo*. It was rather surprising to find that *methH* mutants show simultaneous impairement of both CH_3THF; homocysteine and betaine:homocysteine methyltransferases and in one of them both activities are more thermolabile then in the wild type. On the other hand these findings account for the failure of *methH* mutants to grow on betaine. Interestingly both activities are coordinately enhanced when mycelium is grown in the presence of betaine.

On the basis of *in vitro* assays one would expect that both *methH* mutants have sufficient enzyme to allow growth even in the absence of methionine. It was observed, however, that in transformed mammalian cells a similar decrease in CH_3THF homocysteine activity caused methionine requirement (17).

If the impairement of the studied enzyme is not responsible for auxotrophy one has to assume that another enzyme methylating homocysteine is involved which is affected in *methH* strains. This seems unlikely as both *methH* alleles are normal single gene mutations. Indead our results are most easily compatible with a hypothesis that there is only one homocysteine methylating enzyme which can utilized several methyl donors or there are two enzymes which have the same protein component. It is worth noting, that the enzyme described in *Pseudomonas denitrificans* (10) can utilize both betaine and dimethylacetothetin as methyl donors for methylation of homocysteine.

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