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Enzymatic Lesions in Methionine Mutants of *Aspergillus nidulans*: Role and Regulation of an Alternative Pathway for Cysteine and Methionine Synthesis

ANDRZEJ PASZEWSKI* AND JERZY GRABSKI

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Rakowiecka 36, 02-532 Warsaw, Poland

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In *Aspergillus nidulans* the pathway involving cystathionine formation is the main one for homocysteine synthesis. Mutants lacking cystathionine γ-synthase or β-cystathionase are auxotrophs suppressible by: (i) mutations in the main pathway of cysteine synthesis (*cysA1*, *cysB1*, and *cysC1*), (ii) mutations causing stimulation of cysteine catabolism (*su101*), and (iii) mutations in a presumed regulatory gene (*suAmeth*). A relative shortage of cysteine in the first group of suppressors causes a derepression of homocysteine synthase, the enzyme involved in the alternative pathway of homocysteine synthesis. A similar derepression is observed in the *suAmeth* strain. Homocysteine synthesized by this pathway serves as precursor for cysteine and methionine synthesis. A mutant with altered homocysteine synthase is a prototroph, indicating that this enzyme is not essential for the fungus.

In spite of the research on the methionine-requiring mutants and their suppressors in *Aspergillus nidulans* (2, 10, 15, 26, 28), it is still not clear whether in this organism homocysteine, the immediate precursor of methionine, is synthesized as in *Neurospora* via cystathionine (13) by the following sequence of reactions:

O-acetylhomoserine + cysteine →
cystathionine + acetic acid (1)

cystathionine + water →
homocysteine + pyruvate + NH_3 (2)

or by direct sulfhydrylation:

O-acetylhomoserine + H_2S →
homocysteine + acetic acid (3)

which seems to be the main pathway for homocysteine synthesis in *Saccharomyces cerevisiae* (4).

We have found previously that *A. nidulans* has the enzymes catalyzing reactions 2 and 3, and both of these enzymes are repressed when the mycelium is grown in the presence of methionine or homocysteine (20). This observation suggested that both pathways might be of physiological importance in the fungus. Results obtained previously (21) suggested that the cystathionine pathway is the main one for methionine synthesis in *Aspergillus*, since a mutant lacking β-cystathionase proved to be methionine dependent.

Here we identify the lesions present in other *meth* mutants (10) and also give results bearing on the mechanisms by which certain suppressor mutations (10) are able to suppress mutations in three different *meth* genes.

The results confirm that reactions 1 and 2 constitute the main pathway for methionine synthesis in *A. nidulans* since mutants blocked at these steps are auxotrophs (Fig. 1). Evidence is also provided that the second, minor pathway involving reaction 3 can substitute for the main one when homocysteine synthase, the enzyme catalyzing this reaction, is derepressed. This is found in strains with a block in the main pathway of cysteine synthesis or a mutation in a regulatory-like gene with pleiotropic effects on various cysteine- and methionine-synthesizing enzymes.

MATERIALS AND METHODS

Biological material. Strains used in this work are listed in Table 1. Mutant *cysD1* lacks homocysteine synthase (21), mutant *cysA1* lacks serine transacetylase (22), mutants *mecA1* and *mecB10* are devoid of cystathionine β-synthase and γ-cystathionase, respectively (21, 23, 24), and *sB3* lacks sulfate permease (1). For nutritional and biochemical experiments, the strains listed in column 3 of Table 1 were used. Strain *pyroA4y*, referred to hereafter as the wild type, was used as the reference strain in the experiments.

TABLE 1. *Biological material*[a]

Strain	Source	Strains derived by outcrossing
methG1, pabaB23, biA1	Department of Genetics, University of Glasgow	*methG1, pyroA4, y*
methB3, pyroA4, y		
methH2, ActA, biA1, galE9		*methH2, pyroA4, y*
pyroA4, malA1, y		
SB3, proA2, pabaA2, biA1, lysB5		
methD10, adF9, y	Gajewski and Litwińska (10)	*methD10, pyroA4, y*
methA17, adF9, y		*methA17, pyroA4, y*
methE31, adF9, y		*methE31, pyroA4, y*
methG55 (methF55), biA1 Acr1, paba22, pyroA4, y		*methG55, pyroA4, y*
su18, proA2, pabaA2, biA1		
suAmeth (su25), methB28, proA2, pabaA2		*suAmeth, pyroA4, y*
su101, methB3, pyroA4, y	Ayling (2)	*su101, pyroA4, y*
cysB1 (su102), methB3, pyroA4, y		*cysB1, pyroA4, y*
cysC1 (su103), methB3, pyroA4, y		*cysC1, pyroA4, y*
cysD1, riboD5, y	Paszewski and Grabski (21)	
cysA1, proA2, pabaA3	Pieniążek et al. (22)	
mecB10, biA1, phenA2, anA1	Pieniążek et al. (23)	
mecA1, biA1, anA1		

[a] Symbols: *ad*, adenine; *bi*, biotin; *meth*, methionine; *pyro*, pyridoxine; *paba*, *para*-aminobenzoic acid; *lys*, lysine; *Acr*, resistance to acridine; *Act*, resistance to actidione; *mal*, inability to utilize maltose; *gal*, inability to utilize galactose; *y*, yellow conidia; *su*, suppressor (these mutations are suppressors of methionine mutants). Original symbols given by the authors are in parentheses. Gene symbols follow the suggestions of Clutterbuck (5).

Various double mutants obtained by outcrossing are described below along with the experiments in which they were used.

Media, culture conditions, and genetic techniques. For nutritional tests, a solid minimal medium, described by Cove (6), supplemented with different sulfur-containing and methyl donor substances, was used. Liquid minimal medium and culture conditions were as described previously (20). Crossing methods and heterocaryon tests were as described by Pontecorvo et al. (25).

Biochemical techniques. Enzyme extracts were prepared by the method of Paszewski and Grabski (20, 21). Homocysteine synthase was assayed as described previously (20); the homocysteine formed was determined by the method of Kredich and Tomkins (14). Cysteine synthase (EC 4.2.99.7) and cystathionine β-synthase (EC 4.2.1.22) were assayed according to Pieniażek et al. (24), and adenosine 5'-triphosphate (ATP)-sulfurylase (EC 2.7.7.4) and sulfite reductase (EC 1.8.1.2) were assayed by the method of de Vito and Dreyfuss (8). The sulfide formed was determined according to Siegel (29). β-Cystathionase (EC 4.4.1.1), γ-cystathionase (EC 4.4.1.8), and sulfate permease were measured by the method described previously (21). Isolation and identification of ^{35}S-labeled amino acids were carried out by the method of Paszewski and Grabski (21).

Protein was estimated by the method of Lowry et al. (16), with bovine serum albumin as the standard.

Reagents. L-Cystathionine was a gift from M. Flavin, hypotaurine was from B. Jolles-Bergeret, and acetothetine, propiothetine, and *S*-methylmethionine were from F. Schlenk. ATP, glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate, L-serine, L-homoserine, DL-homocysteine thiolactone-hydrochloride, L-cysteine, *S*-methylcysteine, and *S*-methylisothiourea were obtained from Sigma Chemical Co. (St. Louis, Mo.). Pyridoxal-5-phosphate, L-methionine, and betaine were purchased from Merck (West Germany). ^{35}S-labeled sodium sulfate was from the Institute of Nuclear Research, Swierk, Poland. Sulfocysteine was a gift from T. Nakamura.

O-acetyl-L-serine and *O*-acetyl-L-homoserine were synthesized as described by Wiebers and Garner (31). [^{35}S]cystathionine was prepared by growing the *cysA1mecB1* strain, which accumulates cystathionine (21), on radioactive sulfate. The radioactive cystathionine was isolated by preparative thin-layer chromatography on cellulose plates as described previously (21).

All inorganic chemicals were of reagent grade.

RESULTS

Growth responses of meth mutants to sulfur and methyl donors. The results of

growth tests involving methionine-requiring mutants bearing lesions in six loci described previously are given in Table 2. All but one mutant, *methE31*, were leaky. Strain *methE31* responded to *O*-acetylhomoserine. This property suggests that it is blocked at the homoserine acetylation step (Fig. 1) and indicates that *O*-acetylhomoserine is an intermediate in methionine synthesis in *A. nidulans* as in yeast (7) and *Neurospora* (19). All mutants, again with the exception of strain *methE31*, responded to *S*-methylisothiourea. On the other hand, strain *methE31* was the only mutant that could grow on cystathionine.

Mutants *methH2* and *methD10* did not respond to homocysteine and are thus impaired in the methylation of homocysteine or in the ability to make a suitable methyl donor. Strain *methD10* grew on the medium supplemented with choline and betaine, indicating that *Aspergillus* has an enzyme with betaine-homocysteine methyltransferase activity such as has been observed in *Pseudomonas denitrificans* (30) and in animal tissues (9, 11). Further characteristics of these two mutants will be presented elsewhere.

Identification of the enzymatic blocks in meth mutants. Enzymatic assays showed that two mutants, *methG55* and *methG1*, had only traces of β-cystathionase activity, whereas homocysteine synthase activity was at a normal level in both mutants (Table 3). Autoradiography of sulfur amino acids isolated from *methG55* showed considerable accumulation of cystathionine (Fig. 2: I3 and II1). A similar accumulation of cystathionine was observed in mutant *methG1*, confirming the lack of β-cystathionase activity.

Although strain *methG55* (formerly *methF55*) complemented with strain *methG1* (10), it failed to complement with at least three of five *methG* alleles tested. Cross *methG1* × *methG55* yielded no methionine prototrophs among over 700 progeny. Therefore, *methG55* should be classified as a *methG* allele. Consequently, we considered *methF* not to be a valid locus. Strains *methA17* and *methB3* accumulate less cystathionine than the wild type and thus may have a block in cystathionine γ-synthase (Table 4). Since we had difficulty in detecting cystathionine γ-synthase in the wild type, we decided to study cystathionine levels in vivo. The results (Table 4) indicate that the addition of *O*-acetylhomoserine to the growth medium caused a considerable accumulation of cystathionine in the wild type, whereas it had no effect on the level of this compound in *methA17*

TABLE 2. *Growth response of A. nidulans meth⁻ mutants to the sulfur and methyl donors*

Mutant	Growth[a]																
	None[b]	L-Serine	L-Homoserine	L-Cysteine	*S*-methylcysteine	*O*-acetylhomoserine	L-Cystathionine	*S*-methylisothiourea	DL-Homocysteine thiolactone hydrochloride	L-Methionine	Dimethylthetin	Dimethylpropiothetin	Choline	Betaine	*S*-methylmethionine
methA17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
methB3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
methD10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
methE31	−	+	−	−	−	+	+	−	+	+	−	−	−	−	−
methG55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
methG1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
methH2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Wild type	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] Growth was measured after 48 h. Symbols (diameter of colony): +, 3 mm; ++, 3 to 6 mm; +++, 10 to 15 mm; ++++, 15 mm; −, no growth.
[b] Supplement to minimal medium (1 mM).

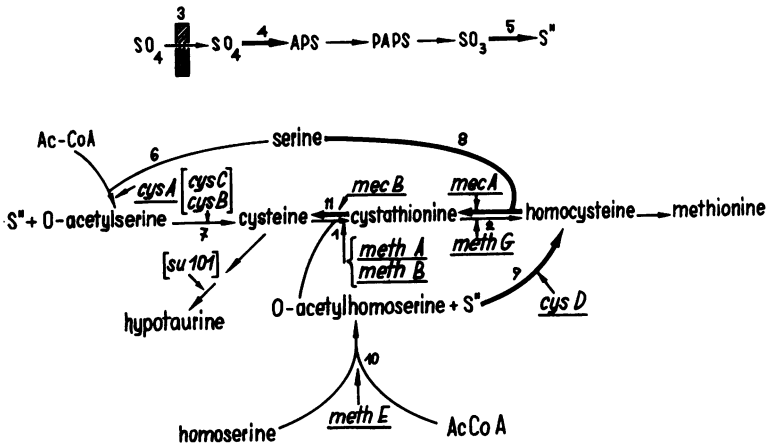

FIG. 1. *Outline of sulfur amino acid metabolism in A. nidulans. Enzymes catalyzing reactions marked with heavy lines are were found derepressed in strains cysA, cysB, cysC, and suAmeth. These four mutations suppress lesions in meth mutants lacking cystathionine γ-synthase (EC 4.2.9.9.9) (1) and β-cystathionase (EC 4.4.1.8) (2). The symbols cysB, cysC, and su101 are given in brackets since the biochemical character of these mutants has not yet been determined. 3, Sulfate permease; 4, ATP-sulfurylase (EC 2.7.7.4); 5, sulfite reductase (EC 1.8.1.2); 6, serine acetyltransferase (EC 2.3.1.30); 7, cysteine synthase (EC 4.2.99.8); 8, cystathionine β-synthase (EC 2.1.22); 9, homocysteine synthase; 10, homoserine acetyltransferase (EC 2.3.1.31); 11, γ-cystathionase (EC 4.4.1.1).*

TABLE 3. *Activities of β-cystathionase and homocysteine synthase in the wild type and the methionine-requiring mutants of A. nidulans*[a]

Strain	Sp act[b]	
	β-Cystathionase	Homocysteine synthase
methA17	1.81	24.5
methB3	1.60	20.5
methD10	2.30	35.5
methE31	1.58	17.3
methG55	0.0–0.1	23.4
methG1	0.0–0.1	23.8
methH2	1.88	24.9
Wild type	1.88	34.0

[a] Mycelia were grown in minimal medium supplemented with 0.2 mM L-methionine for 16 h at 32 C.
[b] Nanomoles per minute per milligram of protein.

and *methB3*. As expected, the addition of *O*-acetylhomoserine to the culture of *methE31* promoted synthesis of cystathionine and enabled this mutant to produce methionine. The consequent cysteine utilization resulted in a reduction of the level of hypotaurine, a catabolite of cysteine. The double mutants *methA17-methG55* and *methB3methG55* form 28- and 9-fold less cystathionine, respectively, than does strain *methG55* (Fig. 2), showing that the lesions *methA17* and *methB3* are epistatic to *methG55*, a further indication that they lead to loss of cystathionine γ-synthase. Unexpectedly, these two mutants did not grow on cystathionine. However, the introduction of the *sB3* mutation (lack of sulfate permease) enabled them to grow on cystathionine, in contrast to *methG* strains (Table 5). In addition, the wild type did not take up cystathionine when grown on the minimal medium containing 2 mM sulfate, but on transfer to the sulfur-free medium for 6 h it took up cystathionine at the rate of 0.7 nmol/min per mg (dry weight). When the wild type and *methA17* grown on 0.4 mM L-methionine were transferred to the sulfur-free medium for 6 h, only the former strain showed partial recovery of cystathionine uptake (0.11 nmol/min per mg [dry weight]). These observations imply the interference of some sulfur compound in cystathionine uptake by strains *methA17* and *methB3*.

The operation of the second pathway of methionine synthesis is indicated by the four to five times lower content of [^{35}S]methionine in the double mutants *methA17cysD1* and *methB3cysD1* than in strains *methA17* and *methB3* grown under the same experimental conditions (Table 6); the *cysD1* mutation results in loss of homocysteine synthase (21).

Classification of the suppressor type 1 loci and their specificity towards meth mutants. Methionine type 1 suppressors with normal morphology, isolated by Gajewski and Litwińska (10) and by Ayling (2), were tested in

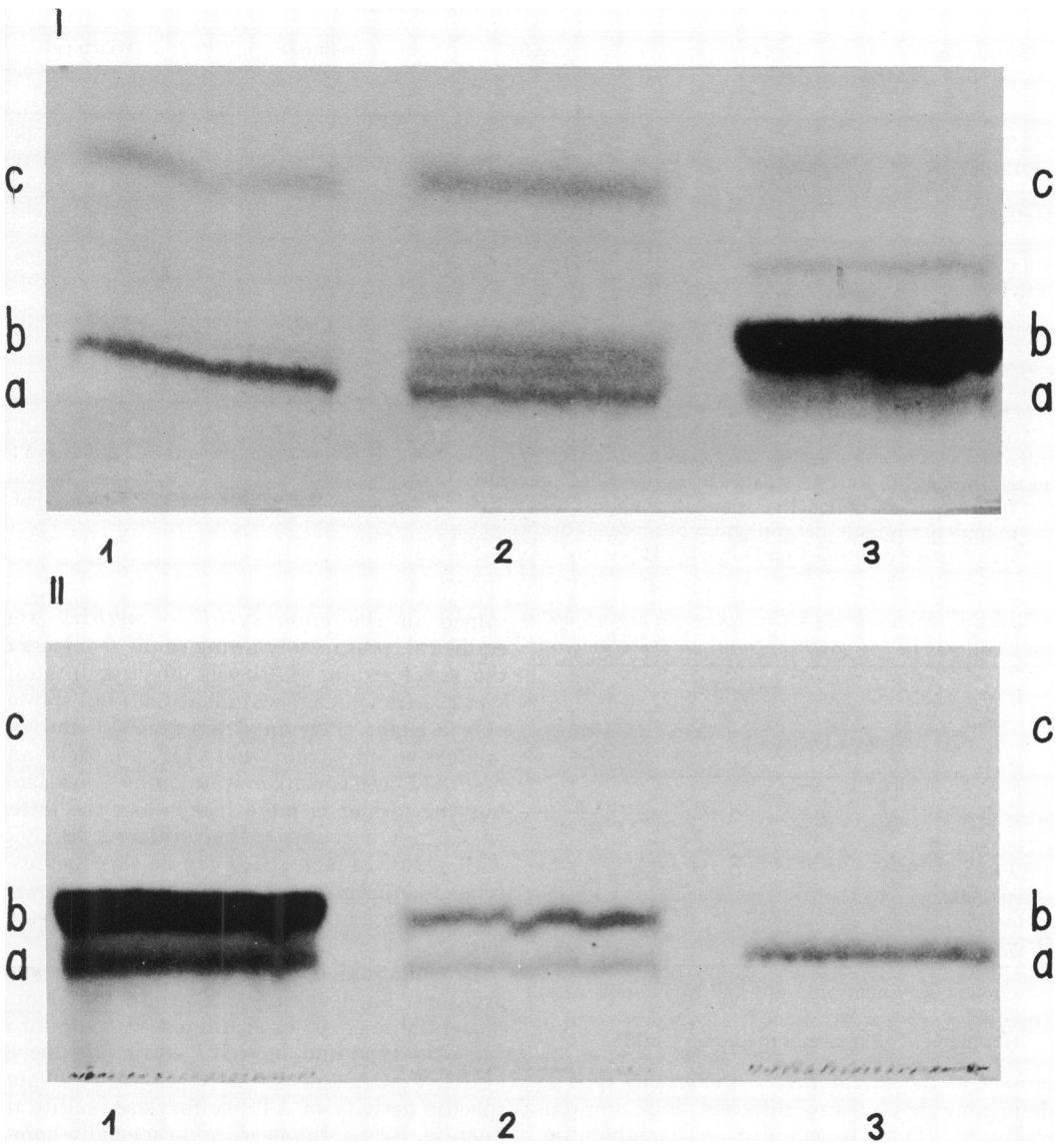

FIG. 2. *Autoradiography of ^{35}S-labeled compounds: fraction retained on Dowex 50 H^+ from the water extracts of methA17 (I1), methA17methG55 (I2), methG55 (I3), and methG55 (II1), methB3methG55 (II2), and methB3 (II3). Mycelia were grown in minimal medium supplemented with 0.1 mM* L-*methionine for 13 h, transferred to minimal medium containing 0.2 mM $Na_2{}^{35}SO_4$ (9.9×10^8 to 10.5×10^8 counts/min per mmol), and grown for an additional 8 h. Isolation and separation of amino acids were as described by Paszewski and Grabski (25). For each strain, material corresponding to 10 mg of mycelial dry weight was analyzed. Only the lower parts of the chromatograms are shown. The spot between glutathione and cystathionine (I2) and above glutathione (I1) was not identified in these experiments, but most likely it is cystine. (a) Glutathione, oxidized; (b) cystathionine; (c) hypotaurine.*

TABLE 4. *Accumulation of ^{35}S-labeled amino acids in mycelia of the wild type and methA17, methB3, and methE31 strains grown in the presence of $^{35}SO_4$ with (+) or without (−) 1 mM O-acetylhomoserine*[a]

Amino acid[b]	Accumulation[c] in strain:							
	methA17		*methB3*		*methE31*		Wild type	
	−	+	−	+	−	+	−	+
Glutathione	1,037	860	985	868	485	1,593	1,623	748
Cystathionine	246	290	175	175	43	1,460	369	631
Hypotaurine	152	196	292	238	385	55	238	133
Methionine	149	114	96	101	0	150	290	245
Total radioactivity[d]	1,988	1,723	1,871	1,611	1,805	5,189	3,219	2,458
Glutathione/cystathionine	4.2	2.96	5.6	4.96	11.3	1.09	4.09	1.18

[a] Mycelia were grown in minimal medium supplemented with L-methionine (0.2 mM) at 32 C for 13 h, transferred to minimal medium containing 0.2 mM $Na_2{}^{35}SO_4$ (3.2×10^5 counts/min per mmol), and grown for an additional 9 h. Further treatment as described previously (25).

[b] Only the main radioactive spots are included. Some cystine was always present in the glutathione spot.

[c] Counts per minute per milligram of mycelial dry weight.

[d] Radioactivity retained on Dowex 50 H^+.

TABLE 5. *Effect of the sB mutation on growth of methA, methB, and methG strains on cystathionine*

Strain	Growth[a]			
	None[b]	L-Cysteine	L-Cystathionine	L-Methionine
sB3	+	++++	++++	++++
methA17	+	+	+	++++
sB3methA17	−	−	++++	++++
methB3	+	+	+	++++
sB3methB3	−	−	++++	++++
methG55	+	+	+	++++
sB3methG55	−	−	−	++++

[a] Growth was measured after 48 h; symbols as in Table 1.

[b] Supplement to minimal medium (1 mM).

crosses of the *suXmethB* × *suYmethB* type to determine how many different loci are represented. The appearance of methionine-requiring segregants in the progeny of these crosses indicated that suppressors were nonallelic. It was found that *cysC1* and *su18* are allelic and that the remaining three, *su101*, *cysB1*, and *suAmeth*, represent different, readily recombining loci. The effect of suppressors on various *meth* mutants is presented in Table 7. None of the suppressors acted on *methE31*. *suAmeth* was found to be the least specific, since it was found to also suppress mutations *methH2* and *methD10* (10). The results obtained in the cross *su101* × *methH2* were equivocal, presumably owing to the leakiness of the latter strain. *su101* was effective in strain *methB3* (in which it was identified) but showed only a slight effect in strain *methG1* and was ineffective in strain *methA17*. *methB3* and *methA17* both control cystathionine γ-synthase, but the former is more leaky than the latter, which may account for their different behavior with *su101*. In fact, *su101* can be classified as a type 1 suppressor only in combination with *methB3* (among the *meth* mutations tested here).

Biochemical characterization of the suppressors. The levels of cysteine and methionine biosynthetic enzymes examined were similar in the wild type and in *su101* strains (Table 8). *cysB1*, *cysC1*, and *suAmeth* show an elevated specific activity of ATP-sulfurylase, sulfite reductase, β-cystathionase, and especially homocysteine synthase. The addition of methionine to the growth medium resulted in repression of these enzymes in the *cysB1* and *cysC1* but not in *suAmeth* strains. The repression was pleiotropic (although probably not involving cysteine synthase), but its extent varied from 50 to 100% depending on the activity enzyme studied.

Examination of the pattern of ^{35}S-labeled amino acids accumulated in the suppressors grown in the presence of radioactive sulfate reveals marked differences between suppressor

TABLE 6. *Accumulation of ^{35}S-labeled amino acids in the mycelia of methA17, methA17cysD1, methG55, and methG55cysD1 strains grown in the presence of $^{35}SO_4$*[a]

Amino acid	Accumulation in strain:							
	methA17		*methA17cysD1*		*methG55*		*methG55cysD1*	
	Counts/min per mg[b]	%	Counts/min per mg	%	Counts/min per mg	%	Counts/min per mg	%
Glutathione	580	67.3	634	68.5	1,394	29.6	1,367	32.7
Cystathionine	126	14.7	154	16.6	3,098	65.9	2,684	64.3
Hypotaurine	96	11.2	126	13.5	87	1.8	104	2.5
Methionine	53	6.2	15	1.6	123	2.6	24	0.5
Total radioactivity	1,889		1,771		10,357		11,402	

[a] For experimental details see the footnotes to Table 4.
[b] Counts per minute per milligram of mycelial dry weight.

TABLE 7. *Specificity of four suppressor mutations towards lesions in different methionine-requiring mutants in A. nidulans*

Suppressor	Specificity[a] of methionine-requiring mutant:					
	meth-A17	*meth-B3*	*meth-D10*	*meth-E31*	*meth-G1*	*meth-H2*
su101	−	+	−	−	/+/	?
cysB1	+	+	−	−	+	−
cysC1	+	+[b]	−	−	+[b]	−
suAmeth	+[c]	+[c]	+[c]	−	+[c]	+[c]

[a] Symbols: −, no suppression; +, suppression; /+/, slight suppression.
[b] Ayling (2).
[c] Gajewski and Litwińska (10).

strains and the wild-type strain (Fig. 3). In strain *su101*, hypotaurine constituted the main radioactive component. It amounts to about 50% of the radioactivity retained on Dowex 50 H^+. On the other hand, in *cysB1* and *cysC1* strains, 60 to 70% of the radioactivity was found in cystathionine and a relatively small amount in glutathione, which is the main sulfur-containing compound in the wild type. Mycelium of *suAmeth* contained an abundance of sulfur amino acids, consistent with the probable impaired regulatory system in this mutant (Table 8).

The levels of cystathionine β-synthase and γ-cystathionase were found to be higher in the suppressors accumulating cystathionine (Table 9), confirming our previous observations (21). The activity of cystathionine β-synthase in strain *su101* was also slightly increased. Thus, it seems that at least a part of the cystathionine formed in *cysB1*, *cysC1*, and *suA25meth* was synthesized by the pathway involving homocysteine synthase and cystathionine β-synthase (Fig. 1).

Nature of the suppressors. Strains *cysC1* and *cysB1* were found to be very similar, both in enzymatic and autoradiographic studies, to strain *cysA1*, which carries a suppressor of the *methG55* mutation (21). This suggested that these two strains might be blocked in the same pathway for cysteine synthesis. We crossed these two mutants with strains *cysD1* (lacking homocysteine synthase), *mecA1* (lacking cystathionine β-synthase), and *mecB10* (lacking γ-cystathionase), mutants that have only one pathway left for de novo cysteine synthesis (Fig. 1). In all crosses, the double mutants obtained were cysteine-requiring auxotrophs. Some of them also responded to other sulfur amino acids, but none responded to *O*-acetylserine (Table 10). The results of these experiments imply that mutations *cysB1* and *cysC1* prevent synthesis of cysteine from *O*-acetylserine. This conclusion is strongly supported by the fact that the double mutant *cysB1mecA1* did not form any glutathione or cystathionine from sulfate (Fig. 4). On the other hand, methionine was formed as well as some other unidentified substances most likely derived from the homocysteine synthesized by homocysteine synthase.

In strain *suAmeth*, no enzymatic block has been detected either biochemically or nutritionally. Unlike strains *cysB* and *cysC*, neither the sulfate assimilation pathway enzymes nor the homocysteine synthase was repressed when strain *suAmeth* was grown in the presence of methionine or cysteine. Crosses with double mutants also carrying *mecA* and *cysD* mutations did not give auxotrophic segregants. It is

TABLE 8. *Activities of methionine biosynthetic enzymes in the wild type and suppressor meth strains of A. nidulans*[a]

Strain	Sp act[b]											
	Sulfate permease		ATP-sulfurylase		Sulfite reductase		Cysteine synthase		β-Cysta-thionase		Homocysteine synthase	
	MM[c]	Meth[d]	MM	Meth	MM	Meth	MM	Meth	MM	Meth	MM	Meth
Wild type	8.1	0.0	56.2	22.5	1.4	0.04	16.7	15.4	1.83	0.79	41.0	18.8
su101	11.0	0.0	60.1	24.8	0.41	0.54	14.6	12.6	1.8		48.0	19.0
cysB1	11.3	0.0	104.0	28.4	2.4	0.5	20.9	11.9	2.52	2.2	133.0	27.4
cysC1	13.6	0.0	94.0	28.7	2.8	0.96	24.2	19.3	4.3	3.3	144.2	25.3
suAmeth	10.1	7.6	92.6	94.2	2.5	3.05	27.6	25.7	4.1	4.0	107.3	128.2

[a] Mycelium grown for 13 h at 32 C was used in the assay of sulfate permease and sulfite reductase; that grown for 16 to 18 h was used for the remaining enzyme estimations.

[b] Nanomoles of product formed per minute per milligram of protein, except in the case of sulfate permease, which is nanomoles of sulfate accumulated per minute per milligram (dry weight).

[c] MM, Minimal medium.

[d] L-Methionine, 2 mM. Methionine was used in preference to the probable regulatory effector cysteine (25) since its concentration can be more easily controlled.

possible, therefore, that the product of this gene has a pleiotropic regulatory function.

Strain *su101* showed a distinctly lower level of glutathione (cysteine) with the concomitantly increased amount of hypotaurine. This indicates a distortion in cysteine catabolism.

DISCUSSION

The data presented here unequivocally proved that methionine synthesis in *A. nidulans* as in *N. crassa* involves cystathionine formation as an intermediate (Fig. 1). This conclusion is strongly supported by the fact that the *cysD1* mutant with impaired homocysteine synthase is a prototroph. Thus, this enzyme is not essential for this organism, in contrast to the analogous enzyme in yeast (4).

The fact that all mutants able to synthesize *O*-acetylhomoserine can grow on *S*-methylisothiourea, from which methylmercaptan can be generated, suggests formation of methionine in *A. nidulans* by the following reaction, as demonstrated in extracts of *Neurospora* (18):

O-acetylhomoserine + CH_3SH →

methionine + acetic acid

Since *S*-methylisothiourea does not support growth of the double mutant *methG55cysD1*, it can be presumed that the above reaction is catalyzed by homocysteine synthase.

Mutations *cysB1* and *cysC1*, which block the main pathway for cysteine synthesis as indicated by genetic and autoradiographic data, suppress lesions in mutants lacking cystathionine, γ-synthase, or β-cystathionase due to the derepression of homocysteine synthase. The results confirm our previous finding of a regulatory role of cysteine. Also the results obtained with an ethionine-resistant mutant, *etr-1*, and a suppressor of methionine mutations, *sume-2*, in *Neurospora* (13, 17) are consistent with the regulatory role of cysteine in this organism.

It is difficult to explain the appearance of cysteine synthase activity in the extracts from the *cysB1* and *cysC1* strains. The biochemical nature of these two mutants remains to be determined.

A much weaker suppression effect was found in the case of mutation *su101*, which seems to cause an enhanced cysteine catabolism, resulting in the accumulation of hypotaurine. A relative shortage of cysteine may cause *O*-acetylhomoserine, normally utilized for cystathionine synthesis, to be available for homocysteine synthesis catalyzed by homocysteine synthase (Fig. 1). The K_m for *O*-acetylhomoserine in this reaction is about 20 mM (20), so that the effectiveness of homocysteine synthesis by this pathway may depend considerably on the intracellular concentration of *O*-acetylhomoserine.

Among the suppressors studied here, *suAmeth* seems to be the most interesting since it exhibits properties of a regulatory gene. It resembles in several aspects the *scon*[c] mutant of *N. crassa* isolated by Burton and Metzenberg (3).

Little is known about regulation of the main pathway of homocysteine synthesis in *Neurospora* and *Aspergillus* which involves cystathionine γ-synthase and β-cystathionase. Kerr and Flavin (12) found that the former enzyme is strongly inhibited by *S*-adenosylmethionine and activated by certain derivatives

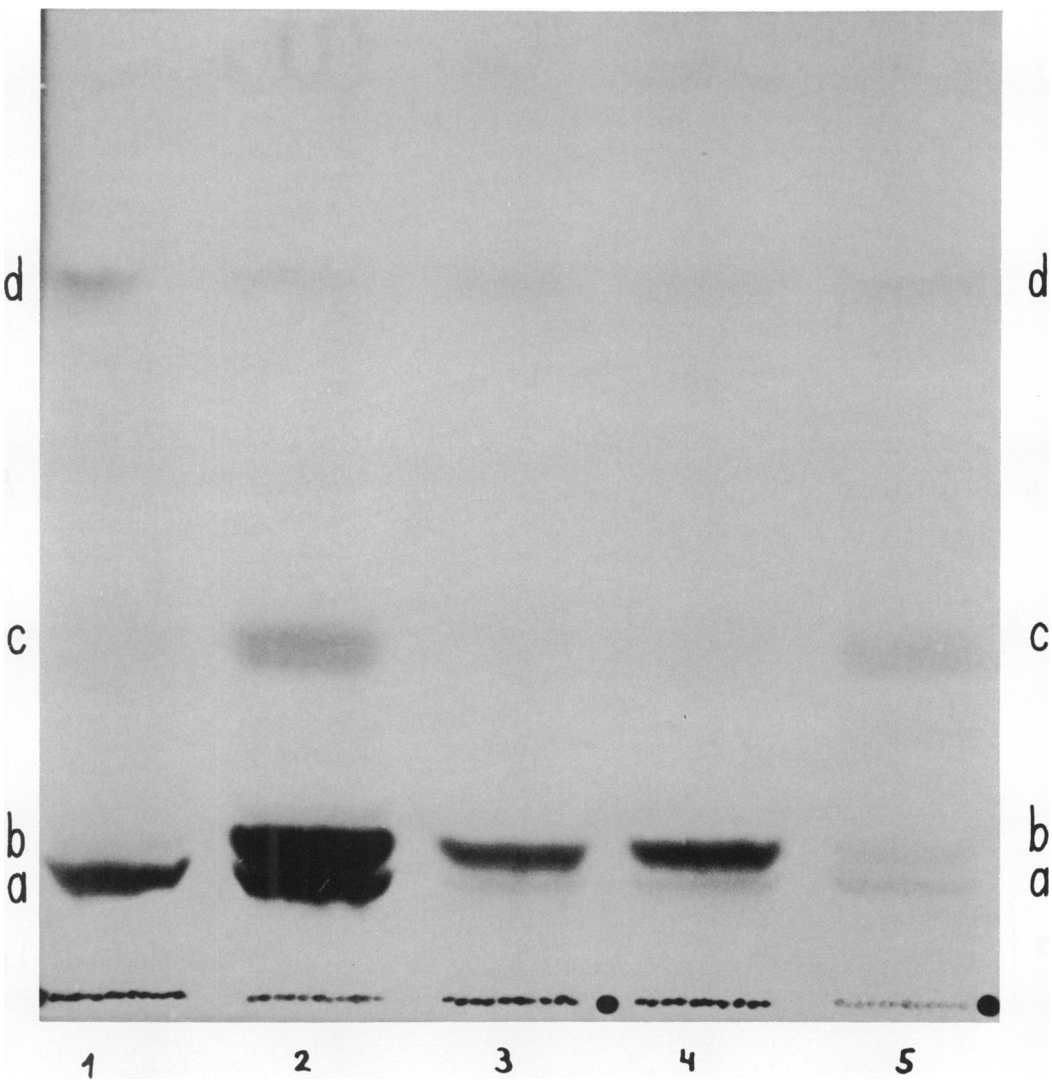

FIG. 3. *Autoradiography of S-labeled compounds retained on Dowex 50 H^+ from water extracts of the strains: wild type (1), suAmeth (2), cysC1 (3), cysB1 (4), and sup101 (5). Mycelia were grown in minimal medium for 13 h, transferred to minimal medium containing 0.2 mM $Na_2{}^{35}SO_4$ (8×10^8 counts/min per mmol), and grown for an additional 7 h. The mycelia were harvested and the amino acids were isolated as described previously (25). For each strain, material corresponding to 7 mg of mycelial dry weight was applied on the plate. (a) Glutathione oxidized, (b) cystathionine, (c) hypotaurine, (d) methionine.*

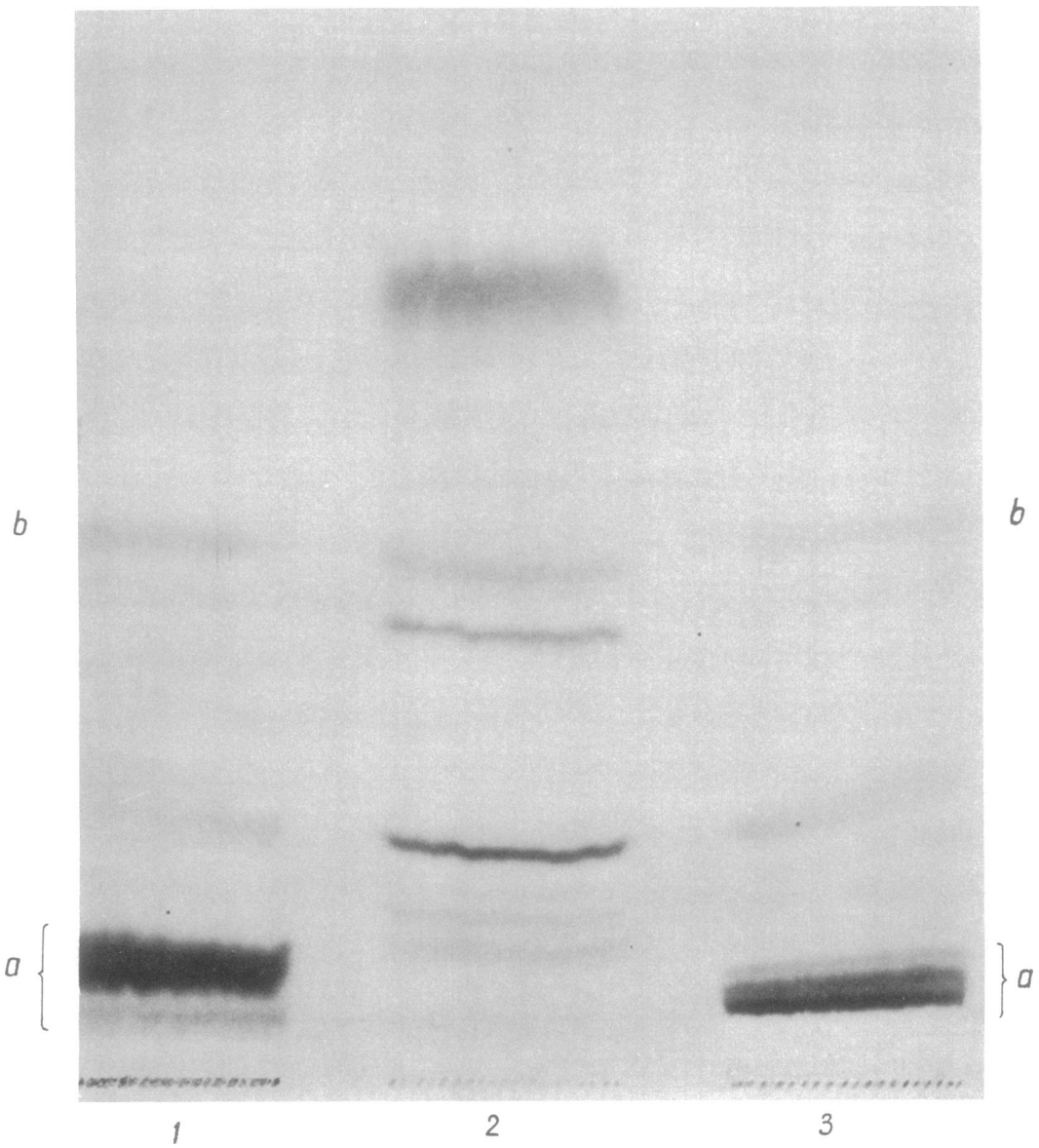

FIG. 4. *Autoradiography of S-labeled compounds retained on Dowex 50 H^+ from the water extracts of the following strains: cysB1 (1), cysB1mecA1 (2), and mecA1 (3). Mycelia were grown in minimal medium supplemented with* L-*cysteine (1 mM) for 13 h, and transferred to minimal medium containing 0.2 mM $Na_2{}^{35}SO_4$ (8×10^8 counts/min per mmol), and grown for an additional 8 h. The mycelia were harvested and the amino acids were isolated as described previously (21). For each strain, material corresponding to 5 mg of mycelial dry weight was applied on the plate. (a) Glutathione, cysteine, and cystathionine region; (b) methionine.*

TABLE 9. *Activity of cystathionine β-synthase and γ-cystathionase in the wild type and suppressor strains*

Strain	Activity (nmol/min per mg of protein)	
	Cystathionine β-synthase	γ-Cystathionase
Wild type	3.74	0.46
su101	7.28	0.49
cysB1	10.6	1.24
cysC1	11.2	1.28
suAmeth	10.7	1.33

TABLE 10. *Growth response to methionine and its precursors of double mutants carrying cysA1, cysB1, and cysC1 mutations with either mecA, mecB, or cysD mutations*[a]

Strain	Growth				
	O-acetylserine[b]	Cysteine	Cystathionine	Homocysteine	Methionine
cysA1cysD1	+	+	+	+	+
cysB1cysD1	−	+	+	+	+
cysB1mecA1	−	+	+	−	−
cysB1mecB10	−	+	−	−	−
cysC1cysD1	−	+	+	+	+
cysC1mecA1	−	+	+	−	−
cysC1mecB10	−	+	−	−	−

[a] Single mutant strains *cysA1*, *cysB1*, *cysC1*, *cysD1*, *mecA1*, and *mecB10* are all prototrophs.

[b] Supplement to minimal medium (1 mM).

of folic acid (27), but the authors found no evidence that this enzyme is repressed during growth on methionine-supplemented medium. On the other hand, in *A. nidulans* grown in the presence of methionine or homocysteine, β-cystathionase is repressed by about 50 to 60%. It has also been observed that strains that accumulate cystathionine, owing to the derepression of homocysteine synthase and cystathionine β-synthase, show an elevated level of β-cystathionase (Tables 8 and 9). This might indicate either induction of β-cystathionase by cystathionine or its derepression resulting from a decrease of the homocysteine pool under conditions of enhanced synthesis of cystathionine. There is evidence suggesting that this enzyme is repressed by homocysteine (21).

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