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ORIGINAL ARTICLE

The level of sulfane sulfur in the fungus *Aspergillus nidulans* wild type and mutant strains

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Abstract The interdependence of the sulfane sulfur metabolism and sulfur amino acid metabolism was studied in the fungus *Aspergillus nidulans* wild type strain and in mutants impaired in genes encoding enzymes involved in the synthesis of cysteine (a precursor of sulfane sulfur) or in regulatory genes of the sulfur metabolite repression system. It was found that a low concentration of cellular cysteine leads to elevation of two sulfane sulfurtransferases, rhodanase and cystathionine γ-lyase, while the level of 3-mercaptopyruvate sulfurtransferase remains largely unaffected. In spite of drastic differences in the levels of biosynthetic enzymes and of sulfur amino acids due to mutations or sulfur supplementation of cultures, the level of total sulfane sulfur is fairly stable. This stability confirms the crucial role of sulfane sulfur as a fine-tuning regulator of cellular metabolism.

Keywords Sulfane sulfur · *Aspergillus nidulans*

Abbreviations

CBL	Cystathionine β-lyase
CGL	Cystathionine γ-lyase
GSH	Glutathione reduced
GSSG	Glutathione oxidized
MPST	3-Mercaptopyruvate sulfurtransferase

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Introduction

Compounds containing labile sulfane sulfur (sulfur atoms at oxidation state 0 or −1) bound to another sulfur atom are widely distributed in living organisms and play an important physiological role. They include hydrodisulfides ($R–S_2H$), polysulfides ($R–S_n–R$), polythionates ($^-SO_3–S_n–SO_3^-$), thiosulfate ($S_2O_3^{2-}$), polysulfonates ($R–S_2O_2^-$) as well as protein-bound sulfur (Beinert 2000). Sulfane sulfur is formed in the non-oxidative pathway of cysteine degradation (Fig. 1) involving three sulfur-transferring enzymes: 3-mercaptopyruvate sulfurtransferase (MPST; EC 2.8.1.2), cystathionine γ-lyase (CGL; EC 4.4.1.1) and rhodanese (thiosulfate sulfurtransferase; EC 2.8.1.1) found in different cellular compartments of plants, fungi, bacteria and animals (Westley 1973). All of them contain sulfhydryl groups in their active sites. These groups bind a transferred atom of sulfur from persulfides in the case of rhodanese and 3-mercaptopyruvate sulfurtransferase (Nagahara and Nishino 1996) or from polysulfides in the case of cystathionine γ-lyase (Yamanishi and Tuboi 1981).

3-Mercaptopyruvate sulfurtransferase catalyses the transfer of sulfur atom from 3-mercaptopyruvate (a single donor) to various acceptors which often produce sulfane sulfur-containing compounds (Fig. 1) (Westley et al. 1983; Toohey 1989). CGL, the last enzyme in the transsulfuration pathway of cysteine biosynthesis from methionine (Fig. 2), can also catalyze β-elimination of cystine and cysteine yielding, respectively, cysteine hydrodisulfide and H_2S, as well as pyruvate and ammonia (Fig. 1) (Yamanishi and Tuboi 1981; Yamagata et al. 2002). This enzyme is also involved in sulfane sulfur transfer in the cell (Cooper and Pinto 2005). Rhodanese catalyzes the transfer of a sulfur atom from sulfane sulfur containing compounds to various acceptors like cyanide and thiols to produce less toxic

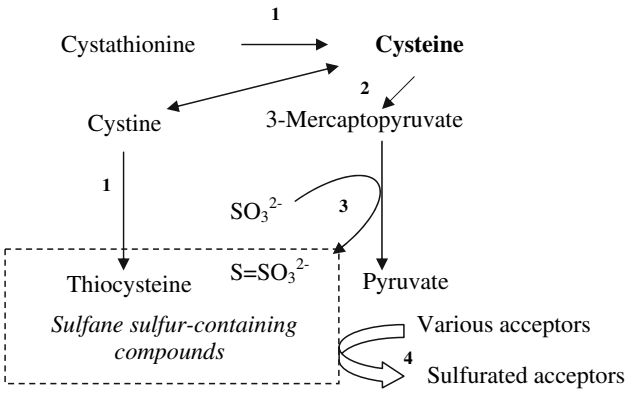

Fig. 1 Sulfane sulfur generation and utilization. Enzymes catalyzing particular reactions: *1* cystathionine γ-lyase, *2* aminotransferase, *3* 3-mercaptopyruvate sulfurtransferase, *4* rhodanese

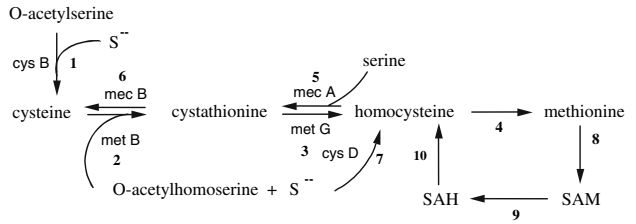

Fig. 2 An outline of sulfur amino acid biosynthesis and interconversion in *Aspergillus nidulans. Symbols of genes* encoding particular enzymes are in italic. *1* cysteine synthase, *2* cystathionine γ-synthase, *3* cystathionine β-lyase, *4* methionine synthase, *5* cystathionine β-synthase, *6* cystathionine γ-lyase, *7* homocysteine synthase, *8* S-adenosylmethionine synthetase, *9* various methyltransferases, *10* S-adenosylhomocysteine hydrolase. S-adenosinehomocysteine (*SAH*), S-adenosinemethionine (*SAM*)

molecules, thiocyanate and persulfides, respectively. Sulfurtransferases participate in the synthesis of an essential component of many different sulfur-containing biomolecules like $(Fe–S)_n$ clusters (Ogasawara et al. 1995; Tse Sum Bui et al. 2000), vitamins (thiamine, biotin) (Marquet 2001) and molybdopterin (Matthies et al. 2004). They are also involved in sulfate assimilation (Donadio et al. 1990; Chang and Vining 2002) and cyanide detoxification (Porter and Baskin 1995; Porter et al. 1996; Baskin et al. 1999; Wróbel et al. 2004). It has also been demonstrated that sulfane sulfur atoms may be transferred to tRNA nucleotides by MPST and rhodanese. The formation of thionucleosides in tRNA is probably important in the translation process (Mueller et al. 2001). Furthermore, MPST and CGL transfer sulfane sulfur atoms to –SH groups in active sites of many enzymes, in some cases activating them, in others leading to their inactivation (Yamanishi et al. 1983; Toohey 1989; Ogasawara et al. 1997). In addition, sulfane sulfur plays a role as an antioxidant. It has been shown that some sulfane sulfur-containing compounds cause inhibition of cytochrome P-450, which is crucial in lipid peroxidation, generating reactive oxygen species and converting xenobiotics to radical species (Ogasawara et al. 1998, 1999).

Sulfane sulfur metabolism has been studied mostly in mammals (Nagahara and Nishino 1996; Beinert 2000; Wróbel et al. 2004; Stipanuk et al. 2006), plants (Papenbrock 2002; Meyer et al. 2003; Bauer et al. 2004) and bacteria (Fernández et al. 2000; Sekowska et al. 2000), and there are only a few reports concerning fungi. In *Aspergillus nidulans cnxF* mutants the failure to synthesize molybdopterin (and the molybdenum cofactor), resulting in the loss of activity of the molybdoenzymes (nitrate reductase and xanthine dehydrogenase), is presumably due, at least in part, to the lack of the sulfur transfer system (Appleyard et al. 1998). Sulfur metabolism and the regulation of the desulfurylation pathway of cysteine were studied in *Saccharomyces cerevisiae* (Maclean et al. 2000; Chan and Appling 2003), although the level of sulfane sulfur has not been investigated.

Since cysteine is the primary source of sulfane sulfur in the cell, one could expect the existence of some interrelation between the regulations of sulfane sulfur and sulfur amino acid metabolisms. To examine such a possibility we have used the filamentous fungus *A. nidulans*. It seems to be a unique organism for such studies due its rich repertoire of sulfur amino acid metabolic pathways (Fig. 2) as well as mutants impaired in regulatory genes involved in the sulfur metabolite repression system (SMR). SMR shuts off the sulfate assimilation pathway when cysteine is in excess (Paszewski et al. 2000). SMR comprises negatively acting *scon* genes. Mutations in the *scon* genes lead to permanent derepression of sulfate assimilation and overproduction of sulfur amino acids (Natorff et al. 1998; Piotrowska et al. 2000). Another component of SMR is the *metR* gene, encoding a transcription factor specific for activation of several sulfur metabolism genes (Natorff et al. 2003). The SCON proteins are components of a SCF-type ubiquitin ligase complex (Patton et al. 1998), which inactivates the METR protein when cysteine is in excess. *Scon* mutants are prototrophs while *metR* mutants are methionine-requiring auxotrophs.

It should be noted that *A. nidulans* synthesizes cysteine de novo from *O*-acetylserine (Fig. 2, step 1). An alternative pathway of cysteine synthesis involves homocysteine synthase, cystathionine β-synthase and cystathionine γ-lyase (Fig. 2 steps 7, 5 and 6, respectively). The latter pathway operates when the first one is impaired. In effect, single mutants like *cysB* or *mecB* are prototrophs while the double mutant *cysB, mecB* is a cysteine requiring auxotroph. In such a situation, homocysteine made from *O*-acetylhomoserine and sulfide serves as a precursor of both cysteine and methionine. This is the reason why *cysB* mutations suppress lesions in the *metB* and *metG* genes which cause methionine auxotrophy.

In this study we examined the effects of mutations and growth condition affecting sulfur amino acid metabolism on the levels of cysteine, glutathione and cystathionine, as well as on sulfurtransferases and total cellular sulfane sulfur level. We have found that in spite of marked differences in the pools of these sulfur compounds in the studied strains, the level of sulfane sulfur was fairly stable, what may suggest an important role of sulfane sulfur in the cellular metabolism.

Materials and methods

Materials

L-cystathionine was from Calbiochem (San Diego, USA); pyridoxal 5-phosphate, NADH, lactate dehydrogenase, dithiothreitol, *N*-ethylmaleimid, GSH, GSSG, L-cysteine, L-cystine, D,L-cystathionine, bathophenanthrolinedisulfonic acid (BPDS), 2,4-dinitrofluorobenzene and PTFE filter were obtained from Sigma (Chemical Company, St Louis, MO, USA); sodium 3-mercaptopyruvate and trifluoroacetic acid (TFA) were from Fluka Chemie GmbH (Buchs, Switzerland); potassium cyanide, potassium phosphate were obtained from Merck (Darmstadt, Germany) and *N*-methyl-L-lysine from Bachem (Bubendorf, Switzerland).

Methods

Strains

The following strains of *A. nidulans* from our collection which carry standard markers (Clutterbuck 1984) were used: *pyroA4, yA2* (used as a reference wild type strain in the experiments); ***mecB10****, nicA2, yA2*; ***cysB10****, pyroA4, yA2*; ***metG55****, pyroA4, yA2*; ***mecB10****,* ***metG55****, pyroA4, yA2*; ***sconB2****, pyroA4, yA2* and ***metR****, pyroA4, yA2*. The genes related to sulfur metabolism are in bold and are shown in Fig. 2. *pyro*—pyridoxine, *cys*—cysteine, *nic*—nicotinic acid, *met*—methionine, *y*—yellow conidia, *mec*—methionine catabolism, *scon*—sulfur controller.

Media, culture conditions and mycelial extracts preparation

Aspergillus nidulans strains were grown in liquid minimal medium (MM) containing 2 mM sulfate (Paszewski and Grabski 1973) supplemented according to their nutritional requirements. L-Methionine was added when needed at the indicated concentrations. The cultures were started by inoculation of 100 ml of medium in a 300-ml conical flask with 3–5 ml of a heavy conidial suspension and grown in a rotary shaker (200 rpm) at 30°C for 18–20 h. Mycelia were collected by filtration, washed with water and blotted on filter paper. They were used immediately for enzyme extracts, or frozen at −78°C and stored until used. Extracts were prepared by grinding mycelial pads in a mortar with an appropriate buffer and powdered glass. The resulting slurry was centrifuged at 15,000×*g* and the supernatant was used for enzyme assays.

Enzyme assays

For determination cystathionine γ-lyase activity extracts were prepared in 0.1 M potassium phosphate buffer, pH 7.8 and passed through Sephadex G-25 (coarse) equilibrated with the same buffer. The reaction mixture contained 1.2 µmol L-cystathionine, 0.6 µmol pyridoxal 5-phosphate, 100 µmol potassium phosphate buffer, pH 7.8, 200 µl of mycelial extract (0.2–0.4 mg protein) and water in a total volume of 0.5 ml. Cystathionine was omitted in the control. Incubation was carried out at 37°C for 30 min followed by addition of 1 ml of ninhydrin reagent (Gaitonde 1967). Tubes were heated at 100°C for 5 min and A_{560} was read against control. The amount of cysteine formed was calculated from cysteine calibration curve prepared under the same conditions.

3-Mercaptopyruvate sulfurtransferase activity was assayed according to the method of Valentine and Frankenfeld (1974) with some modifications as described by Wróbel et al. (2004).

Rhodanese activity was assayed by Sőrbo's method (1955), following the procedure described by Wróbel et al. (2004).

The activities of MPST and rhodanese were expressed as nanomoles of pyruvate or SCN per minute per milligram of protein, respectively.

Sulfur-containing compounds determination

Sulfane sulfur was determined by the method of Wood (1987), based on cold cyanolysis and colorimetric detection of ferric thiocyanate complex ions.

Frozen mycelia were ground in 10% w/v PCA/1 mM BPDS (3 ml g^{-1} of mycelial pads) in a mortar with powdered glass. The resulting slurry was centrifuged 10 min at 4°C at 1,400×*g* and the supernatant was stored at −76°C until used for HPLC analyses.

The reduced (GSH) and oxidized (GSSG) glutathione, cysteine, cystine and cystathionine were determined by a reversed-phase HPLC method of Dominick et al. (2001). A modified elution gradient was introduced to allow for simultaneous separation and quantitation of cystathionine in samples. Samples were separated on a 4.6 mm × 250 mm Luna C_{18} (5u) column with a Phenomenex Security Guard column filled with the same packing

material. The chromatographic system consisted of LC-10 Atvp Shimadzu pumps, four channel degassers, column oven and a Shimadzu SIL-10 Advp autosampler. Chromatographic peaks were measured by a Shimadzu SPD-M10 Avp-diode array detector. Class VP 7.2.1 version software was used to control system operation and facilitate data collection. A mobile phase consisting of solvent A (water/0.1%TFA) and solvent B (acetonitrile/0.1% TFA) was used for elution of samples. After injection the column was eluted with 20% B followed with 35 min linear gradient to 55% B and 10 min isocratic period at 55% B, then a 15 min linear gradient to 100% B and 10 min isocratic period. The column was then re-equilibrated to the initial conditions for 15 min.

Stock solutions were prepared for standard curves as follows: 2.4 µM N^{ϵ}-methyllysine, 1.2 µM L-cysteine, 1.2 µM L-cystine, 1.2 µM GSH, 1.2 µM GSSG, and 2.2 µM D,L-cystathionine. All stock solutions were prepared in 10% PCA/1 mM BPDS, except for N^{ϵ}-methyllysine which was prepared in water. A separate stock solution of the internal standard, N^{ϵ}-methyllysine, was prepared by 1:10 dilution of the 2.4 µM solution. Standard curves were generated using solutions of 13–75 nmol of each compound per milliliter of supernatant obtained from mycelia. All HPLC solvents were HPLC grade. Samples were filtered through a 0.20-µm PTFE filter. Analyses of 20 µl of sample were performed at a flow rate of 1.0 ml/min at 20°C with diode array detection at 365 nm.

Protein was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

Results and discussion

The levels of the three key enzymes in sulfane sulfur metabolism, CGL, MPST and rhodanese, in the wild type and various *A. nidulans* mutant strains impaired in sulfur amino acid synthesis and their interconversion are shown in Fig. 3. When the values obtained for the wild type strain grown in sulfate containing MM are taken as a reference, it appears that the MPST levels are similar in all the studied strains, within the standard deviation (Fig. 3). Rhodanese was slightly elevated in the *sconB* strain, but more significantly in the *cysB* mutant defective in cysteine synthase, suggesting that the enzyme level may be regulated by cysteine concentration.

Pronounced differences were observed in CGL levels. The enzyme was enhanced in the wild type grown in the presence of methionine (WT^a), as well as in the *cysB* and *sconB* strains (Paszewski et al. 1977; Natorff et al. 1993). CGL activity increased in the *metR* mutant, indicating that

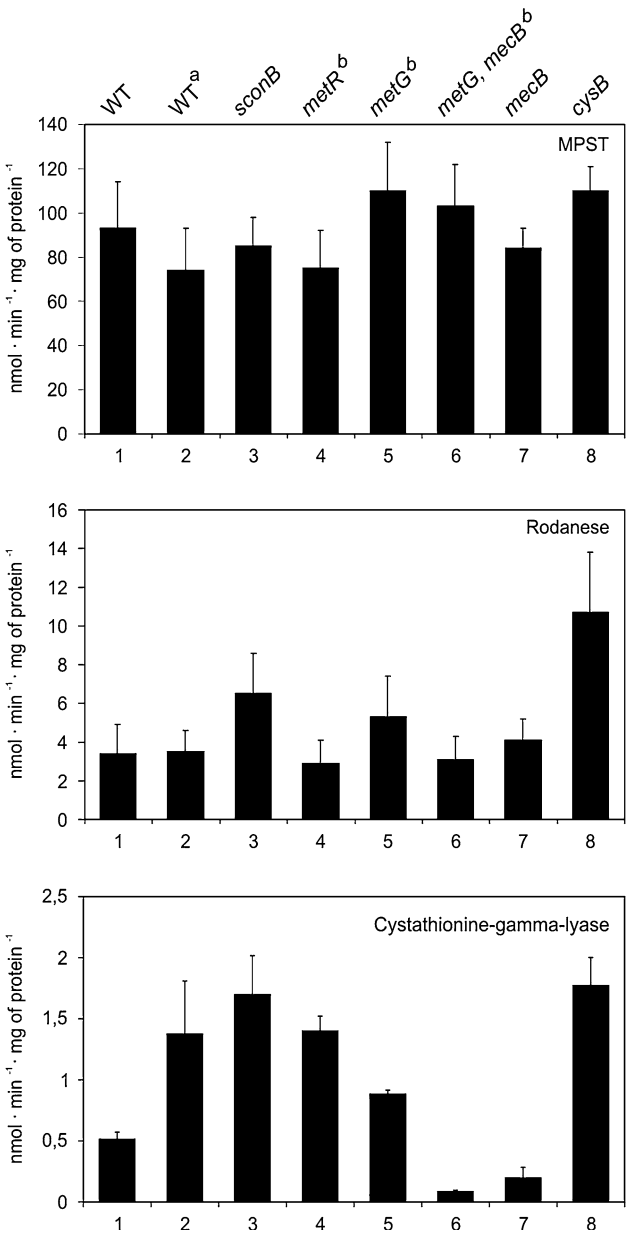

Fig. 3 Activities of sulfurtransferases expressed as nanomoles·per min·per milligram of protein in the wild type (*WT*) and mutant strains of *Aspergillus nidulans*. The strains were grown in MM or in MM medium supplemented with **a** 5 mM L-methionine or **b** 0.25 mM L-methionine. *sconB*, *metR*, *metG*, *mecB*, *cysB*—mutant strains impaired in regulatory genes involved in the sulfur metabolite repression system, described in the Sect. "Introduction" and shown in Fig. 2

the *mecB* gene expression is not dependent on the METR transcription factor.

The *mecB* strain defective in CGL grows at the wild-type rate on MM with sulfate as a sole sulfur source. Therefore, when *cysB*-encoded cysteine synthase is active, CGL is dispensable for growth of the fungus both as a cysteine synthesizing enzyme and as sulfurtransferase. When methionine (or homocysteine) is in abundance, CGL

plays a role in cysteine synthesis, because the sulfate assimilation pathway is repressed under these conditions. It is worth noting that the *mecB* strain still possesses some CGL activity that is practically absent in the double mutant *mecB, metG* (Fig. 3). This suggests that cystathionine β-lyase (CBL) encoded by *metG* also has some γ-lyase activity, at least in vitro, albeit evidently not sufficient to complement the cysteine requirement of the *cysB, mecB* mutant.

The levels of sulfur compound pools that may influence the level of sulfane sulfur are shown in Fig. 4. It is evident that the amounts of cysteine, cystathionine and glutathione which are efficient sources of cysteine vary between strains to a much greater degree than the levels of sulfurtransferases (Fig. 4). The *cysB* and *mecB* strains, both of them being prototrophs and each impaired in one of the cysteine-forming enzymes, contain less cysteine than the wild type strain grown in MM (Table 1). The *cysB* strain also exhibits a relatively high GSH/GSSG ratio, similar to that observed in the methionine requiring strains grown in the presence of a low concentration of methionine (Table 1). There is a marked increase in cystine in the wild type strain grown in the presence of 5 mM methionine (Table 1). Moreover, we have observed that under these conditions, the level of homoserine was also about tenfold higher than in the sulfate-grown control (not shown), which may indicate an inhibition of homoserine acetyltransferase by methionine.

The variation in cystathionine content between the strains is even more pronounced (Fig. 3). Not surprisingly, it is high in the *metG*-carrying strains impaired in the CBL. Cystathionine level is also enhanced in the wild type and *metR* grown in the presence of methionine, as well as in the *sconB* mutant, which is derepressed in sulfate assimilation and synthesis of sulfur-containing amino acids. The level of cystathionine is also high in the *cysB* strain, which has less cysteine and glutathione (Table 1). In this strain, cysteine is synthesized by the alternative pathway, in which cystathionine β-synthase is more active than CGL, resulting in cystathionine accumulation (Natorff et al. 1993). A decreased cysteine pool in the *cysB* strain indicates that the alternative pathway of its synthesis is less effective than the main one. A decreased cysteine pool in the *cysB* mutant does not lead to significant lowering of the level of sulfane sulfur.

In general, it appears that in spite of the marked differences in the pools of sulfur compounds observed among strains with various defects in sulfur amino acid metabolism, the level of sulfane sulfur remains stable (Fig. 4). This level is dependent neither on cysteine concentration in the cell nor on the pathway, by which the amino acid is

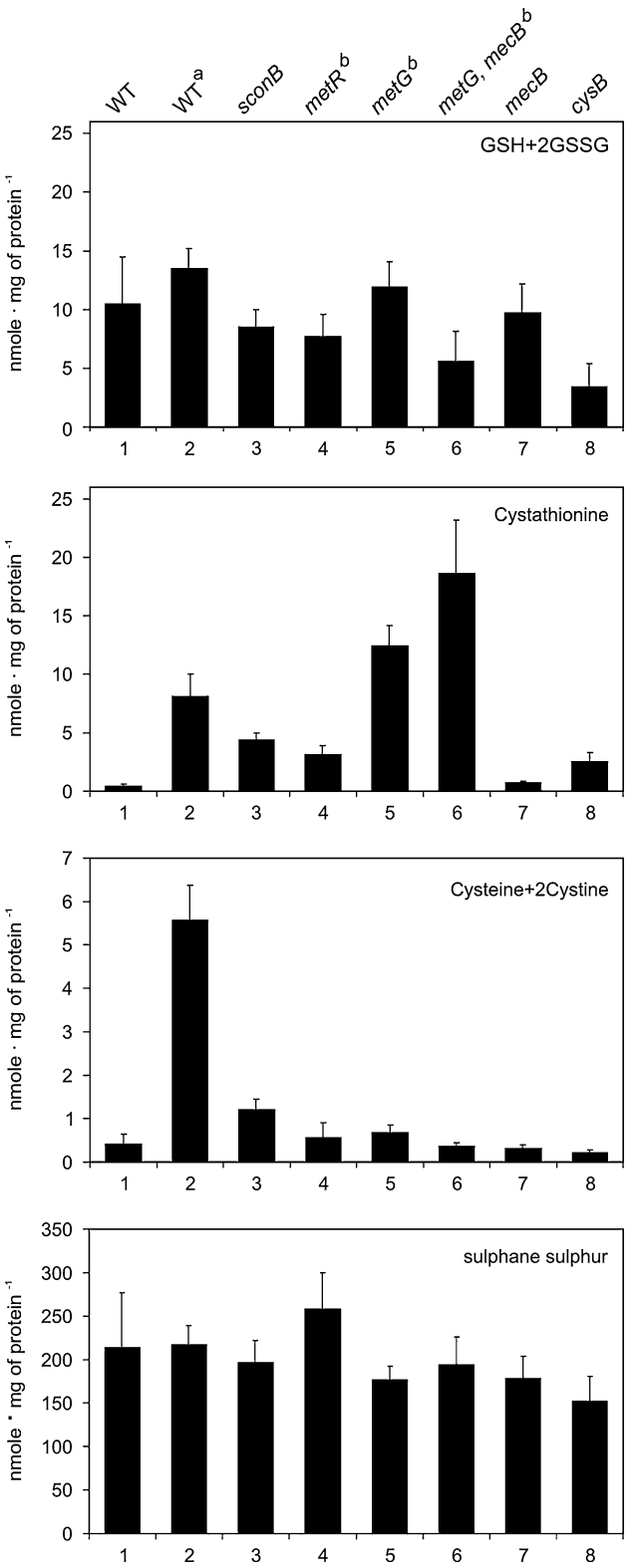

Fig. 4 Levels of "total" cysteine (CSH + CSSC × 2), "total" glutathione (GSH + GSSG × 2), cystathionine and sulfane sulfur in the wild type and mutant strains of *Aspergillus nidulans*. Growth conditions and strains as in Fig. 3

Table 1 Content of reduced and oxidized cysteine and glutathione in the studied strains

Strain	GSH[c]	GSSG[c]	GSH/GSSG[c]	Cysteine[c]	Cystine[c]	Cysteine/cystine
WT	1.3 ± 0.6	4.6 ± 1.7	0.3	0.42 ± 0.23	ND	ND
WT[a]	3.7 ± 0.3	4.9 ± 0.7	0.75	0.56 ± 0.22	2.5 ± 0.3	0.2
sconB	2.9 ± 0.9	2.8 ± 0.3	1	0.82 ± 013	0.19 ± 0.06	4.3
metR[b]	0.9 ± 0.1	3.4 ± 0.9	0.3	0.56 ± 0.34	ND	ND
metG[b]	1.5 ± 0.2	5.2 ± 1.0	0.3	0.56 ± 0.15	0.06 ± 0.01	9.3
mecB, metG [b]	0.6 ± 0.03	2.6 ± 0.5	0.25	0.27 ± 0.05	0.05 ± 0.01	5.4
mecB	0.9 ± 0.1	4.4 ± 1.2	0.2	0.29 ± 0.09	0.01	29
cysB	1.0 ± 0.4	1.2 ± 0.8	0.8	0.17 ± 0.05	0.02 ± 0.01	8.5

ND not detected

[a] MM + 5 mM methionine

[b] MM + 0.25 mM methionine

[c] nanomole per·milligram of protein

formed. The results also indicate that sulfane sulfur metabolism is not regulated by the SMR system, which controls the expression of genes encoding the sulfate assimilation pathway enzymes and homocysteine synthase.

This remarkable homeostasis of sulfane sulfur points to its vital importance for the cell. It seems unsurprising, however, in view of the multiple roles this sulfur plays in cell metabolism, being an activator in some processes and an inhibitor in others. These functions require the stabilization of sulfane sulfur concentration that is consistent with its role as a fine-tuning regulator of cellular metabolism.

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References

Appleyard MVCL, Sloan J, Kana'n GJM, Heck IS, Kinghorn JR, Unkles SE (1998) The *Aspergillus nidulans cnxF* gene and its involvement in molybdopterin biosynthesis: molecular characterization and analysis of *in vivo* generated mutants. J Biol Chem 273:14869–14876

Baskin SI, Porter DW, Rockwood GA, Romano JA, Patel HC, Kiser RC, Cook CM, Ternay AL (1999) *In vitro* and *in vivo* comparison of sulfur donors as antidotes to acute cyanide intoxication. J Appl Toxicol 19:173–183

Bauer M, Dietrich C, Nowak K, Sierralta WD, Papenbrock J (2004) Intracellular localization of sulfurtransferases from *Arabidopsis thaliana*. Plant Physiol 135:916–926

Beinert H (2000) A tribute to sulfur. Eur J Biochem 267:5657–5664

Chan SY, Appling DR (2003) Regulation of *S*-adenosylmethionine levels in *Saccharomyces cerevisiae*. J Biol Chem 278:43051–43059

Chang Z, Vining LC (2002) Biosynthesis of sulfur-containing amino acids in *Streptomyces venezuelae* ISP5230: roles for cystathionine β-synthase and transsulfuration. Microbiology 148:2135–2147

Clutterbuck AJ (1984) Gene symbols in *Aspergillus nidulans*. Genet Res 21:291–296

Cooper AJ, Pinto JT (2005) Aminotransferase, L-amino acid oxidase and beta-lyase reactions involving L-cysteine S-conjugates found in allium extracts. Relevance to biological activity? Biochem Pharmacol 69:209–220

Dominick PK, Cassidy PB, Roberts JC (2001) A new and versatile method for determination of thiolamines of biological importance. J Chromatogr B Biomed Sci 761:1–12

Donadio S, Shafiee A, Hutchinson CR (1990) Disruption of a rhodanese-like gene results in cysteine auxotrophy in *Saccharopolyspora erythraea*. J Bacteriol 172:350–360

Fernández M, von Doesburg W, Rutten GAM, Marugg JD, Alting AC, von Kranenburg R, Kuipers OP (2000) Molecular and functional analyses of the *metC* gene of *Lactococcus lactis*, encoding Cystathionine β-lyase. Appl Environ Microbiol 66:42–48

Gaitonde MK (1967) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem J 104:627–633

Lowry OH, Rosenbrough NJ, Farr AL, Randall RI (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Maclean KN, Janosık M, Oliveriusova J, Kery V, Kraus JP (2000) Transsulfuration in *Saccharomyces cerevisiae* is not dependent on heme: purification and characterization of recombinant yeast cystathionine β-synthase. J Inorg Biochem 81:161–171

Marquet A (2001) Enzymology of carbon-sulfur bond formation. Curr Opin Chem Biol 5:541–549

Matthies A, Rajagopalan KV, Mendel RR, Leimkühler S (2004) Evidence for the physiological role of a rhodanese-like protein for the biosynthesis of the molybdenum cofactor in humans. Proc Natl Acad Sci USA 101:5946–5951

Meyer T, Burow M, Bauer M, Papenbrock J (2003) *Arabidopsis* sulfurtransferases: investigation of their function during senescence and in cyanide detoxification. Planta 217:1–10

Mueller EG, Palenchar PM, Buck CJ (2001) The role of the cysteine residues of ThiI in the generation of 4-thiouridine in tRNA. J Biol Chem 276:33588–33595

Nagahara N, Nishino T (1996) Role of amino acid residues in the active site of rat liver mercaptopyruvate sulfurtransferase. cDNA cloning, overexpression, and site-directed mutagenesis. J Biol Chem 271:27395–27401

Natorff R, Balińska M, Paszewski A (1993) At least four regulatory genes control sulphur metabolite repression in *Aspergillus nidulans*. Mol Gen Genet 238:185–192

Natorff R, Piotrowska M, Paszewski A (1998) The *Aspergillus nidulans* sulphur regulatory gene *sconB* encodes a protein with WD40 repeats and an F-box. Mol Gen Genet 257:255–263

Natorff R, Sieńko M, Brzywczy J, Paszewski A (2003) The *Aspergillus nidulans metR* gene encodes a bZIP protein which activates transcription of sulphur metabolism genes. Mol Microbiol 49:1081–1094

Ogasawara Y, Isoda S, Tanabe S (1995) Reconstitution of an iron-sulfur cluster with bound sulfur: a possible source of acid-labile sulfur in biological systems. Biol Pharm Bull 18:1045–1048

Ogasawara Y, Suzuki T, Ishii K, Tanabe S (1997) Modification of liver cytosol enzyme activities promoted in vitro by reduced sulfur species generated from cystine with gamma-cystathionase. Biochim Biophys Acta 1334(1):33–43

Ogasawara Y, Isoda S, Tanabe S (1998) A labile sulfur in trisulfide affects cytochrome P-450 dependent lipid peroxidation in rat liver microsomes. Toxicol Lett 99:191–198

Ogasawara Y, Isoda S, Tanabe S (1999) Antioxidant effects of albumin-bound sulfur in lipid peroxidation of rat liver microsomes. Biol Pharm Bull 22:441–445

Papenbrock J (2002) Recent results of the sulfurtransferase/rhodanese protein family. Recent Res Develop Biochem 3:13–31

Paszewski A, Grabski J (1973) Studies on cystathionine beta-synthase and O-acetylhomoserine sulfhydrylase as the enzymes of alternative methionine biosynthetic pathways in *Aspergillus nidulans*. Acta Biochim Polon 20:159–167

Paszewski A, Prazmo W, Landman-Balińska M (1977) Regulation of homocysteine metabolizing enzymes in *Aspergillus nidulans*. Mol Gen Genet 155:109–112

Paszewski A, Natorff R, Piotrowska M, Brzywczy J, Sienko M, Grynberg M, Pizzinini E, Turner G (2000) Regulation of sulfur amino acid biosynthesis in *Aspergillus nidulans*: physiological and genetical aspects. In: Brunold C et al (eds) Sulfur nutrition and sulfur assimilation in higher plants. Paul Haupt Publishers, Bern, pp 93–105

Patton EE, Willems AR, Sa D, Kuras L, Thomas D, Creig KL, Tyers M (1998) CDC 53 is a scaffold protein for multiple Cdc 34/Skp 1/ F-box protein complexes that regulate cell division and methionine biosynthesis in yeast. Genes Dev 12:692–705

Piotrowska M, Natorff R, Paszewski A (2000) The *Aspergillus nidulans* gene *sconC* involved in the regulation of sulphur metabolism, belongs to the *SKP1* family. Mol Gen Genet 264:276–282

Porter DW, Baskin SI (1995) Specificity studies of 3-mercaptopyruvate sulfurtransferase. J Biochem Toxicol 10:287–292

Porter DW, Nealley EW, Baskin SI (1996) In vivo detoxification of cyanide by cystathionase γ-lyase. Biochem Pharmacol 52:941–944

Sekowska A, Kung H-F, Danchin A (2000) Sulfur metabolism in *Escherichia coli* and related bacteria: facts and fiction. J Mol Microbiol Biotechnol 2:145–177

Sőrbo B (1955) Rhodanase. Methods Enzymol 2:334–337

Stipanuk MH, Dominy JE Jr, Lee J-I, Coloso RM (2006) Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. J Nutr 136:1652S–1659S

Toohey JL (1989) Sulphane sulphur in biological systems: a possible regulatory role. Biochem J 264:625–632

Tse Sum Bui B, Escalettes F, Chottard G, Florentin D, Marquet A (2000) Enzyme-mediated sulfide production for the reconstitution of [2Fe-2S] clusters into apo-biotin synthase of *Escherichia coli*, Sulfide transfer from cysteine to biotin. Eur J Biochem 267:2688–2694

Valentine WN, Frankenfeld JK (1974) 3-Mercaptopyruvate sulfurtransferase (E.C.2.8.1.2): a simple assay adapted to human blood cells. Clin Chim Acta 51:205–210

Westley J (1973) Rhodanese. Adv Enzymol Relat Areas Mol Biol 39:327–368

Westley J, Adler H, Westley L, Nishida C (1983) The sulfurtransferases. Fundam Appl Toxicol 3:377–382

Wood IL (1987) Sulfane sulfur. Methods Enzymol 43:25–29

Wróbel M, Jurkowska H, Śliwa L, Srebro Z (2004) Sulfurtransferases and cyanide detoxification in mouse liver, kidney and brain. Toxicol Mech Methods 14:331–337

Yamagata S, Isaji M, Yamane T, Iwana T (2002) Substrate inhibition of L-cysteine α,β-elimination reaction catalyzed by L-cystathionine γ-lyase of *Saccharomyces cerevisiae*. Biosci Biotechnol Biochem 66:2706–2709

Yamanishi T, Tuboi S (1981) The mechanism of the L-cystine cleavage reaction catalyzed by rat liver gamma-cystathionase. J Biochem (Tokyo) 89:1913–1921

Yamanishi T, Kubota I, Tuboi S (1983) Mechanism of the activation of δ-aminolevulinate synthetase in *Rhodopseudomonas spheroides* by rat liver mitochondrial fraction. J Biochem (Tokyo) 94:181–188