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SHORT PAPER

The basis for an apparent auxotrophy for reduced sulphur metabolites in *s*F⁻ mutants of *Aspergillus nidulans*

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SUMMARY

*s*F⁻ mutants of *Aspergillus nidulans* are subject to toxicity by a metabolite derived from sulphate but are not blocked in cysteine biosynthesis. Bearing in mind the unavoidable presence of sulphate in agar-solidified media, their apparent auxotrophy for thiosulphate, L-cysteine or L-methionine stems from the ability of these reduced sulphur-containing metabolites to repress, directly or indirectly, the syntheses of sulphate permease and the enzymes of sulphate assimilation, thereby preventing synthesis of the toxic metabolite.

Cysteine biosynthesis in the ascomycete fungus *Aspergillus nidulans* (see Fig. 1) has been the subject of considerable biochemical and genetical analysis (e.g. Arst, 1968; Gravel, Käfer, Niklewicz-Borkenhagen & Zambryski, 1970; Pieniazek, Kowalska & Stepien, 1973; Pieniazek, Stepien & Paszewski, 1973; Pieniazek, Bal, Balbin & Stepien, 1974; Paszewski & Grabski, 1974, 1975; Bal, Maleszka, Stepien & Cybis, 1975; Stepien, Pieniazek, Bal & Morzycka, 1975). Nevertheless, the only genetically characterized mutants which might be considered to lack sulphite reductase, the enzyme catalysing the reduction of sulphite to sulphide, are the *s*F⁻ mutants described by Gravel *et al.* (1970). These mutants respond to thiosulphate, L-cysteine and L-methionine but not to sulphate or sulphite. An enzyme having sulphite reductase activity from *A. nidulans* has been characterized biochemically (Yoshimoto, Nakamura & Sato, 1967).

*s*F is located in linkage group VII between the *prn* gene cluster, involved in L-proline catabolism, ($\sim$15 cM away) and *panto*B, involved in D-pantothenate biosynthesis, ($<$1 cM away) (Arst & MacDonald, 1975). The *nim*0 gene, defined by a temperature-sensitive amitotic mutation (Morris, 1975), is also tightly linked to *s*F. Therefore a cross of partial genotype *nim*0-18 *prn*A-1 × *s*F-211 *panto*B-100 was analysed. By scoring for the unselected markers amongst *nim*0⁺ *s*F⁺ and *nim*0⁺ *panto*B⁺ recombinants, the map order *prn*A——*s*F——*panto*B——*nim*0 was obtained.

Unlike mutants blocked in the conversion of sulphate to sulphite, *s*F-211 strains have an inhibited, rather than a sulphur-starved, morphology on solid media containing sulphate as the sole sulphur source. As agar contains sulphated polysaccharides, sulphate cannot be excluded from agar-solidified media and is therefore routinely present whether or not another sulphur source is added. Confirmation that *s*F⁻ strains suffer from sulphate toxicity comes from the growth properties of *s*F-211 *su*A-25*meth* double mutants. The *su*A-25*meth* mutation results in derepression of the syntheses of sulphate permease and the enzymes of sulphate assimilation, probably because of loss of a negatively acting regulatory molecule (Paszewski & Grabski, 1975; Lukaszkiewicz & Paszewski, 1976).

Double mutants carrying both *s*F-211 and *su*A-25*meth* grow poorly on any sulphate-containing medium, irrespective of the presence of another sulphur source. Because of the *su*A-25*meth* mutation, reduced sulphur compounds which can normally cause repression because of conversion to the effector(s) L-cysteine and/or L-homocysteine (Paszewski & Grabski, 1974) do not diminish sulphate uptake or conversion (if necessary) of sulphate to a toxic metabolite.

Although the near lethality of the *s*F-211 *su*A-25*meth* genotype in the presence of sulphate establishes that toxicity of sulphate or a sulphate-derived metabolite is at least partly responsible for the *s*F-211 phenotype, it does not distinguish between two possible origins of the toxicity. Firstly, a block in the cysteine biosynthetic pathway might lead to accumulation of a toxic intermediate. Alternatively, *s*F-211 might not interfere with sulphate assimilation but might simply render the organism subject to toxicity by sulphate or some metabolite derived from sulphate. These two possibilities are easily distinguished by their predictions of the patterns of epistasy in doubly mutant strains carrying *s*F-211 and a lesion in sulphate assimilation. A block in the pre-sulphite part

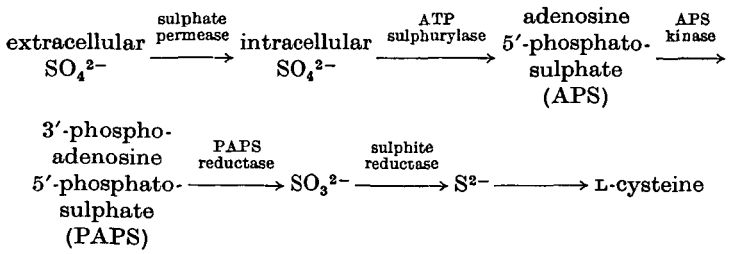

Fig. 1. The sulphate assimilation pathway leading to cysteine biosynthesis.

of the sulphate assimilation pathway should relieve the toxicity if it prevents accumulation of the toxic metabolite, but it should not affect the requirement for a reduced sulphur metabolite if *s*F-211 blocks cysteine biosynthesis. However, if sulphate toxicity be the sole basis for the *s*F$^-$ phenotype, then any mutation relieving the toxicity should be completely epistatic to *s*F-211. The results confirm the second alternative. Double mutants carrying *s*F-211 and a mutation leading to loss of any of the three enzymes necessary for conversion of sulphate to sulphite – ATP sulphurylase (*s*C-12), APS kinase (*s*D-50), or PAPS reductase (*s*A-1 or *s*E-15) (Arst, 1968; Gravel *et al.* 1970) – respond to sulphite and are indistinguishable from the respective single mutants carrying the wild type *s*F allele. Thus *s*F-211 does not lead to loss of sulphite reductase or any other activity indispensable for cysteine biosynthesis.

The identity of the toxic metabolite remains elusive. The fact that *s*F-211 *s*C-12, *s*F-211 *s*D-50, *s*F-211 *s*A-1, and *s*F-211 *s*E-15 double mutants are resistant to sulphate and respond to sulphite establishes firstly that sulphate must undergo metabolic conversion to become toxic and secondly that this conversion involves the pre-sulphite part of the sulphate assimilation pathway. The toxic metabolite cannot be sulphite, nor can sulphite serve as its precursor. This conclusion seems, at first sight, in contradiction with the abilities of *s*A-1 and *s*E-15, which prevent conversion of PAPS to sulphite, to protect *s*F-211 strains against sulphate toxicity. However, Gravel *et al.* (1970) have shown that levels of choline-O-sulphate (COS), in whose synthesis PAPS acts as the sulphate donor (Kaji & Gregory, 1959; Orsi & Spencer, 1964), are substantially reduced in *s*A$^-$ and *s*E$^-$ mutants. Presumably, the accumulating PAPS exerts a very potent feedback inhibition on one or more of the preceding metabolic steps (i.e. sulphate transport, ATP sulphurylase, APS kinase). The same regulatory role of PAPS could enable *s*A-1 and *s*E-15 to relieve sulphate toxicity to *s*F-211 strains. It is therefore possible that a sulphated compound

for which PAPS serves as the sulphate donor be the toxic metabolite. COS can probably be eliminated as a possibility, however. Under conditions in which it supplements choline auxotrophies and must therefore be taken up (Arst, 1968, 1971; Gravel, 1976), exogenous COS is toxic to *s*F-211 strains only in so far as the sulphate produced by its hydrolysis is toxic (i.e. *s*F-211 *s*C-12, *s*F-211 *s*D-50, *s*F-211 *s*A-1, and *s*F-211 *s*E-15 double mutants are not inhibited by COS).

Whatever the identity of the toxic metabolite, however, it is clear that the apparent auxotrophy of *s*F-211 strains results not from a lesion in cysteine biosynthesis but from a need to restrict sulphate assimilation. Thiosulphate, L-cysteine and L-methionine all accomplish this. Sulphite is ineffective because it is too poor a sulphur source to result in repression. Similarly, D-methionine, another derepressing sulphur source (at least at low concentrations such as 200 μM (Arst, 1968)), does not protect *s*F-211 strains against toxicity although it does supplement methionine auxotrophs and *s*C$^-$, *s*D$^-$, *s*A$^-$ and *s*E$^-$ strains.

Note added in proof

The *s*B-3 mutation, resulting in loss of the sulphate permease (Arst, 1968; Lukaszkiewicz, & Paszewski, 1976) protects *s*F-211 strains against sulphate toxicity (in the presence of a non-repressing sulphur source such as sulphite or D-methionine). Unlike *s*-C-12, *s*D-50, *s*A-1, and *s*E-15, the *s*B-3 mutation does not protect *s*F-211 strains against choline-O-sulphate toxicity. This is consistent with the fact that *s*B-3 single mutants but not the single mutants blocked in the conversion of intracellular sulphate to sulphite are able to utilize COS as a source of sulphur (Arst, 1968). However, *csu*A-6, resulting in loss of the hydrolytic enzyme choline-O-sulphatase (Arst, 1971; Gravel, 1976), does not protect *s*F-211 strains against COS toxicity (monitored in *s*B-3 *csu*A-6 *s*F-211 triple mutants so that *s*B-3 can protect against the toxicity of sulphate present in the medium). Moreover, *csu*A-6 does not enhance sulphate toxicity in *s*F-211 strains. This confirms that COS is not the metabolite responsible for sulphate toxicity to *s*F-211 strains and demonstrates that the toxicity of COS is dependent upon its conversion to sulphate.

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REFERENCES

Arst, H. N., Jr. (1968). Genetic analysis of the first steps of sulphate metabolism in *Aspergillus nidulans*. *Nature* **219**, 268–270.

Arst, H. N., Jr. (1971). Mutants of *Aspergillus nidulans* unable to use choline-O-sulphate. *Genetical Research* **17**, 273–277.

Arst, H. N., Jr. & Macdonald, D. W. (1975). A gene cluster in *Aspergillus nidulans* with an internally located *cis*-acting regulatory region. *Nature* **254**, 26–31.

Bal, J., Maleszka, R., Stepien, P. & Cybis, J. (1975). Subcellular mislocation of cysteine synthase in a cysteine auxotroph of *Aspergillus nidulans*. *Federation of European Biochemical Societies Letters* **58**, 164–166.

Gravel, R. A. (1976). Choline-O-sulphate utilization in *Aspergillus nidulans*. *Genetical Research* **28**, 261–276.

Gravel, R. A., Käfer, E., Niklewicz-Borkenhagen, A. & Zambryski, P. (1970). Genetic and accumulation studies in sulfite-requiring mutants of *Aspergillus nidulans*. *Canadian Journal of Genetics and Cytology* **12**, 831–840.

Lukaszkiewicz, Z. & Paszewski, A. (1976). Hyper-repressible operator-type mutant in sulphate permease gene of *Aspergillus nidulans*. *Nature* **259**, 337–338.

Morris, N. R. (1975). Mitotic mutants of *Aspergillus nidulans*. *Genetical Research* **26**, 237–254.

Orsi, B. A. & Spencer, B. (1964). Choline sulphokinase (sulphotransferase). *Journal of Biochemistry* (*Tokyo*) **56**, 81–91.

Paszewski, A. & Grabski, J. (1974). Regulation of S-amino acids biosynthesis in *Aspergillus nidulans*. Role of cysteine and/or homocysteine as regulatory effectors. *Molecular and General Genetics* **132**, 307–320.

Paszewski, A. & Grabski, J. (1975). Enzymatic lesions in methionine mutants of *Aspergillus nidulans*: role and regulation of an alternative pathway for cysteine and methionine synthesis. *Journal of Bacteriology* **124**, 893–904.

Pieniazek, N. J., Bal, J., Balbin, E. & Stepien, P. P. (1974). An *Aspergillus nidulans* mutant lacking serine transacetylase: evidence for two pathways of cysteine biosynthesis. *Molecular and General Genetics* **132**, 363–366.

Pieniazek, N. J., Kowalska, I. M. & Stepien, P. P. (1973). Deficiency in methionine adenosyltransferase resulting in limited repressibility of methionine biosynthetic enzymes in *Aspergillus nidulans*. *Molecular and General Genetics* **126**, 367–374.

Pieniazek, N. J., Stepien, P. P. & Paszewski, A. (1973). An *Aspergillus nidulans* mutant lacking cystathionine β-synthase: identity of L-serine sulfhydrylase with cystathionine β-synthase and its distinctness from O-acetyl-L-serine sulfhydrylase. *Biochimica et Biophysica Acta* **297**, 37–47.

Stepien, P. P., Pieniazek, N. J., Bal, J. & Morzycka, E. (1975). Cysteine biosynthesis in *Aspergillus nidulans*. *Acta Microbiologica Polonica A* **7**, 201–210.

Yoshimoto, A., Nakamura, T. & Sato, R. (1967). Isolation from *Aspergillus nidulans* of a protein catalysing the reduction of sulfite by reduced viologen dyes. *Journal of Biochemistry* (*Tokyo*) **62**, 756–766.

Kaji, A. & Gregory, J. D. (1959). Mechanism of sulfurylation of choline. *Journal of Biological Chemistry* **234**, 3007–3009.