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THE REGULATORY *cysK* MUTANT OF *S. TYPHIMURIUM*

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A triazole-resistant mutant, *cysK1358*, showing novel properties was isolated. Biochemical analysis of this strain suggests a regulatory character of the *cysK1358* mutation.

The *cysK1358* mutation has a pleiotropic effect: the expression of the other gene, *cysA*, is also altered.

The growth of *S. typhimurium* is inhibited by 1,2,4-triazole (Bogusławski *et al.*, 1967), and our previous studies indicated that 1,2,4-triazole prevents induction of the cysteine biosynthetic enzymes by *O*-acetyl-L-serine.

The triazole-resistant strain, *trzA*, bears a mutation in the structural gene for *O*-acetylserine sulfhydrylase *A*, and this locus was designated *cysK*. More recent studies have shown that *O*-acetylserine sulfhydrylase *A* catalyses the reaction between *O*-acetylserine and 1,2,4-triazole giving 1,2,4-triazolyl-1-alanine as a product (Kredich *et al.*, 1975). Thus, the resistance of *cysK* strains to 1,2,4-triazole appears to be due to the low activity of *O*-acetylserine sulfhydrylase and the consequent inability of 1,2,4-triazole to decrease the level of *O*-acetylserine.

The present communication describes a mutant strain of *S. typhimurium* with an altered expression of the *cysK* gene. This strain, *cysK1358*, carries a mutation which renders repressed the two cysteine enzymes: *O*-acetylserine sulfhydrylase *A* and sulphate permease.

MATERIALS AND METHODS

Bacterial strains and phages. The *S. typhimurium* strains used are listed in Table 1. The phage P22 mutant *int-4* was used for transduction (Smith & Levine, 1967).

Culture media, culture conditions and procedures for preparation of cell-free extracts were as described previously (Hulanicka *et al.*, 1972).

Genetic procedure. Phage lysates were prepared, and transduction was performed as previously (Hulanicka *et al.*, 1972).

Table 1

Strains of Salmonella typhimurium used

Strain	Genotype	Origin
LT2	wild-type	From stock collection
	hisD23gal50 HfrB2	P. Hartman
TK1104	*hisD23gal50cysK1358* HfrB2	Spontaneous mutant resistant to 1,2,4-triazole
	pyrF146	From stock collection
	cysA20	From stock collection
DW25	*cysB1352*	N.M. Kredich
TK1028	*cysA20*$^+$*cysK1358*	Recombinant from *cysA20*×TK1104
SB1690	*trpB223ptsI*	From J. L. Cordaro
TK1101	*pyrF146cysA*	By Pardee's method from *pyrF146*
TK1109	*pyrF146cysA*$^+$*cysK1358*	Transductants from TK1028 lysate× ×TK1101
TK1110	*pyrF146cysA*$^+$*cysK1358*$^+$	
TK1123	*pyrF146*$^+$*cysB1352cysA*$^+$*cysK1358*	Transductants from DW25 lysate× ×TK1109
TK1124	*pyrF146*$^+$*cysB1352*$^+$*cysA*$^+$*cysK1358*	
TK1125	*pyrF146*$^+$*cysB1352cysA*$^+$*cysK*1358$^+$	Transductant from DW25 lysate× ×TK1110

Enzyme assays. The activity of sulphate permease was determined as described by Karbonowska *et al.* (1977).

Sulphite reductase was assayed by the method of Siegel & Kamin (1971).

O-Acetylserine sulfhydrylase activity was determined according to Kredich (1971). One unit of *O*-acetylserine sulfhydrylase is defined as the amount of enzyme catalysing formation of 1 μmole of cysteine per minute.

Antibodies against *O*-acetylserine sulfhydrylase *A* were prepared, and immunological determination of this enzyme was performed as described by Hulanicka *et al.* (1974).

Protein was determined by the biuret method (Gornall *et al.*, 1949).

Chemicals. O-Acetylserine was prepared by the method of Sakami & Toennies (1942). Other chemicals used were commercial products of reagent grade. Carrier-free sodium [^{35}S]sulphate was purchased from the Institute for Nuclear Research (Świerk, Poland).

RESULTS

A triazole resistant mutant *trzA* with novel properties was isolated during our studies on the mechanism of triazole inhibition. In this mutant, synthesis of *O*-acetylserine sulfhydrylase *A* is repressed, irrespective of the sulphur source in the medium.

Preliminary mapping of this mutation by conjugation proved its location to be in the *cysA-pts* region. Since the presence of *gal50* mutation in the *hisD23gal50-*

cysK1358 HfrB2 strain renders cells resistant to P22 phage, one triazole-resistant recombinant from conjugation mating with *cysA20* was used for phage propagation of P22 - L4 mutant.

The triazole-resistant mutation obtained is 55% cotransducible with the *cysA* and 90% with the *pts* genes (Table 2). The same genetic linkage was found for the *trzA* mutants, designated *cysK* (Hulanicka *et al.*, 1974). These values of cotransduction strongly suggest that triazole resistance of the mutant results from a mutation in the *cysK* gene coding for *O*-acetylserine sulfhydrylase *A*, or close to this locus.

Table 2

Cotransduction of cysK1358 with pts and cysA

Transductants were selected as either *pts*$^+$ or *cysA*$^+$ recombinants and scored for their triazole resistance or sensitivity by replicate plating. The *cysK1358* strain was used as a phage donor.

Recipient	Total transductants	*trz*$^-$ transductants	Cotransduction (%)
cysA20	200	110	55
trpB223ptsI	200	180	90

Because of biochemical properties of this *trzA* mutation (see below) we propose to denote the novel mutation *cysK1358*.

The effect of cysK1358 mutation on the expression of cysteine genes. The location and triazole resistance of the *cysK1358* mutation prompted us to assay the activity of *O*-acetylserine sulfhydrylase and examine its properties in this mutant. Crude extracts of the wild-type and mutant strains grown on different sulphur sources were studied. In agreement with the results of Kredich (1971), a high activity of *O*-acetylserine sulfhydrylase was found in the wild-type cells grown on a poor source of sulphur such as L-djenkolate, and a low activity in the cells grown on a good sulphur source such as cysteine. Growth on sulphate led to an intermediate activity of this enzyme. However, in the triazole-resistant strain *cysK1358* the activity of *O*-acetylserine sulfhydrylase in the cells grown on different sulphur sources was practically the same, ranging from 0.36 to 0.55 units/mg protein. These values are similar to those found for the wild-type cells grown on cysteine (0.67 unit/mg protein). Moreover, the lack of *O*-acetylserine sulfhydrylase derepression in the *cysA*$^+$*cysK1358* transductant indicates that the phenotype of the parental strain results from a single mutation (Table 3).

The same level of the enzyme on different sulphur sources suggests a regulatory rather than a structural type of this mutation. To check this supposition, a quantitative immunochemical determination of *O*-acetylserine sulfhydrylase *A* was performed. Two methods were used for the assay of the mutant protein cross-reacting with the antibodies against the wild-type *O*-acetylserine sulfhydrylase *A*. In the "direct" assay the extracts of the mutant and the wild-type enzyme were titrated separately. In the "indirect" assay, the mutant protein was added to the assay mixture containing the same amount of the wild-type enzyme. As shown in Fig. 1, the nega-

Table 3

The activity of O-acetylserine sulfhydrylase of mutants and wild-type strains grown on different sulphur sources

The activity of enzyme was expressed in units/mg protein.

Source of sulphur	*hisD23gal50cysK1358* HfrB2	*pyrF146cysA*$^+$ *cysK1358*	*hisD23gal50* HfrB2
L-Cysteine	0.36	0.32	0.67
L-Djenkolate	0.55	0.31	14.60
Sulphate	0.48	0.37	5.20

tive slope of titration curves of the mutant and the wild-type enzyme is the same. The results suggest the presence of the wild-type *O*-acetylserine sulfhydrylase *A* in the strains bearing the *cysK1358* mutation.

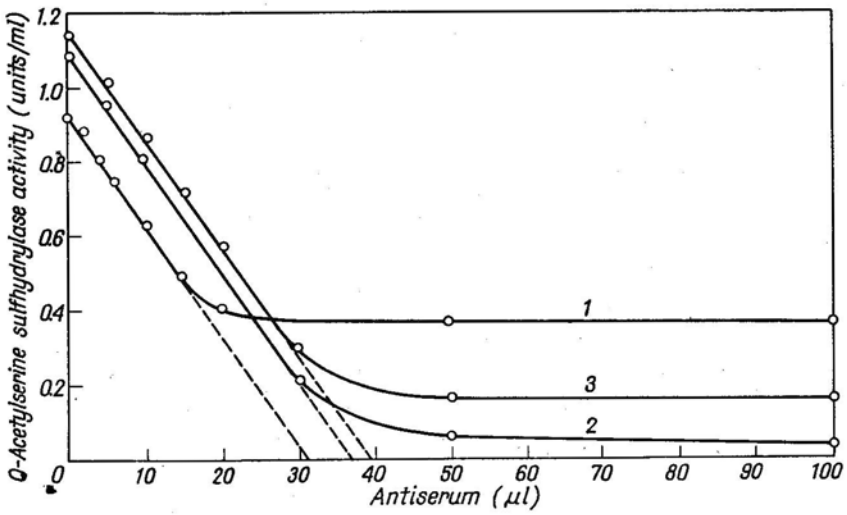

Fig. 1. Immunochemical assay of *O*-acetylserine sulfhydrylase of the wild type and the *cysK1358* mutant. Each point represents the enzyme activity remaining after preincubation of the extract with the indicated volume of antiserum. The broken line (— — —) is an extrapolation of the initial, linear portion of the titration curve. 1, *pyrF146cysA*$^+$*cysK1358* (TK1109); 2, *pyrF146cysA*$^+$-*cysK1358*$^+$ (TK1110); 3, TK1109+TK1110.

As can be seen from Table 4, in the *cysK1358* strain the regulation of *cysA* gene, located near the *cysK1358* mutation, is altered. The level of sulphate permease is repressed and cannot be derepressed by cysteine starvation, similarly as in the case of *O*-acetylserine sulfhydrylase. Sulphite reductase is regulated in the same way as in the wild-type cells, namely growth on cysteine causes repression, whereas cysteine starvation in the cells grown on L-djenkolate results in derepression of the enzyme (Table 4).

Table 4

Sulphate permease and sulphite reductase in cysK1358 and cysK1358⁺ strains

For conditions of the assays see Methods.

Source of sulphate	*pyrFcysA⁺cysK1358*		*pyrFcysA⁺cysK1358⁺*	
	Sulphite reductase (μmole/mg protein/min)	Sulphate permease (pmole/mg dry wt./min)	Sulphite reductase (μmole/mg protein/min)	Sulphate permease (pmole/mg dry wt./min)
L-Cystine	0.006	0.080	0.007	0.090
L-Djenkolate	0.020	0.090	0.024	0.431

The effect of the constitutive cysB1352 mutation on expression of the cysK1358 allele. The *cysB* locus is a regulatory gene of the cysteine regulon and the prototrophic *cysB1352* mutant shows the constitutive expression of the cysteine enzymes.

It seemed interesting to check whether the presence of the constitutive mutation *cysB1352* can affect the altered regulation of sulphate permease and *O*-acetylserine sulfhydrylase in the *cysK1358* mutant. The double mutant carrying both these mutations was constructed, and the extracts of this strain grown on different sulphur sources were assayed for the cysteine enzymes. The results presented in Table 5 show that *cysB1352* has no effect on the level of sulphate permease and *O*-acetylserine sulfhydrylase. Both enzymes were repressed and their activity was similar to that found in the *pyrFcysK1358* strain. This means that the *cysK1358* mutation is epistatic to the *cysB1352* mutation.

Table 5

The effect of cysB1352 mutation on cysteine enzymes in the cysK1358 and cysK1358⁺ strains

Strains	*O*-Acetylserine sulfhydrylase (units/mg protein)		Sulphate permease (picomole/mg dry wt./min)		Sulphite reductase (μmole/mg protein/min)	
	L-Cysteine	L-Djenkolate	L-Cysteine	L-Djenkolate	L-Cysteine	L-Djenkolate
cysB1352	15.1	18.5	0.60	0.62	0.040	0.050
pyrF⁺cysB1352⁺cysA⁺cysK1358	0.47	0.52	0.034	0.053	0.009	0.032
pyrF⁺cysB1352cysA⁺cysK1358	0.87	0.54	0.039	0.037	0.032	0.038
pyrF⁺cysA⁺cysK1358	0.81	0.49	0.056	0.056	0.006	0.034
pyrF⁺cysA⁺cysK1358⁺	0.48	20.0	0.017	0.82	0.007	0.032

Sulphite reductase is the only enzyme studied which is subject to regulation by the *cysB1352* mutation, and is expressed constitutively, similarly as in the *cysB1352* strain.

DISCUSSION

The repressed non-regulated level of *O*-acetylserine sulfhydrylase *A* in the *cysK1358* mutant suggests that *cysK1358* mutation is regulatory rather than structural. Immunological titration of *O*-acetylserine sulfhydrylase *A* of the *cysK1358* strain indicates the presence of the wild-type enzyme, which confirms a regulatory character of the *cysK1358* mutation.

In all *cysK* mutants known so far, the level of *O*-acetylserine sulfhydrylase, although it was low, was still dependent on sulphur source in the medium. Probably, the lack of regulation of the expression of the *cysK* gene in the *cysK1358* mutant is caused by a mutation in a regulatory site of this locus, presumably in the initiator. The *cysK1358* mutation has a pleiotropic effect since the expression of the gene *cysA* is also altered.

The simplest explanation to account for these data is that both genes *cysA* and *cysK* are transcribed jointly, as it takes place during transcription of genes forming one operon.

CysB locus is a regulatory gene of cysteine regulon and the prototrophic *cysB1352* mutant shows a constitutive expression of cysteine enzymes (Kredich, 1971). The presence of this constitutive mutation had no effect on the expression of the *cysK* and *cysA* genes, whereas the others were derepressed.

The mutation in a regulatory site of promoter or initiator should be located at one extreme end of the gene (Reznikoff, 1972). Unfortunately, in the *cysK* mutants it is not possible to perform a fine mapping, since the mutation in the *cysK* structural gene does not lead to cysteine requirement (Hulanicka *et al.*, 1974). Basing on the above the *cysK1358* mutation seems to be similar to the operator-down mutations of histidine operon isolated by Ely (1974) and Ely *et al.* (1974).

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CysK-REGULATOROWY MUTANT *SALMONELLA TYPHIMURIUM*

Streszczenie

Wyizolowano odpornego na triazol mutanta *cysK1358* o nowych właściwościach. Biochemiczna analiza szczepu *cysK1358* sugeruje regulatorowy charakter mutacji *cysK1358*. Mutacja ta ma właściwości plejotropowe, gdyż w tym szczepie ekspresja drugiego genu, *cysA*, jest również zmieniona.

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