Molec. gen. Genetics 148, 143–148 (1976)

MGG



# A Mutation Affecting Expression of the Gene Coding for Serine Transacetylase in *Salmonella typhimurium*

M. Danuta Hulanicka

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Nicholas M. Kredich

Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

**Summary.** A 1,2,4-triazole resistant mutant of *S. typhimurium* has been isolated, in which serine transacetylase activity is seven times higher than in wild type. Partially purified serine transacetylase from a strain carrying the *trz-312* mutation has kinetic properties which are virtually identical to those of the wild type enzyme and binds to *O*-acetylserine sulfhydrylase A to form a cysteine synthetase complex which is also indistinguishable from that found in wild type. Thus the increased activity of serine transacetylase associated with *trz-312* appears to result from increased quantities of a kinetically normal, enzyme protein. Resistance to 1,2,4-triazole is probably due to the ability of *trz-312* strains to synthesize *O*-acetyl-L-serine at a rapid enough rate to compensate for that utilized by the *O*-acetylserine triazolylase reaction.

Genetic mapping experiments, using P1-mediated transduction, show that *trz-312* is 91–99% linked to *cysE*, the structural gene for serine transacetylase. The results of three point crosses indicate that this mutation is located at one extreme end of the *cysE* locus, as would be expected for a promotor mutation.

## Introduction

The growth of *Salmonella typhimurium* is inhibited by 1,2,4-triazole (Boguslawski et al., 1967). Previous studies have shown that 1,2,4-triazole interferes with cysteine biosynthesis, and prevents the induction of sulfate permease, ATP-sulfurylase and adenosine-5′-phosphosulfate kinase by *O*-acetyl-L-serine in cells grown on sulfate as the sole sulfur source (Hulanicka et al., 1972). Furthermore, 1,2,4-triazole resistant mutants of the types *trzA* and *trzB* have been shown to be insensitive to this anti-inducer property of 1,2,4-triazole. More recent studies have shown that the enzyme *O*-acetylserine sulfhydrylase A catalyzes a reaction between *O*-acetyl-L-serine and 1,2,4-triazole, giving 1,2,4-triazolyl-1-alanine as a product (Kredich et al., 1975). This enzyme ordinarily carries out the final step of cysteine biosynthesis, in which *O*-acetyl-L-serine and sulfide react to form L-cysteine and acetate. The available evidence indicates that 1,2,4-triazole inhibits cysteine biosynthesis, and thus cell growth, by depleting cellular levels of *O*-acetyl-L-serine, which compound serves not only as a cysteine precursor but also as an internal inducer of cysteine biosynthetic enzymes (Kredich, 1971).

We have demonstrated that *trzA* strains bear a mutation in the structural gene for *O*-acetylserine sulfhydrylase A, and this locus has been designated *cysK* (Hulanicka et al., 1974). *trzB* strains have very low or absent levels of *O*-acetylserine sulfhydrylase A and carry mutations in or very near the *cysK* locus, which are relatively unstable, and which may give rise to non-transmissible plasmids (Hulanicka and Klopotowski, 1971). The resistance of *trzA* and *trzB* strains to 1,2,4-triazole appears to be due either to their low activities of *O*-acetylserine sulfhydrylase A and the consequent inability of 1,2,4-triazole to deplete *O*-acetyl-L-serine levels, or to a preferential loss of triazolylase activity, or to both (Kredich et al., 1975).

This communication describes a mutant strain of *S. typhimurium* with an altered expression of *cysE*, the structural gene for serine transacetylase, which enzyme catalyzes the synthesis of *O*-acetyl-L-serine from L-serine and acetyl-CoA (Kredich and Tomkins, 1966). This strain carries a mutation in or near the *cysE* locus itself, has seven times normal levels of serine transacetylase, and is resistant to 1,2,4-triazole.

## Materials and Methods

*Organisms.* All bacteria used in these studies were derivatives of *S. typhimurium* LT2 (Table 1). The *galE*, P1-sensitive strains used

**Table 1.** Description of bacterial strains

| Strain | Genotype | Source or method of construction[a] |
|---|---|---|
| TK1000 | wild type LT2 of N.D. Zinder | B.N. Ames |
| – | *pyrE125* | P.E. Hartman |
| HfrB2 | HfrB2 *hisD23 gal-50* | P.E. Hartman |
| DW148 | HfrB2 *hisD23 gal-50 trz-312* | Spontaneous mutant from HfrB2, resistant to 1,2,4-triazole |
| DW158 | *trz-312* | *pyrE*$^+$, 1,2,4-triazole resistant recombinant from *pyrE125* × DW148 |
| DW24 | *cysE396* | (Kredich, 1971) |
| TK1106 | *trz-312* | Cysteine prototroph, resistant to 1,2,4-triazole from DW24 × DW158 |
| TK1065 | *pyrE125 cysE1709* | Sensitive to phage P1[b] |
| TK1066 | *pyrE125 cysE1711* | ,, |
| TK1017 | *pyrE125 cysE1714* | ,, |
| TK1019 | *pyrE125 cysE1720* | ,, |
| TK1092 | *pyrE125 cysE1722* | ,, |
| TK1091 | *pyrE125 cysE1723* | ,, |
| TK1021 | *pyrE125 cysE1725* | ,, |
| TK1022 | *pyrE125 cysE1726* | ,, |
| TK1023 | *pyrE125 cysE1728* | ,, |
| TK1024 | *pyrE125 cysE1730* | ,, |
| TK1025 | *pyrE125 cysE1731* | ,, |
| TK1026 | *pyrE125 cysE1732* | ,, |

[a] Transductions used to construct strains were performed with the L4 derivative of phage P22. The recipient is shown on the left and the donor on the right

[b] These strains were derived from their parental *pyrE125 cysE* strains as spontaneous mutants which are resistant to phage FO and transducible by phage P1. All are sensitive to D-galactose, and presumably carry mutations in *galE* (Mojica-A, 1975)

for genetic mapping were derived as phage FO resistant mutants (Mojica-A, 1975) from a series of *pyrE125 cysE* strains originally isolated by Michael Becker. Phage P1CM *clr-100* was obtained from John Roth. This P1 strains carries a chloramphenicol resistance marker and a heat sensitive repressor (Rosner, 1972).

*Media and Culture Conditions.* The minimal medium used was the medium C of Vogel and Bonner (1956) in which $MgSO_4$ was replaced by an equimolar amount of $MgCl_2$, and the sulfur source was provided as either 1.0 mM L-cystine, 0.5 mM L-djenkolic acid or 0.1 mM $Na_2SO_4$ (Hulanicka and Klopotowski, 1972). Supplements consisted of 0.5% glucose and, when appropriate for the growth of auxotrophs, 0.2 mM uracil and 0.1 mM of the required amino acid. Solid media were prepared by the addition of agar and additional glucose to final concentrations of 1.5% and 2.0% respectively. Recipient bacteria in transductions were grown in nutrient broth for P22 and in Luria broth (Luria and Burrows 1957) for P1.

Except as specifically noted, all liquid cultures were grown with rotary shaking at 37°. Cell densities were estimated by measuring turbidity at 650 nm. Bacteria for enzyme studies were harvested from exponentially growing cultures by centrifugation.

*Genetic Methods.* Phage P22 transduction was performed as described previously (Hulanicka and Klopotowski, 1972), using the L4 strain of Smith and Levine (1967). Three point crosses were carried out by transduction with phage P1CM *clr-100* in the manner described by Mojica-A (1975) for *S. typhimurium*. This procedure involves the use of *galE*, P1-sensitive strains as recipients and phage prepared by heat induction of temperature sensitive lysogens. Resistance to 1,2,4-triazole was scored by replicating colonies onto minimal agar plates containing 10 mM of this inhibitor and any other appropriate nutrients. $Cys^-$ strains cannot be scored for 1,2,4-triazole resistance, owing to reversal of inhibition by cysteine and *O*-acetyl-L-serine.

*Enzyme Studies.* *O*-Acetylserine sulfhydrylase and serine transacetylase activities were determined as described previously (Kredich et al., 1969). For inhibitor studies in which serine transacetylase was assayed in the presence of L-cysteine, and alternative method was used (Kredich and Tomkins, 1966), based on the loss of absorbance at 232 nm caused by cleavage of the thioester bond of acetyl-CoA (Stadtman, 1953). Sulfite reductase was assayed as hydroxylamine reductase by the method of Siegel and Kamin (1971). An enzyme unit is defined as that amount catalyzing the formation of one μmole of product per min, except for hydroxylamine reductase for which the oxidation of one μmole of NADPH per min comprises an enzyme unit.

Cysteine synthetase was partially purified and separated from *O*-acetylserine sulfhydrylase A by a modification of a previously described method (Kredich et al., 1969). A crude extract from approximately 5 g of bacterial paste was diluted to a protein concentration of 20 mg/ml, and one fourth volume of 5% protamine sulfate (neutralized with NaOH) was slowly added with stirring. After 30 min at 23° the mixture was clarified by centrifugation and 225 mg ammonium sulfate per ml was added to the supernatant. Precipitated protein was removed by centrifugation, and to the supernatant was added 80 mg ammonium sulfate per ml. After collection by centrifugation, this precipitate was dissolved in a small volume of 0.1 M Tris-HCl, pH 7.6 containing 0.5 M NaCl, 1 mM 2-mercaptoethanol, 0.5 mM $Na_2EDTA$, and applied to a 90 cm × 1.8 $cm^2$ column of Sephadex G-150 at 23°, equilibrated with the same buffer. Fractions were assayed for serine transacetylase and *O*-acetylserine sulfhydrylase activities. Using this proce-

dure, all the serine transacetylase appears in the void volume bound to *O*-acetylserine sulfhydrylase A as cysteine synthetase, and is easily separated from the lower molecular weight, free *O*-acetylserine sulfhydrylase A.

Immunochemical titrations of *O*-acetylserine sulfhydrylase A were performed as described previously (Hulanicka et al., 1974). Protein was determined by the biuret method (Gornall et al., 1949), using bovine serum albumin as a standard.

*Chemicals.* *O*-Acetyl-L-serine was prepared by the method of Sakami and Tonnies (1942). 1,2,4-Triazole was obtained from Sigma, and other chemicals were commercial products of reagent grade.

## Results

During studies on the mechanism of 1,2,4-triazole inhibition, a 1,2,4-triazole resistant mutant, DW148, with novel properties was isolated. Unlike previously studied mutants (Hulanicka et al., 1974), DW148 was noted neither to have abnormal levels of *O*-acetylserine sulfhydrylase A activity, nor to be constitutively derepressed for cysteine biosynthetic enzymes. Furthermore, preliminary genetic mapping by conjugation established that the *trz-312* mutation in DW148 resulting in 1,2,4-triazole resistance is located in the *pyrE-cysE* region of the *S. typhimurium* chromosome, rather than in *cysK* or *cysB*.

**Table 2.** Properties of wild type and DW158 serine transacetylases[a]

| Property | TK1000 (wild type) | DW158 (*trz-312*) |
|---|---|---|
| Km, apparent for L-serine[b] | 1.1 mM | 1.0 mM |
| Km, apparent for acetyl-CoA[b] | 0.15 mM | 0.17 mM |
| Ki, apparent for L-cysteine[b] | 1.0 μM | 1.0 μM |
| Ratio of O-acetylserine sulfhydrylase/serine transacetylase activities in cysteine synthetase | 0.8–1.2 | 0.8–1.3 |
| Ratio of O-acetylserine sulfhydrylase activities after and before preincubation of cysteine synthetase with 1 mM *O*-acetyl-L-serine | 1.6 | 1.8 |
| Immunotitration of free *O*-acetylserine sulfhydrylase A (units/ml)[c] | 392 | 435 |

[a] All studies except the immunotitration of free *O*-acetylserine sulfhydrolase A were performed on partially purified cysteine synthetase

[b] Non-varied substrate concentrations were 1.0 mM for L-serine and 0.10 mM for acetyl-CoA

[c] Immunotitration data are expressed as units of enzyme activity neutralized by 1 ml of standard rabbit antiserum prepared against highly purified *O*-acetyl-serine sulfhydrylase A

*Serine Transacetylase of trz-312 Strains.* Crude extracts of DW148 were found to have 5 to 10 fold higher levels of serine transacetylase activity than those found in its parental strain *HfrB2* or in wild type. A similar increase in enzyme activity was observed in TK1106, a 1,2,4-triazole resistant strain obtained from a cross in which DW148 was used as a phage donor. The simultaneous acquisition of 1,2,4-triazole resistance and increased levels of serine transacetylase in this $cysE^+$ transductant of a *cysE* strain suggests that both phenomena result from a single mutation located in or near the *cysE* locus.

A higher specific activity of an enzyme may result from either a mutation in its structural gene, giving rise to a kinetically altered, abnormal enzyme, or from a mutation in a regulatory gene leading to an increase rate of synthesis of normal enzyme. (Reznikoff, 1972). In order to distinguish between these two possibilities, certain kinetic and physico-chemical properties of the serine transacetylase from the *trz-312* strain, DW158, were examined, and compared with those of the wild type enzyme.

Wild type serine transacetylase has the convenient property of binding to *O*-acetylserine sulfhydrylase A to form a complex, cysteine synthetase, with a molecular weight considerably greater than that of either protein alone (Kredich et al., 1969). Cysteine synthetase ordinarily comprises all of the serine transacetylase in extracts of wild type cells and, owing to its greater molecular size, can be separated from excess free *O*-acetylserine sulfhydrylase A by gel filtration chromatography. The ratio of the two enzyme activities in cysteine synthetase can then be used to detect the presence of a kinetically altered enzyme (Becker and Tomkins, 1969). When partially purified cysteine synthetase from DW158 was compared with a similar preparation from wild type, the ratio of *O*-acetylserine sulfhydrylase activity/serine transacetylase activity was found to be approximately one in both strains (Table 2). Furthermore, the total yield of cysteine synthetase per mg of crude extract protein applied to the column was approximately 25 times greater from DW158 than from wild type, indicating that the mutant strain contains increased quantities of a kinetically normal serine transacetylase protein.

Preincubation of cysteine synthetase with *O*-acetyl-L-serine causes dissociation of the complex and a resultant increase in the *O*-acetylserine sulfhydrylase activity of the preparation (Kredich et al., 1969). The *O*-acetylserine sulfhydrylase activity of partially purified cysteine synthetase was increased by a factor of 1.6 for wild type and 1.8 for DW158 after preincubation with 1 mM *O*-acetyl-L-serine (Table 2).

Kinetic studies (Table 2) on partially purified serine transacetylase (as cysteine synthetase) gave apparent Km values for L-serine of 1.1 mM for wild type

**Table 3.** Activities of cysteine biosynthetic enzymes in TK1000 (wild type) and TK1106 (*trz-312*) grown on different sulfur sources[a]

| Sulfur Source | Serine Transacetylase (units/mg) | | O-Acetylserine Sulfhydrylase (units/mg) | | Sulfite Reductase (units/mg) | |
|---|---|---|---|---|---|---|
| | TK1000 | TK1106 | TK1000 | TK1106 | TK1000 | TK1106 |
| Djenkolate | 0.020 | 0.14 | 15.1 | 16.7 | 0.060 | 0.030 |
| Sulfate | 0.018 | 0.15 | 6.7 | 5.3 | 0.018 | 0.016 |
| Cystine | 0.024 | 0.16 | 0.2 | 0.3 | 0.002 | 0.001 |

[a] The results express specific activities in crude extracts

and 1.0 mM for the enzyme from DW158. The apparent Km values for acetyl-CoA were 0.15 mM for wild type and 0.17 mM for DW158. Furthermore, the extent of feedback inhibition by L-cysteine was identical for both enzymes with an apparent Ki value of 1.0 μM.

*O*-Acetylserine sulfhydrylase A immunotitration assays, using the free enzyme obtained from Sephadex G-150 gel filtration, gave nearly identical values for the wild type and DW158 enzymes. Thus, unlike most previously described 1,2,4-triazole resistant strains (Hulanicka et al., 1974), the *O*-acetylserine sulfhydrylase A in DW158 appears to be unaltered in specific activity.

*Effect of trz-312 on Other Cysteine Biosynthetic Enzymes.* 1,2,4-Triazole resistant strains of the type *trzA* and *trzB* have been shown to have more derepressed levels of sulfite reductase activity than wild type when grown on sulfate as a sole sulfur source (Hulanicka and Klopotowski, 1972). This phenomenon is thought to be secondary to elevated intracellular concentrations of the internal inducer, *O*-acetyl-L-serine, which in turn result from the partial loss of *O*-acetylserine sulfhydrylase activity in these mutants. Since a similar phenomenon might also be expected to occur in a strain with an increased capactiy for *O*-acetyl-L-serine synthesis, it was of interest to measure sulfite reductase and *O*-acetylserine sulfhydrylase activities in the *trz-312* strain TK1106 grown on different sulfur sources. The results of these studies (Table 3) demonstrated no significant increases in the activities of these enzymes in the mutant when compared to wild type. Indeed, the sulfite reductase activity in djenkolate-grown TK1106 was only 50% of that found in wild type, a finding for which we have no good explanation. As usual, serine transacetylase activity was independent of the sulfur source used for growth in both strains, but was always about seven times greater in TK1106.

*Genetic Mapping of trz-312.* Genetic mapping experiments demonstrated 85 to 95% linkage by phage P22-mediated transduction between the *trz-312* mutation of TK1106 with various mutations in *cysE* (data not shown). Using phage P1-mediated transduction in P1-sensitive derivatives of these strains, three point crosses were performed in order to determine if *trz-312* is located at one extreme end of the *cysE* locus, as would be expected of an operator or promotor mutation. The results of these crosses are given in Table 4, and show a *trz-312* to *cysE* linkage of 91 to 99%. Among $cysE^+ trz^+$ recombinants, the linkage of *cysE* with *pyrE125* uniformly is found to be

**Table 4.** Genetic mapping of *trz-312* by P1 mediated transduction[a]

| Recipient | Total | Recombinants | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $pyr^+ trz^+$ | $pyr^+ trz^-$ | $pyr^- trz^+$ | $pyr^- trz^-$ | $\%pyr^+$ | $\%trz^-$ | $p$[b] |
| TK1017 | 760 | 2 | 402 | 6 | 350 | 53 | 98.9 | <0.2 |
| TK1019 | 541 | 13 | 252 | 36 | 240 | 49 | 90.9 | <0.001 |
| TK1021 | 766 | 3 | 452 | 14 | 297 | 59 | 97.8 | <0.001 |
| TK1022 | 536 | 1 | 248 | 7 | 280 | 46 | 98.5 | <0.1 |
| TK1023 | 541 | 1 | 240 | 4 | 296 | 45 | 99.4 | <0.3 |
| TK1024 | 742 | 0 | 276 | 4 | 462 | 37 | 99.5 | <0.2 |
| TK1025 | 455 | 0 | 212 | 3 | 240 | 47 | 99.3 | <0.15 |
| TK1026 | 455 | 0 | 204 | 11 | 230 | 46 | 97.5 | <0.005 |
| TK1065 | 1,034 | 1 | 544 | 11 | 478 | 53 | 98.8 | <0.005 |
| TK1066 | 789 | 0 | 360 | 9 | 420 | 46 | 98.9 | <0.01 |
| TK1091 | 552 | 1 | 260 | 21 | 270 | 47 | 96.0 | <0.001 |
| TK1092 | 676 | 0 | 304 | 32 | 340 | 45 | 95.3 | <0.001 |

[a] All crosses were performed with a P1CM *clr-100* lysate from a lysogen of TK1106 carrying $pyrE^+ cysE^+$ *trz-312*. Recipients were *pyrE125 cysE* with selection for $cysE^+$ and scoring for *pyrE* and 1,2,4-triazole resistance. The genotype $trz^-$ or *trz-312* is resistant while $trz^+$ is sensitive to this inhibitor

[b] Each p value was calculated by a chi-square contingency test and expresses the probability that the distribution of recombinant classes might occur by chance alone

considerably less than that found in $cysE^+ trz^-$ recombinants. This is the result expected if *trz-312* is situated between *pyrE125* and a given *cysE* mutation, since the creation of $cys^+$ $trz^+$ $pyrE^+$ recombinants would then require a quadruple cross-over event. The p values for the probability that the differences noted in recombinant classes are due to chance alone are $<0.01$ for seven of 12 different *cysE* recipients and range from $<0.10$ to $<0.30$ for the other five. Therefore, while not conclusive, the data strongly support the notion that the *trz-312* mutation is located at one extreme end of the *cysE* locus.

## Discussion

Our results show that strains bearing *trz-312* contain increased amounts of serine transacetylase protein. Since immunologic data demonstrate a wild type specific activity for the *O*-acetylserine sulfhydrylase A from DW158, the normal ratio of enzyme activities found in the cysteine synthetase complex from this strain can result only from a serine transacetylase of wild type specific activity. This property and the finding of essentially identical apparent Km and Ki values for wild type and DW158 enzymes, leave little doubt that the increase in serine transacetylase activity noted in the mutant strain represents an elevated level of catalytically normal, enzyme protein. Although we cannot rule out the possibility of a decreased rate of enzyme degradation or inactivation in DW158, we feel that its high level of serine transacetylase probably is caused by an increased rate of enzyme synthesis.

Several possible types of mutation leading to an increased rate of enzyme synthesis must be considered: 1) a mutation resulting in gene amplification as noted in the case of glycyl-tRNA synthetase (Folk and Berg, 1971; Hill and Combriatto, 1973); 2) the presence of the structural gene for an enzyme on an extrachromosomal element; 3) an altered state of gene repression resulting from either a defective repressor or an altered operator site (Smith, 1971); 4) an altered promoter site for a structural gene as described for the *lac* operon (Arditti et al., 1973), in the operon for glucose-6-phosphate dehydrogenase (Fraenkel and Banerjee, 1971); in the glycerol kinase operon (Berman-Kurtz et al., 1971) and in the gene for D-amino acid dehydrogenase (Wild and Klopotowski, 1975).

The stability of the 1,2,4-triazole resistance phenotype of strains bearing *trz-312* and the close linkage of this mutation to chromosomal markers by conjugation make the first two alternatives unlikely. Furthermore, amplification of a chromosomal region usually alters the linkage between flanking markers (Beeftinck et al., 1974), a phenomenon which we have not observed in transductions involving *trz-312* (data not shown).

The very close linkage of *trz-312* to the *cysE* locus itself is consistant with the former being an alteration in either an operator or promoter site. This mutation also might lie in a repressor gene, adjacent to and controlling *cysE*; however, in the absence of any evidence for regulation of *cysE* in wild type (Kredich, 1971), this hypothesis and the related one, involking *trz-312* as a defective *cysE* operator, seem unlikely. We conclude, therefore, that *trz-312* is probably a mutation in the promoter site of the *cysE* locus, which leads to an increased rate of serine transacetylase synthesis by enhancing the affinity of the promotor for RNA polymerase. If our hypothesis is correct, the genetic data placing *trz-312* between *pyrE125* and all other *cysE* mutations tested, establish the direction of *cysE* transcription as counter-clockwise on the *S. typhimurium* chromosome (Sanderson, 1972).

Growth inhibition by 1,2,4-triazole appears to result from the *O*-acetylserine sulfhydrylase A-catalyzed reaction of this compound with *O*-acetyl-L-serine to give 1,2,4-triazole-1-alanine (Kredich et al., 1975). The consumption of *O*-acetyl-L-serine in this reaction deprives the cell not only of this direct precursor of cysteine, but also of the inducer for enzymes necessary for the synthesis of sulfide, that substance which must compete with 1,2,4-triazole in the first place. The metabolic block caused by the low levels of *O*-acetylserine sulfhydrylase A present in *trzA* and *trzB* mutants no doubt results in the accumulation of *O*-acetyl-L-serine, which in turn leads to the more derepressed levels of cysteine biosynthetic enzymes observed in the absence of 1,2,4-triazole. Resistance to this inhibitor is due to the low *O*-acetylserine triazolylase activities found in these strains.

The results of previous studies indicate that 1,2,4-triazole inhibited cultures of wild type *S. typhimurium* synthesize *O*-acetyl-L-serine at two to three times the rate necessary for a normal growth rate (Kredich et al., 1975). However, more than 97% of the *O*-acetyl-L-serine synthesized during growth in the presence of 4 mM 1,2,4-triazole is converted to 1,2,4-triazolyl-1-alanine, which appears in the culture medium. Since inhibited cultures appear to be L-cysteine starved and should be free of significant feedback inhibition of serine transacetylase (Kredich et al., 1969), the rate of 9 nmoles/min, mg cell protein measured in inhibitor treated cultures (Kredich et al., 1975) probably represents the maximum in vivo rate of *O*-acetyl-L-serine synthesis with the substrate concentrations present under such conditions. This value is reasonably close to the in vitro specific activ-

ity of 20 nmoles/min, mg protein noted in wild type, crude extracts, where serine transacetylase is assayed using approximately half-saturating concentrations of L-serine and acetyl-CoA. We feel that the 1,2,4-triazole resistance found in strains bearing *trz-312* most likely is due to their capacity for synthesizing *O*-acetyl-L-serine at a rapid rate enough to compensate for the loss of this vital metabolite *via* the *O*-acetylserine triazolylase reaction. The reversal of 1,2,4-triazole inhibition of wild type by exogenous L-serine supports this hypothesis, if one assumes intracellular L-serine concentrations ordinarily to be far below those necessary for maximum serine transacetylase activity.

The results given here, showing only wild type levels of cysteine biosynthetic enzymes in 1,2,4-triazole-free cultures of TK1106, can be explained in terms of the efficiency of feedback inhibition of serine transacetylase by L-cysteine. Theoretically, one would predict the increased amounts of serine transacetylase in *trz-312* strains to cause at least a small increase in the steady state levels of *O*-acetyl-L-serine and L-cysteine; however, the ready conversion of the former compound to the latter by a normal amount of *O*-acetylserine sulfhydrylase A, together with feedback inhibition of serine transacetylase by L-cysteine, probably results in only a minimal increase in *O*-acetyl-L-serine concentration, which is insufficient to change appreciably the state of repression of cysteine biosynthetic enzymes.

*Acknowledgements.* This work was supported by U.S. Public Health Service research grant AM12828 from the National Institute of Arthritis, Metabolism and Digestive Diseases. One of us (NMK) is an Investigator of the Howard Hughes Medical Institute.

## References

Arditti, R., Grodzicker, T., Beckwith, J.: Cyclic adenosine monophosphate-independent mutants of the lactose operon of *Escherichia coli*. J. Bact. **114**, 652–655 (1973)

Becker, M.A., Tomkins, G.M.: Pleiotropy in a cysteine-requiring mutant of *Salmonella typhimurium* resulting from altered protein-protein interaction. J. biol. Chem. **244**, 6023–6030 (1969)

Beeftinck, F., Cunin, R., Glandsdorff, N.: Arginine gene duplications in recombination proficient and deficient strains of *Escherichia coli* K12. Molec. gen. Genet. **132**, 241–253 (1974)

Berman-Kurtz, M., Lin, E.C.C., Richey, D.P.: Promotor-like mutant with increased expression of the glycerol kinase operon of *Escherichia coli*. J. Bact. **106**, 724–731 (1971)

Boguslawski, J., Walczak, W., Klopotowski, T.: Reversal by serine and potentiation by glycine of the inhibitory effect of 1,2,4-triazole on the growth of *Salmonella typhimurium*. Acta biochim. polon. **14**, 133–141 (1967)

Folk, W.R., Berg, P.: Duplication of the structural gene for glycyl-transfer RNA synthetase in *Escherichia coli*. J. molec. Biol. **58**, 595–610 (1971)

Fraenkel, D.G., Banerjee, S.: A mutation increasing the amount of a constitutive enzyme in *Escherichia coli*, glucose-6-phosphate dehydrogenase. J. molec. Biol. **56**, 183–194 (1971)

Gornall, A.G., Bardawill, C.J., David, M.M.: Determination of serum proteins by means of the biuret reaction. J. biol. Chem. **177**, 751–766 (1949)

Hill, C.W., Combriato, G.: Genetic duplications induced at very high frequency by ultraviolet irradiation in *Escherichia coli*. Molec. gen. Genet. **127**, 197–214 (1973)

Hulanicka, D., Klopotowski, T.: Formation of plasmids carrying the *cysA* region of *Salmonella* chromosome. FEBS Proc. Meet., Abstract 872 (1971)

Hulanicka, D., Klopotowski, T.: Mutants of *Salmonella typhimurium* resistant to triazole. Acta biochim. polon. **19**, 251–260 (1972)

Hulanicka, D., Klopotowski, T., Smith, D.A.: The effect of triazole on cysteine biosynthesis in *Salmonella typhimurium*. J. gen. Microbiol. **72**, 291–301 (1972)

Hulanicka, M.D., Kredich, N.M., Treijan, D.M.: The structural gene for O-acetylserine sulfhydrylase A in *Salmonella typhimurium*. J. biol. Chem. **249**, 867–872 (1974)

Kredich, N.M.: Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*. J. biol. Chem. **246**, 3474–3484 (1971)

Kredich, N.M., Becker, M.A., Tomkins, G.M.: Purification and characterization of cysteine synthetase, a bifunctional protein complex, from *Salmonella typhimurium*. J. biol. Chem. **244**, 2428–2439 (1969)

Kredich, N.M., Foote, L.J., Hulanicka, M.D.: Studies on the mechanism of inhibition of *Salmonella typhimurium* by 1,2,4-triazole. J. biol. Chem. **250**, 7324–7331 (1975)

Kredich, N.M., Tomkins, G.M.: The enzymic synthesis of L-cysteine in *Escherichia coli* and *Salmonella typhimurium*. J. biol. Chem. **241**, 4955–4965 (1966)

Luria, S.E., Burrows, J.W.: Hybridization between *Escherichia coli* and *Shigella*. J. Bact. **74**, 461–476 (1957)

Mojica-A, T.: Transduction by phage P1CM *clr-100* in *Salmonella typhimurium*. Molec. gen. Genet. **138**, 113–126 (1975)

Reznikoff, W.S.: The operon revisited. Ann. Rev. Genet. **6**, 133–156 (1972)

Rosner, J.L.: Formation, induction and curing of bacteriophage P1 lysogens. Virology **48**, 679–689 (1972)

Sakami, W., Toennies, G.: The investigation of amino acid reactions by methods of non-aqueous titrimetry. J. biol. Chem. **144**, 203–217 (1942)

Sanderson, K.E.: Linkage map of *Salmonella typhimurium*, edition IV. Bact. Rev. **36**, 558–586 (1972)

Siegel, L.M., Kamin, H.: TPNH-Sulfite reductase (*Escherichia coli*). Meth. Enzymol. **17B**, 539–545 (1971)

Smith, D.A.: S-Amino acid metabolism and its regulation in *Escherichia coli* and *Salmonella typhimurium*. Advanc. Genetics **16**, 141–165 (1971)

Smith, H.O., Levine, M.: A phage P22 gene controlling integration of prophage. Virology **31**, 207–216 (1967)

Stadtman, E.R.: The enzymatic synthesis of acyl-coenzyme A compounds. J. cell. comp. Physiol. **41** (Suppl. 1), 89–107 (1953)

Wild, J., Klopotowski, T.: Insensitivity of D-amino acid dehydrogenase synthesis to catabolic repression in *dadR* mutants of *Salmonella typhimurium*. Molec. gen. Genet. **136**, 64–73 (1975)

Vogel, H.J., Bonner, D.M.: Acetylornithinase of *Escherichia coli*. J. biol. Chem. **218**, 97–106 (1956)

Communicated by H. Böhme

*Received and accepted July 6, 1976*