The Structural Gene for O-Acetylserine Sulphydrylase A in Salmonella typhimurium

IDENTITY WITH THE trzA LOCUS*

(Received for publication, July 30, 1973)

M. DANUTA HULANICKA,† NICHOLAS M. KREIDICH,§ and DAVID M. TREIMAN
From the Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

SUMMARY

Mutants of Salmonella typhimurium, resistant to 1,2,4-triazole and mapping in the trzA and trzB loci, have been found to have low to virtually absent levels of O-acetylserine sulphydrylase activity while remaining prototrophic for cysteine. Kinetic, chemical, and immunochemical studies of the highly purified enzymes from two trzA mutants prove that both strains bear mutant alleles for O-acetylserine sulphydrylase A. A third trzA mutant contains a heat-labile enzyme. Little or no material cross-reacting with antibody to wild type O-acetylserine sulphydrylase A could be detected in three trzB mutants or in the trzA deletion examined. Since trzA is clearly the structural gene for O-acetylserine sulphydrylase A, we suggest that the designation, cysK, seems more appropriate for this locus. The lack of any demonstrable growth requirement for cysteine in trz mutants, which completely lack O-acetylserine sulphydrylase A, indicates that the synthesis of cysteine from O-acetyl-L-serine and sulfide can be catalyzed by another enzyme in S. typhimurium.

In Salmonella typhimurium and Escherichia coli the biosynthesis of L-cysteine from L-serine and exogenous sulfate requires a sulfate permease and six enzymes in a branched convergent pathway (1), or the final step of which involves the reaction of auxotrophs nutritional characterizations of many of the hundreds of cysteine his co-workers (2-6). In addition, the Metabolic Diseases. Grant AM12828 from the National Institute of Arthritis and Polich Academy of Sciences, Warsaw, Poland. -

1 D. M. Hulanicka, and T. Klopotowski (1971), abstract presented at the Seventh Meeting of the Federation of European Biochemical Societies.

2 The abbreviations used are: ICR-191, 3-chloro-7-methoxy-9-(3-chloroethyl)aminopropylamino) acridine dihydrochloride; nitrosoguanidine, N-methyl-N'-nitro-N-nitrosoguanidine.

3 Public Health Service Research Career Development Awardee, Grant OM 42304 from the National Institute of General Medical Sciences.

4 Present address, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

* This investigation was supported by Public Health Service Grant AM12828 from the National Institute of Arthritis and Metabolic Diseases.


Printed in U.S.A.
containing 5 g of glucose per liter, in which either 1.0 mm L-cysteine or 0.5 mm L-djenkolic acid was used as the sole sulfur source (7). Growth on the poor sulfur source, L-djenkolic acid, normally derepresses the rate of synthesis of O-acetylsulfhydrylase A to levels which are 50 to 100 times greater than those found during growth on L-cysteine (7). Sodium lactate at a concentration of 2 g per liter was substituted for glucose for the growth of *p* strains (11). Growth media were supplemented with 50 μg per ml of the appropriate amino acid for the growth of *trp* and *his* strains, and 10 μg per ml of uracil was used to support the growth of the *pyr* strain, DW-132. Solid media were prepared by the addition of agar to liquid media at a concentration of 15 g per liter. All mating experiments were performed using bacteria grown in nutrient broth.

**Genetic Methods**—All bacteria used were nonlysogenic derivatives of *S. typhimurium* LT-2 (Table I). The *trz* (triazole resistant) strains, DW-130, DW-136, and DW-137, were obtained by transducing cysA20 to cys+ with L-4 phage lysates from the appropriate *trz* donors, and then scoring for colonies resistant to 1,2,4-triazole. All but one of the *trz* mutants were isolated and scored as previously described (9) on minimal agar plates containing appropriate nutritional supplements and 10 mm 1,2,4-triazole. Mutagenesis was performed by placing a small crystal of ICR-191 or nitrosoguanidine on a lawn of bacteria, and subsequently isolating colonies from the ring of growth surrounding the inhibition zone produced by the mutagen. Mutant *sli-12* was originally isolated as a nitrosoguanidine-induced mutant resistant to selenite at a concentration of 0.5 mg of sodium selenite per ml. This strain and its derivative, DW-130, were later found to be *trz*. Although replica-plating for either triazole resistance or selenite resistance is very useful as a scoring technique, neither marker is suitable for selection in genetic crosses owing to very high frequencies of spontaneous mutation to resistance. Neither triazole resistance nor selenite resistance can be determined in cys strains, since the sensitivity of nonresistant strains to either inhibitor is overcome by sulfate, sulfide, cysteine, and L-djenkolic acid (12, 13).

The criteria of Hulanicka and Klopotowski (9) were used to differentiate *cysA* and *trzB* mutants from one another. These investigators have shown that although both types of *trz* mutants are 40 to 60% cotransducible with *cysA*, *trzB* transductants, unlike *trzA* transductants, are unstable and readily segregate to *cysA*. Furthermore, *trzA* mutants are closely linked to *purC* (90%) and *cysA* (95%) by conjugation, while *trzB* mutants show a lesser linkage with *cysA* (10 to 20%) and no linkage at all with *purC*.

Phosphatransferase system deficient mutants (*p*ts) were scored by their inability to grow on a citrate-free minimal salts medium containing glucose, 5 g per liter, as sole carbon source. Such mutants grow well on lactate (11). Bacteriophage lysates were prepared by infecting log-phase bacteria in nutrient broth with the integration-deficient L-4 strain of phage P-22 (14) at a multiplicity of 0.1. Incubation was continued for 8 to 18 hours after which intact bacteria and debris were removed by low speed centrifugation. Lysates were stored at 4° over chloroform. Transduction was carried out as previously described (9). Conjugation was performed by mixing log phase cultures of Hfr and F− strains in a 1:10 ratio and then plating on selective plates.

**Immunologic Determinations**—Micro-double-diffusion tests were done at room temperature for 16 hours in 1% agarose, 0.2 m NaCl, 0.05 m Tris-HCl, pH 7.6. After development the slides were rinsed, dried, and stained with a 20 mg per ml solution of Coomassie blue in methanol-water-glacial acetic acid (5:8:1).

The methods used for the quantitative immunochromatographic determination of material cross-reacting with O-acetylsulfhydrylase A are based on the finding by Becker and Tomkins (8) that a short preincubation of the enzyme with antibody results in an almost quantitative loss of catalytic activity. Mixtures containing approximately 0.2 unit of O-acetylsulfhydrylase A, 0.02 mg of bovine serum albumin and varying amounts of antiserum in 0.2 ml of 0.1 m Tris-HCl, pH 7.6 were incubated at 23° for 30 min and then assayed for enzyme activity. Using highly purified wild type O-acetylsulfhydrylase A, a plot of enzyme activity remaining versus the volume of antiserum added gives a line that is straight until approximately 70% of the initial activity has been lost. The deviation of this line from linearity at higher antibody concentrations is probably due to the equilibrium between free and bound enzyme, and a small residual activity of the enzyme-antibody complex (8). The negative slope of the linear portion of this plot was taken as the amount of enzyme which can be inactivated per volume of serum. We find that a given antiserum gives the same antibody content (±10%) whether assayed with purified enzyme or with crude extracts of wild type *S. typhimurium*, derepressed for O-acetylsulfhydrylase by growth on L-djenkolic acid as a sole sulfur source or repressed by growth on L-cysteine.

An antibody unit is defined as that amount which by this assay will inactivate 1 enzyme unit of purified wild type O-acetylsulfhydrylase A. Using a specific activity of 1200 enzyme units per mg for our best preparation of O-acetylsulfhydrylase A (approximately 98% pure), one antibody unit will therefore inactivate 0.83 μg of this enzyme.

Two methods were used for the quantitative assay of mutant proteins cross-reacting with wild type O-acetylsulfhydrylase A.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Designations and derivations of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-172</td>
<td>wild type, trz+</td>
</tr>
<tr>
<td>cysA20</td>
<td>cys+20</td>
</tr>
<tr>
<td>purC7</td>
<td>pur+</td>
</tr>
<tr>
<td>HfrB2</td>
<td>HfrB2, hisD23, gal-50</td>
</tr>
<tr>
<td>TK-306</td>
<td>HfrK5, hisD3512, trzA45</td>
</tr>
<tr>
<td>TK-307</td>
<td>HfrK5, hisD3512, trzB46</td>
</tr>
<tr>
<td>sli-12</td>
<td>trzA301</td>
</tr>
<tr>
<td>DW-130</td>
<td>cys+, trzA301</td>
</tr>
<tr>
<td>DW-132</td>
<td>purF146, trpA100, trzA302</td>
</tr>
<tr>
<td>DW-136</td>
<td>cys+, trzA46</td>
</tr>
<tr>
<td>DW-137</td>
<td>cys+, trzB46</td>
</tr>
<tr>
<td>DW-151</td>
<td>HfrB2, hisD23, gal-50, trzB913</td>
</tr>
<tr>
<td>DW-152</td>
<td>HfrB2, hisD23, gal-50, trzB914</td>
</tr>
<tr>
<td>SB-1690</td>
<td>trpB228, ptsB34</td>
</tr>
<tr>
<td>SB-2560</td>
<td>trpB228, del (trzA, ptsH1, err 49)</td>
</tr>
</tbody>
</table>
drylase A. In the "direct assay," the mutant enzyme, in the form of either a purified protein or a crude extract, was used as the sole source of O-acetylserine sulfhydrylase activity in an antibody inactivation assay. Using an antibody preparation standardized against wild type enzyme (at concentrations of up to one hundred times those used with the wild type enzyme), the negative slope of the resultant plot was then used to calculate the specific activity of the mutant enzyme. In our calculations we have assumed that equimolar amounts of the wild type and mutant enzymes are inactivated to an equal extent by a given amount of antibody, and therefore express mutant enzyme specific activities either as enzyme units per mg of O-acetylserine sulfhydrylase A protein or as the percent of wild type specific activity.

The "indirect assay" was used for mutants having little or no O-acetylserine sulfhydrylase A activity detectable by the direct assay. In this procedure, mutant crude extract protein was added to an antibody inactivation assay mixture containing the usual amount of wild type enzyme. Using the same assumptions which were made for the direct assay, the negative slope of the resultant plot was then taken as the average specific activity of the mixture of mutant and wild type proteins. Since the O-acetylserine sulfhydrylase A activity of the mutant protein was negligible compared to wild type activity in such assays, the amount of mutant cross-reacting protein added to the incubation mixture was calculated as follows:

\[
\text{Average specific activity} = \frac{\text{wild type enzyme activity}}{\text{mutant enzyme protein}}
\]

\[
\text{Mutant enzyme protein} = \frac{\text{average specific activity} - \text{wild type enzyme protein}}{\text{mutant enzyme activity}}
\]

Concentrations of mutant cross-reacting material as low as 1 \(\mu\)g per ml can be estimated with about 20% accuracy by the indirect assay.

Other Methods—Antibodies to O-acetylserine sulfhydrylase A were prepared by injecting 1 mg of highly purified wild type enzyme in Freund's complete adjuvant into the foot pads of New Zealand white rabbits at 3- to 8-week intervals. Sera from immunized animals and from control nonimmunized animals were dialyzed against 0.1 M Tris-HCl, pH 7.6 before use. Immunized sera were found to contain 200 to 400 antibody units per ml, while control sera gave less than 0.1 antibody unit per ml.

We have previously described the methods used for the preparation of bacterial extracts and their assay for the enzymes of cysteine biosynthesis (7), the purification and assay of O-acetylserine sulfhydrylase A and the preparation of tryptic peptide maps (15). Peptide maps were stained with ninhydrin by the method of Blackwood (16). Arginine containing peptides were identified with phenanthrenequinone (17), and peptides containing histidine or tyrosine were detected with Pauly reagent (18). Polyacrylamide disc gel electrophoresis was performed using the system of Reisfeld and Small (19), omitting the urea, and the gels were stained with Coomassie blue (20). Protein was assayed by the biuret method (21) using bovine serum albumin as a standard. Sedimentation velocities were determined in a Spinco model E ultracentrifuge equipped with a photoelectric scanner. Bacterial growth curves were done by measuring the turbidity of cultures at 650 nm in a spectrophotometer.

RESULTS

Preliminary experiments revealed that crude extracts of triazole-resistant mutants grown on djenkolate contain low levels of O-acetylserine sulfhydrylase activity, ranging from 0.1 to 21% of that found in the wild type strain. Djenkolate-grown levels of serine transacetylase, sulfite permease, ATP sulfurylase, adenine-5'-phosphosulfate kinase, 3'-phosphoadenosine-5'-phosphosulfate reductase, and sulfite reductase were found to be normal. In order to differentiate between the possibilities of either pleiotropic mutations leading indirectly to low levels of a normal enzyme or true O-acetylserine sulfhydrylase A structural gene mutations, crude extracts of mutant strains were assayed by the direct and indirect immunochemical assays for material cross-reacting with the wild type enzyme.

Assay of a crude extract of the trzA mutant, DW-132, by the direct method revealed that this strain makes wild type levels of a cross-reacting protein with 22% the specific activity of wild type O-acetylserine sulfhydrylase A (Table II). In contrast an extract of DW-136 was found to contain only 39% of the wild type level of a protein with 30% of normal enzyme specific activity. In addition the DW-136 enzyme, unlike wild type O-acetylserine sulfhydrylase A, is rapidly inactivated at 37° (Fig. 1), but not at room temperature. A crude extract of the trzA mutant DW-130 was found to contain a nearly normal amount (by the indirect assay) of a protein with approximately 1% of wild type specific activity.

In contrast, extracts of the trzB strains, DW-137 and DW-151, and the trz deletion, SB-2960, contained no O-acetylserine sulfhydrylase A detectable by either the direct or indirect immunochemical assays. The trzB mutant, DW-152, contained

<table>
<thead>
<tr>
<th>Strain</th>
<th>Enzyme activity per total protein</th>
<th>Enzyme activity per mg cross-reacting material by direct assay</th>
<th>Cross-reacting material per total protein</th>
<th>Double diffusion test with antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>15.4</td>
<td>1200</td>
<td>12.8</td>
<td>Positive</td>
</tr>
<tr>
<td>DW-130</td>
<td>0.24</td>
<td>11</td>
<td>9.9</td>
<td>Positive</td>
</tr>
<tr>
<td>DW-132</td>
<td>3.2</td>
<td>229</td>
<td>12.4</td>
<td>Positive</td>
</tr>
<tr>
<td>DW-136</td>
<td>1.78</td>
<td>354</td>
<td>5.0</td>
<td>Negative</td>
</tr>
<tr>
<td>DW-151</td>
<td>0.27</td>
<td>&lt;5</td>
<td>&lt;0.1</td>
<td>Negative</td>
</tr>
<tr>
<td>DW-152</td>
<td>0.41</td>
<td>&lt;5</td>
<td>0.21</td>
<td>Negative</td>
</tr>
<tr>
<td>SB-2960</td>
<td>0.02</td>
<td>&lt;5</td>
<td>&lt;0.1</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* These values were determined by dividing the O-acetylserine sulfhydrylase activity per mg of total protein in the crude extract by the enzyme activity per mg of cross-reacting material as determined by the direct assay. Since a significant fraction of the total O-acetylserine sulfhydrylase activity in a crude extract of a low activity mutant such as DW-130 is due to a protein other than O-acetylserine sulfhydrylase A, the value of 21.8 \(\mu\)g per mg obtained for that strain is falsely elevated.

* The positive test is one which gives a line of complete identity. In extracts giving a negative test a faint line of complete non-identity was occasionally seen, indicating that our antisera is not monospecific when tested against crude extracts.
FIG. 1. Heat lability of the O-acetylserine sulfhydrylase activity from DW-136. Crude extracts of DW-136 and wild type were incubated at 37° and samples were taken for assay at the times indicated. Initial activities were approximately 1 unit per ml in 0.1 M Tris-HCl, pH 7.6 containing 0.1 mg per ml of bovine serum albumin.

FIG. 2. Polyacrylamide disc gel electrophoresis of the purified O-acetylserine sulfhydrylase A from wild type, DW-130 and DW-132. The double bands with the wild type enzyme have been reported before (23), and are also noted with DW-132. The wild type and DW-132 enzymes are identical in their electrophoretic mobility, while the enzyme from DW-130 migrates slightly more rapidly. The direction of electrophoresis is toward the bottom.

As determined by the direct method (Fig. 3), the purified enzyme from DW-130 was noted to have specific activities of 19 enzyme units per mg of total protein and 20 enzyme units per mg of immunochemically reactive protein (1200 enzyme units per mg of total protein or per mg of immunochemically reactive protein for the purified wild type enzyme). The close agreement of these two values indicates that the mutant protein is just as immunochemically reactive in the direct assay as is the wild type enzyme. The purified enzyme from DW-132 had specific activities of 208 enzyme units per mg of total protein and 340 enzyme units per mg of immunochemically reactive protein (Fig. 3), suggesting that this mutant protein either is slightly more readily inactivated by antibody or was not as pure as the wild type enzyme. The $K_m$ values for O-acetyl-L-serine for the different enzymes were found to be 7 mM for the wild type enzyme, 38 mM for the DW-130, and 28 mM for the DW-132 enzyme. The $K_m$ for sulfide is less than 0.5 mM for all three enzymes.

The ultraviolet and visible spectra of all three proteins are essentially identical with absorption peaks noted at 278 nm and 412 nm. The addition of O-acetyl-L-serine to solutions of the different proteins at pH 7.6 produced the expected decrease in absorbance at 412 nm, while a new absorption maximum at 470 nm appeared (15). The spectrally determined dissociation constant for the O-acetyl-L-serine enzyme complex was found to be 1 $\mu$M for each protein.

Tryptic peptide maps of the DW-132 enzyme could not be distinguished from that of the wild type enzyme, but the peptide map of DW-130 showed a very obvious difference. One arginine-containing peptide was found to be missing from the wild type protein, and a new arginine peptide with a slightly greater cathodal migration was noted (Fig. 4). This new peptide showed a positive reaction with the Pauly reagent, giving the orange color characteristic of histidine rather than of tyrosine. Since the missing wild type peptide does not stain with the Pauly reagent, and the substitution of a histidine for a neutral or acidic amino acid would be consistent with the increased cathodal migration of the new DW-130 peptide, we conclude that the mutant and wild type proteins differ by probably only a single amino acid substitution in which a neutral or acidic residue in

The results of double diffusion tests on extracts of these mutants are also summarized in Table II. Crude extracts of DW-130 and DW-132 gave precipitation lines of complete identity with the wild type enzyme, while no evidence of cross-reacting material was noted using extracts of the trzB mutants, the trz deletion SB-2950, or the trzA mutant DW-136.

In order to further verify the immunochemical data indicating that certain trz mutants produce altered forms of O-acetylserine sulfhydrylase A, the enzyme activities from DW-130 and DW-132 were purified and compared with the wild type enzyme. Polyacrylamide disc gel electrophoresis revealed the mutant enzymes to be at least 90% pure and similar or identical with the wild type enzyme in their electrophoretic behavior (Fig. 2). The $\beta_{20, w}$ values of all three proteins were found to be 4.5.

As determined by the direct method (Fig. 3), the purified enzyme from DW-130 was noted to have specific activities of 19 enzyme units per mg of total protein and 20 enzyme units per mg of immunochemically reactive protein (1200 enzyme units per mg of total protein or per mg of immunochemically reactive protein for the purified wild type enzyme). The close agreement of these two values indicates that the mutant protein is just as immunochemically reactive in the direct assay as is the wild type enzyme. The purified enzyme from DW-132 had specific activities of 208 enzyme units per mg of total protein and 340 enzyme units per mg of immunochemically reactive protein (Fig. 3), suggesting that this mutant protein either is slightly more readily inactivated by antibody or was not as pure as the wild type enzyme. The $K_m$ values for O-acetyl-L-serine for the different enzymes were found to be 7 mM for the wild type enzyme, 38 mM for the DW-130, and 28 mM for the DW-132 enzyme. The $K_m$ for sulfide is less than 0.5 mM for all three enzymes.

The ultraviolet and visible spectra of all three proteins are essentially identical with absorption peaks noted at 278 nm and 412 nm. The addition of O-acetyl-L-serine to solutions of the different proteins at pH 7.6 produced the expected decrease in absorbance at 412 nm, while a new absorption maximum at 470 nm appeared (15). The spectrally determined dissociation constant for the O-acetyl-L-serine enzyme complex was found to be 1 $\mu$M for each protein.

Tryptic peptide maps of the DW-132 enzyme could not be distinguished from that of the wild type enzyme, but the peptide map of DW-130 showed a very obvious difference. One arginine-containing peptide was found to be missing from the wild type protein, and a new arginine peptide with a slightly greater cathodal migration was noted (Fig. 4). This new peptide showed a positive reaction with the Pauly reagent, giving the orange color characteristic of histidine rather than of tyrosine. Since the missing wild type peptide does not stain with the Pauly reagent, and the substitution of a histidine for a neutral or acidic amino acid would be consistent with the increased cathodal migration of the new DW-130 peptide, we conclude that the mutant and wild type proteins differ by probably only a single amino acid substitution in which a neutral or acidic residue in
rise to both triazole and selenite resistance is a relatively rare occurrence. Of approximately 20
same mutation is responsible for resistance to both inhibitors. We have found that a single mutation in the
in the cross between DW-130 and
132 are consequences of mutations in the structural gene for this
between triazole resistance and selenite resistance was observed
were found to have very low levels of 0-acetylserine sulfhydrylase activity, while 10 of the 10 trz+ recombinants tested had wild
transductants and scored for their triazole resistance or sensitivity by replicate plating.

<table>
<thead>
<tr>
<th>Recipient bacteria</th>
<th>Flage closures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DW-130 (cys+, trzA301)</td>
</tr>
<tr>
<td></td>
<td>Total cys+, trz+</td>
</tr>
<tr>
<td>cysA20</td>
<td>100</td>
</tr>
<tr>
<td>SB-1690 (trpB223, ptsI34)</td>
<td>100</td>
</tr>
</tbody>
</table>

The finding of markedly diminished levels of a single specific enzyme activity in a given class of allelic mutants is generally taken as acceptable evidence for the assignment of a structural gene to the enzyme for which it codes. The existence of measurable amounts of O-acetylseline sulfhydrylase activity in trzA mutants, however, together with the observed prototrophy for cysteine of such mutants, suggested the possibility that the trzA locus might only control the extent of expression of an otherwise located and intact structural gene for O-acetylseline sulfhydrylase A. The results of our studies have conclusively demonstrated that certain trzA mutants synthesize altered forms of enzyme, which cross-react with antibody to wild type O-acetylseline sulfhydrylase A. The kinetic, chemical, and immunochemical data obtained from the characterization of the purified enzymes from DW-130 and DW-132 prove that these two strains bear mutant structural genes for O-acetylseline sulfhydrylase A, while the results of quantitative immunochemical assays of crude extracts indicate that both strains contain wild type levels of mutant proteins.

The lability at 37° of the enzyme from DW-130 adds additional evidence that the trzA locus is the structural gene for O-acetylseline sulfhydrylase A. The low levels of cross-reacting material found in this strain may be a reflection of an abnormally rapid in vivo rate of degradation to an immunochemically unreactive product rather than a consequence of a slower rate of synthesis. Our inability to detect cross-reacting material in DW-136 by the agar double diffusion technique, while easily demonstrating immunochemically reactive enzyme by the direct and indirect assays, is probably due to an unusual lability of this enzyme to the conditions of gel diffusion.

The very low to immeasurable levels of cross-reacting material in the three trzB mutants studied suggest that these strains may bear deletions, nonsense mutations, or frameshift mutations in the structural gene for O-acetylseline sulfhydrylase A. The latter possibility is supported by the findings of Hulanicka and Klopotowskii who have shown that the frameshift mutagen, ICR-191, preferentially gives rise to trzB mutants. An alternate explanation for the lack of cross-reacting material in trzB strains is that the structural gene for O-acetylseline sulfhydrylase A may be situated intact but in an inactive form on a plasmid.

The very low level of O-acetylseline sulfhydrylase activity and the absence of immunochemically reactive protein in the deletion SB-2950 is consistent with the assumption that the pts deletion in this strain extends into the trzA locus (11), i.e. the structural gene for O-acetylseline sulfhydrylase A.

The observation of Becker and Tomkins (8), that S. typhimurium contains a second type of O-acetylseline sulfhydrylase, which is antigenically and presumably genetically dis-
tinct from O-acetylserine sulphydrylase A, offers a reasonable explanation for the finding of small amounts of immunochemically unreactive enzyme activity in extracts from wild type bacteria repressed by 0-acetylserine sulfhydrylase A by growth on L-cystine is enzyme activity in extracts from wild type bacteria repressed by excellent technical assistance.

We too find that about 20% of the total 0-acetyl-L-serine and sulfide. Since we have been unable to demonstrate any dependence at all upon cystine for an optimal explanation for the finding of small amounts of immunochemically distinct from 0-acetylserine sulfhydrylase A, offers a reasonable explanation for the finding of small amounts of immunochemically unreactive enzyme activity in all trz mutants, including 0-acetyl-L-serine and sulfide. Since we have been unable to demonstrate any dependence at all upon cystine for an optimal growth rate with strains DW-130 and SB-2950, we can only conclude that this alternate pathway for cysteine biosynthesis is a very efficient one.

Hulanicka et al. (13) have presented evidence suggesting that 1,2,4-triazole inhibits S. typhimurium by interfering with the ability of 0-acetyl-L-serine to induce the biosynthetic pathway. Since these authors have observed that triazole inhibition is easily overcome by exogenous 0-acetyl-L-serine, an obvious explanation for the resistance of 0-acetylserine sulfhydrylase A allows 0-acetyl-L-serine to accumulate to a concentration where it can overcome the triazole effect. It seems very likely, however, that just the lack of sulfide, resulting from a failure to derepress the enzymes necessary for assimilatory sulfate reduction, would also lead to 0-acetyl-L-serine accumulation and hence resistance to triazole in trz+ strains. Clearly, a fuller understanding of triazole resistance must await an elucidation of the mechanism by which triazole interferes with cysteine biosynthesis.

The fact that 0-acetylserine sulphydrylase A is the major 0-acetylserine sulphydrylase in S. typhimurium (8, 15) and is derepressed 50- to 100-fold in cells starved for sulfur (7), together with its ability to bind to serine transacetylase (24), an enzyme with a well established role in cysteine biosynthesis, can be taken as evidence for the involvement of this protein in the cysteine biosynthetic pathway. Therefore, in view of the data presented here, showing that the trzA locus is the structural gene for 0-acetylserine sulphydrylase A, we propose that trzA be referred to as cysK, even though mutations in this gene do not lead to auxotrophy for cysteine.

Acknowledgment—We wish to thank Mrs. Linda J. Foote for her excellent technical assistance.

REFERENCES

3. DRYFUS, J. (1964) J. Biol. Chem. 239, 2292-2297
22. SANDERSON, K. E. (1972) Bacterial Rev. 36, 558-586
The Structural Gene for O-Acetylserine Sulphydrylase A in Salmonella 
typhimurium : IDENTITY WITH THE trzA LOCUS
M. Danuta Hulanicka, Nicholas M. Kredich and David M. Treiman


Access the most updated version of this article at http://www.jbc.org/content/249/3/867

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/249/3/867.full.html#ref-list-1