Pleiotropy in a Cysteine-requiring Mutant of *Salmonella typhimurium* Resulting from Altered Protein-Protein Interaction

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**SUMMARY**

The bifunctional protein complex, cysteine synthetase, in *Salmonella typhimurium* is composed of the enzymes serine transacetylase and O-acetylserine sulfhydrylase. A point mutation (BB1) in the structural gene for serine transacetylase results in diminished catalytic activity of both components of the complex. Enzyme inactivation studies using either specific O-acetylserine sulfhydrylase antiserum or heat show that the isolated O-acetylserine sulfhydrylase components of the mutant are indistinguishable from wild type O-acetylserine sulfhydrylase. When O-acetylserine sulfhydrylase from either wild type or mutant source is combined with serine transacetylase from a common source, their behavior is identical as measured by decrement in enzymic activity, immunological inactivation, and heat inactivation. Thus, when hybrid cysteine synthetases, composed of one component from the wild type and one from the mutant, are investigated, the O-acetylserine sulfhydrylase components of such hybrid complexes behave like those found in the native cysteine synthetase from which the serine transacetylase component was derived. These studies establish that the diminished catalytic activity of the sulfhydrylase of the mutant results from the association of catalytically normal O-acetylserine sulfhydrylase with mutant serine transacetylase. The basis of the pleiotropic phenotype of mutant BB1 is, therefore, an alteration in protein-protein interaction resulting from a structural gene mutation.

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The anabolic enzyme, cysteine synthetase, in *Salmonella typhimurium* is a bifunctional protein complex (Fig. 1) containing two enzymic activities: (a) serine transacetylase, which catalyzes the acetylation of L-serine by acetyl coenzyme A to form O-acetyl-L-serine, and (b) O-acetylserine sulfhydrylase, which catalyzes the sulfhydrylation of O-acetyl-L-serine by inorganic sulfide to form L-cysteine (1, 2).

Whereas all of the serine transacetylase activity in the cell is found in the cysteine synthetase complex, only a small proportion of the O-acetylserine sulfhydrylase activity is so bound. The complexed O-acetylserine sulfhydrylase is referred to as "O-acetylserine sulfhydrylase-STA," and the free O-acetylserine sulfhydrylase is referred to as "O-acetylserine sulfhydrylase-A" (2).

Resolution of the cysteine synthetase complex into its component enzymes is specifically accomplished in solution by the addition of O-acetyl-L-serine (Fig. 1), as estimated by sedimentation velocity and gel filtration studies (1).

By chemical, physical, and kinetic criteria, resolved O-acetylserine sulfhydrylase-STA is identical with O-acetylserine sulfhydrylase-A (1). The cysteine synthetase complex can be reconstituted by mixing resolved serine transacetylase with either of these sulfhydrylase fractions in the absence of O-acetyl-L-serine. The resulting complex has sedimentation, gel filtration, electrophoretic and kinetic properties identical with those of the original cysteine synthesis complex (1).

Certain catalytic properties of O-acetylserine sulfhydrylase are changed when the enzyme is bound to serine transacetylase. While the $K_m$ of the free enzyme for O-acetyl-L-serine is 5 mm and the turnover number is $4.4 \times 10^4$ units per $10^{-4}$ moles of protein-bound pyridoxal phosphate, for O-acetylserine sulfhydrylase...

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**Fig. 1.** Schematic representation of the relationship between the cysteine synthetase complex and its component enzymes. Each molecule of cysteine synthetase is shown to contain 1 molecule of serine transacetylase and 2 molecules of O-acetylserine sulfhydrylase. The latter enzyme itself is composed of 2 identical subunits.
bound to serine transacetylase, these values are 20 and 2.3 × 10^4, respectively (1).

We have set about isolating and characterizing mutations in the components of the cysteine synthetase complex.

The experiments presented here describe the effects of one specific point mutation in the structural gene for serine transacetylase which results in the production of a transacetylase molecule with markedly diminished catalytic activity which binds to normal O-acetylseryl sulfate hydrolase in an abnormal manner. As a consequence, bound wild-type O-acetylseryl sulfate hydrolase also has an unusually diminished catalytic activity. This mutation, then, is pleiotropic because the abnormal gene product interacts with a normal enzyme to alter the activity of the latter.

**EXPERIMENTAL PROCEDURE**

**Materials**

L-Cysteine and N-acetyl-L-cysteine were products of Sigma; O-acetyl-L-serine and O-acetyl-L-threonine were obtained from Yeda Research and Development, Ltd. Streptomycin sulfate was a product of Nutritional Biochemicals, and enzyme-grade ammonium sulfate and acetyl-CoA were products of Mann. Purified wild type serine transacetylase was a gift of Dr. Nicholas M. Krebs. O-Acetyl-NL-homoserine, O-succinyl-NL-serine, and O-succinyl-NL-threonine were donated by Dr. D. Kerr and Dr. S. Guggenheim; 5,5'-dithiobis(2-nitrobenzoic acid) was purchased from Aldrich. Gel filtration and ion exchange resins were obtained from Pharmacia. All other reagents were purchased from commercial sources and were of the highest grade generally available.

Absorbance was measured in a Gilford recording spectrophotometer, model 2000. Centrifugation was done in a Sorvall RC-2 refrigerated centrifuge or an International model PR2 refrigerated centrifuge.

**Protein and Enzyme Determinations**

Protein concentration determinations were made by the method of Lowry, et al. (3). Serine transacetylase and O-acetylseryl sulfate hydrolase concentrations were determined as previously described (4, 5). One unit of activity in either assay is that amount of enzyme catalyzing the reaction of 1 μmole per min of substrate at 25°C. The lower limit of sensitivity of the serine transacetylase assay is 0.002 unit per ml. Concentrations of O-acetylseryl sulfate hydrolase after preliminary incubation with the substrate, O-acetyl-L-serine, were determined on an aliquot of material diluted into a solution containing 0.1 mg per ml of bovine serum albumin and 10 mM O-acetyl-L-serine (final concentration). Determinations of O-acetylseryl sulfate hydrolase in the presence of purified wild type serine transacetylase were carried out in an excess of the latter by incubating the mixture for 15 min at room temperature before assaying.

**Immunological Determinations**

Antiserum was prepared to O-acetylseryl sulfate hydrolase-A as previously described (1). The ring precipitin test and quantitative precipitin reaction were carried out as described by Kabat and Mayer (6).

Inactivation of O-acetylseryl sulfate hydrolase activities by the specific antiserum and by control immunized rabbit serum was studied after incubation of the reaction mixtures for 2 hours at 37°C in capped disposable glass test tubes (10 × 75 mm). Each reaction mixture of 0.25 ml contained 0.1 ml of enzyme extract in 0.1 M Tris-HCl (pH 7.6), a variable volume of antiserum or unimmunized rabbit serum (previously dialyzed against 0.1 M Tris-HCl (pH 7.6) and diluted into this buffer containing 0.5 mg per ml of bovine serum albumin), and 0.1 M Tris-HCl (pH 7.0) with 0.5 mg per ml of bovine serum albumin. Following the incubation, the reaction mixtures were assayed for O-acetylseryl sulfate hydrolase activity. Control incubations without antiserum or without enzyme accompanied each experiment. When inactivation studies were performed in the presence of O-acetyl-L-serine, the enzyme extracts were made 0.75 mM in O-acetyl-L-serine prior to addition of the extract to the antiserum-buffer mixture. The inactivation reaction of either free or bound O-acetylseryl sulfate hydrolase with antiserum was complete in 2 hours.

**Bacterial Strains**

Bacterial strains used for the immunochemical and kinetic studies were wild type S. typhimurium LT2 and BB1, a cysteine auxotroph derived from cys E-396 (7). Simon, Gillespie, Demerec, and Itikawa (8) have shown that cysteine auxotrophs tend to accumulate additional mutations in the genes controlling cysteine biosynthesis, the cys E mutation of cys E-396 was transduced by the method of Loper et al. (9) using a phage P-22 derivative, LA (10), into a pyrimidine auxotroph, pyr E-125 (pyr E and cys E markers cotransduce at a frequency of about 3%), and a nonlysogenic cys E pyr+E recombiant (BB1) was selected. In an identical manner, the following cysteine markers described by Clowes (11) were transduced into pyr E-125, and cys E pyr+E strains BB2 through BB7, respectively, were derived: cys E-11, cys E-17, cys E-2, cys E-6, cys E-8, and cys E-30. Strains BB1 through BB7 were used in complementation studies.

**Preparation of Extracts**

Sixteen liters each of BB1 and wild type LT2 (in 1-liter batches) were grown overnight at 37°C with shaking. The medium was the minimal sulfate-glucose medium of Vogel and Bonner (12) modified by replacing magnesium sulfate with magnesium citrate and adding 1.0 mM n-cysteine as the sole sulfur source. Cells were harvested in late log phase by centrifugation at 13,000 × g for 10 min after which the pellets were washed with water and centrifuged at 20,000 × g for 10 min. The cells were then resuspended in 2 ml of 0.1 M Tris-HCl (pH 7.6) per g of cells, wet weight, and disrupted by sonic oscillation for 90 sec at 4 amps in a Branson Sonifier, model LS75. After centrifugation of the sonically disrupted extract for 1 hour at 37,000 × g, the supernatant layer (crude extract) was brought to pH 7.6 with 3.0 M Tris base, and 0.5 volume of 10% streptomycin sulfate in 0.1 M Tris-HCl (pH 7.6) was added slowly with stirring. After standing for 30 min at 4°C, the material was centrifuged at 37,000 × g for 30 min. The supernatant layer was removed for further treatment.

**RESULTS**

**Characterization of Precipitin Reaction**

Previously described double gel diffusion data (1) showed single precipitin bands resulting from the reaction of rabbit anti-O-acetylseryl sulfate hydrolase-A with either purified O-acetylseryl sulfate hydrolase-A or purified cysteine synthetase. The quantitative precipitin reaction for the antiserum-O-acetylseryl sulfate hydrolase-A reaction (Fig. 2) was used to calibrate the antiserum.
Increasing amounts of purified O-acetylserine sulfhydrylase-A were added to a constant amount of antisera, and following incubation and centrifugation, the supernatant layers were assayed for O-acetylserine sulfhydrylase activity and subjected to the ring precipitin test. The equivalence points estimated by each of these methods are quite similar. The data indicate that the antisera contain a monospecific antibody to O-acetylserine sulfhydrylase.

Resuspension of carefully washed antigen-antibody precipitate yields about 11% of the original O-acetylserine sulfhydrylase activity precipitated; this activity disappears from the supernatant layer on further centrifugation. These experiments indicate that the antigen-antibody complexes have residual enzyme activity.

**Genetics of Cys E Region and Physiology of BB1**

The *cys E* locus has been subdivided into *E*<sub>α</sub> and *E*<sub>β</sub> cistrons by abortive transduction (7), although some of the mutants on which the original complementation study was based were subsequently shown to contain additional mutations in or near the *cys B* region (13). We have confirmed the existence of two complementation groups using the *cys E* markers originally described but prepared “pure” by the method described for deriving BB strains. Thus BB4, BB5, BD6, and BB7 map in one complementation group (*cys E*<sub>α</sub>) whereas BB1, BB2, and BB3 map in the other (*cys E*<sub>β</sub>). In crude extracts from these mutants, as well as from all other *cys E* mutants thus far isolated, serine transacytase activity is markedly diminished or absent. In contrast, levels of O-acetylserine sulfhydrylase activity in extracts of these *cys E* mutants range from about 70 to 100% of the wild type level<sup>1</sup> (1).

Extracts of BB1 contain about 0.5% of wild type serine transacytase activity and about 80% of wild type O-acetylserine sulfhydrylase activity. When crude extracts of BB1 are initially incubated with O-acetyl-L-serine alone, however, the O-acetylserine sulfhydrylase activity of the mutant is equal to that of the wild type similarly treated. Mutant BB1 fails to grow on the minimal salts-glucose medium of Vogel and Bonner (12) but can grow on minimal medium supplemented with either 0.2 mM L-cysteine or 1.0 mM O-acetyl-L-serine. Unlike *cys E* mutants which have no detectable serine transacytase activity and which show no detectable growth when 0.2 mM sulfide and 5.0 mM L-serine are added to the medium, BB1 grows on minimal medium supplemented with these substrates, probably a reflection of the small amount of serine transacylase present. Finally, BB1 shows a reversion frequency to wild type phenotype of about 1 per 10<sup>5</sup> organisms. Several of these revertants have been isolated and have wild type levels of both serine transacylase and O-acetylserine sulfhydrylase activities.

**Isolation of O-Acetylserine Sulfhydrylase Activities**

In order to isolate the sulfhydrylase activities for further studies, the supernatant layer from the streptomycin sulfate precipitation step for each of the extracts prepared as above (see “Experimental Procedure”) was divided into two parts, and each part underwent one of the sequences of steps labeled a and b in Table I. The first part was subjected to ammonium sulfate fractionation and the material precipitating between 0.37 and 0.50 saturation was applied to a column of Sephadex G-200. Fractions of the eluent were assayed for both serine transacylase and O-acetylserine sulfhydrylase activities before and after preliminary incubation with 10 mM O-acetyl-L-serine. The elution profiles for the wild type (LT2) and for the mutant (BB1) extracts are seen in Fig. 3, A and B, respectively. As previously noted for the wild type (Fig. 3A) (2), two peaks of O-acetylserine sulfhydrylase activity were observed, the first associated with the serine transacylase activity in a fixed ratio (cysteine synthetase), and the second free of serine transacylase activity. When the fractions were initially incubated with 10 mM O-acetyl-L-serine, only those from the first peak showed any alteration in O-acetylserine sulfhydrylase activity, in this case a 2-fold increment throughout this peak. The elution profile of the mutant extract (Fig. 3B) also showed two peaks of O-acetylserine sulfhydrylase activity, but only barely detectable serine transacylase activity was found even when assayed in the presence of 10 mM O-acetyl-L-serine. When fractions from the first mutant peak were initially incubated with O-acetyl-L-serine, an increment in O-acetylserine sulfhydrylase activity of approximately 2.9-fold was observed across this peak (compared to 2-fold for the wild type).

The results illustrated in Fig. 3 suggest the following properties of mutant BB1: (a) diminished serine transacylase activity, (b) complex formation between the slightly active serine transacylase and O-acetylserine sulfhydrylase, (c) dissociation of this complex by addition of O-acetyl-L-serine, and (d) abnormal association between O-acetylserine sulfhydrylase and mutant serine transacylase.

The binding of active O-acetylserine sulfhydrylase to mutant serine transacylase is suggested by the appearance of two peaks

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<sup>1</sup> M. A. Becker, unpublished data.
Table I

Preparation of O-acetylserine sulfhydrylase fractions

<table>
<thead>
<tr>
<th>Step</th>
<th>LT2</th>
<th>BB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>No preliminary incubation</td>
<td>Preliminary incubation with 10 ( \text{mM} ) O-acetyl-L-serine</td>
</tr>
<tr>
<td>Crude extract</td>
<td>84</td>
<td>0.44</td>
</tr>
<tr>
<td>Streptomycin sulfate precipitation</td>
<td>118</td>
<td>0.66</td>
</tr>
<tr>
<td>(a) Ammonium sulfate fractionation (0.37–0.50)</td>
<td>4.2</td>
<td>1.89</td>
</tr>
<tr>
<td>Sephadex G-200-120 gel filtration (cysteine synthetase pool)</td>
<td>15.2</td>
<td>4.89</td>
</tr>
<tr>
<td>(b) Ammonium sulfate fractionation (0.40–0.70)</td>
<td>12.2</td>
<td>0.72</td>
</tr>
<tr>
<td>Sephadex G-50 gel filtration</td>
<td>30.2</td>
<td>0.80</td>
</tr>
<tr>
<td>DEAE-Sephadex Chromatography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Acetylserine sulfhydrylase-A pool</td>
<td>37.2</td>
<td>1.84</td>
</tr>
<tr>
<td>O-Acetylserine sulfhydrylase-B pool</td>
<td>37.2</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Table I were applied to matching columns (90 × 2.8 cm²) containing Sephadex G-200 and Sephadex G-120 previously equilibrated with 0.1 M Tris-HCl (pH 7.6) with 1 mM 2-mercaptoethanol. Elution was performed at 25°C in this buffer at a flow rate of 0.2 ml per min, and fractions of 1.0 ml were collected. △—△△△—△△△—△ O-acetylserine sulfhydrylase activity; ○——○ O-acetylserine sulfhydrylase activity after preliminary incubation with O-acetyl-L-serine; ■——■ serine transacetylase activity. Fractions containing cysteine synthetase used in later experiments are indicated as CS Pool.
scribed later have conclusively ruled out an O-acetylserine sulfhydrylase-STA of increased specific activity in BB1.

When the second parts of the supernatant layers from the streptomycin sulfate step (Table I) were fractionated with ammonium sulfate and the materials precipitating between 0.40 and 0.70 saturation were filtered through coarse Sephadex G-50 and subsequently applied to columns of DEAE-Sephadex A-50 ion exchange resin, the patterns resulting from elution with 0 to 0.35 M linear NaCl gradients shown in Fig. 4, A and B, were obtained. Surprisingly, rather than two, there were three identifiable peaks of O-acetylserine sulfhydrylase activity. The first and major peak to be eluted in each case showed no increment in activity upon preliminary incubation with O-acetyl-L-serine, but did show a decrement in O-acetylserine sulfhydrylase activity when purified, resolved, wild type serine transacetylase was added to it. By these criteria, this peak contained O-acetylserine sulfhydrylase-A.

Slightly later in the elution pattern, a small peak of O-acetylserine sulfhydrylase activity appeared. The second peak, which was eluted at a higher salt concentration BB1 than for the wild type, represented cysteine synthetase by the following criteria: increment in O-acetylserine sulfhydrylase activity on preliminary incubation with O-acetyl-L-serine for both extracts and coelution with all of the serine transacetylase activity of the wild type extract.

At still a higher NaCl concentration, a third peak of O-acetylserine sulfhydrylase activity was seen. This activity was not altered by preliminary incubation with O-acetyl-L-serine or by addition of purified, resolved serine transacetylase. This enzymic activity, therefore, has not been described previously and certain of its properties were investigated. It was nondialyzable and heat labile and will be referred to subsequently as "O-acetylserine sulfhydrylase-B." The substrate specificity of this activity was high. Both sulfide and O-acetyl-L-serine were required for the formation of aliphatic thiol. Neither L-serine, N-acetyl-L-serine, O-succinyl-L-serine, O-acetyl-L-homoserine, nor O-acetyl-L-threonine could replace O-acetyl-L-serine as substrate.

For the experiments to be described below, cysteine synthetase was obtained from the initial peak eluted from Sephadex G-200-120 gel as noted in Fig. 3, A and B. O-Acetylserine sulfhydrylase-A was obtained from those fractions of the initial sulfhydrylase peak eluted from DEAE-Sephadex ion exchange columns which showed neither serine transacetylase activity nor altered O-acetylserine sulfhydrylase activity after preliminary incubation with O-acetyl-L-serine, but did show diminished sulfhydrylase activity after incubation with purified serine transacetylase (Fig. 4, A and B). All pooled enzyme fractions were dialyzed against 0.1 M Tris-HCl (pH 7.6) prior to use.

**Immunological Characterization of O-Acetylserine Sulfhydrylase-STA, O-Acetylserine Sulfhydrylase-STA, and O-Acetylserine Sulfhydrylase-B from Wild Type (LT2) and Mutant (BB1)**

The inactivation of O-acetylserine sulfhydrylase-A from both LT2 and BB1 by rabbit anti-O-acetylserine sulfhydrylase-A is seen in Fig. 5. The "specific antigenic activity" of this enzyme was demonstrated by anti-O-acetylserine sulfhydrylase-A. Incubation mixtures were prepared as described under "Experimental Procedure." After incubation at 37° for 2 hours, samples were removed for O-acetylserine sulfhydrylase assay. 

![Fig. 4. Elution patterns of O-acetylserine sulfhydrylase and serine transacytase activities from DEAE-Sephadex A-50. A, wild type LT2; B, mutant BB1. The eluant from the Sephadex G-50 gel filtration step for each extract (b, Table I) was applied to a column (25 x 3.1 cm) containing DEAE-Sephadex A-50 previously equilibrated with 0.1 M Tris-HCl (pH 7.6). Elution was performed at 25° over a 1050 ml of 0 to 0.35 M linear NaCl gradient in 0.1 M Tris-HCl (pH 7.6). Fractions of 9.3 ml were collected.](http://www.jbc.org/)

![Fig. 5. Inactivation of O-acetylserine sulfhydrylase activities by anti-O-acetylserine sulfhydrylase-A. Incubation mixtures were prepared as described under "Experimental Procedure." After incubation at 37° for 2 hours, samples were removed for O-acetylserine sulfhydrylase assay. ■—■, LT2 O-acetylserine sulfhydrylase-A; ◇—◇, BB1 O-acetylserine sulfhydrylase-A; □—□, LT2 cysteine synthetase; △—△, BB1 cysteine synthetase; ●—●, LT2 cysteine synthetase in 15 mM O-acetyl-L-serine; ♦—♦, hybrid cysteine synthetase composed of BB1 O-acetylserine sulfhydrylase-A and LT2 serine transacytase; ○—○, hybrid cysteine synthetase composed of LT2 O-acetylserine sulfhydrylase-A and BB1 serine transacytase.](http://www.jbc.org/)
acetylase activities. serine sulfhydrylase-A; OASS, 0-acetylserine sulfhydrylase.

assayed for both 0-acetylserine sulfhydrylase and serine trans- OASS-STA, O-acetylserinesulfhydrylase-STA; OASS-A, O-acetyl-
rate was 0.15 ml per min. Fractions of 0.5 ml were collected and a The abbreviations used are: STA, serine transacetylase; 

nism was applied and eluted with the equilibrating buffer. Flow 

cysteine synthetases on Sephadex G-100-120. To matched col- BBl 
of dialyzed, concentrated cysteine synthetase from each orga- BBl 

formed in the presence of 15 M O-acetyl-L-serine, the inactivation pattern of O-acetyl-

Thus, the specific antigenic activity of the resolved O-acetyl-

serine sulfhydrylase-STA was identical with that of O-acetyl-

synthetase complex results in twice as much O-acetylserine 
sulfhydrylase activity per antigenic site as is seen in the cysteine synthetase complex itself.

The slope of the line obtained from the immunologic inactiva-
tion of complexed O-acetylserine sulfhydrylase-STA in BB1 cysteine synthetase was one-third that of the wild type enzyme (Fig. 5). However, when BB1 cysteine synthetase was disso-
ciated with O-acetyl-L-serine, the inactivation pattern of O-acetyl-

serine sulfhydrylase-STA was identical with that of O-acetyl-

serine sulfhydrylase-A from either organism (Fig. 5). There was a 3-fold increase in the specific antigenic activity of O-acetylserine sulfhydrylase-STA when it was dissociated from mutant serine transacetylase compared to only a 2-fold change in specific antigenic activity when the wild type complex was dissociated. Since the specific antigenic activity of the resolved O-acetylserine sulfhydrylase-STA from both wild type and mutant is identical with that of O-acetylserine sulfhydrylase-A from both organisms, then the unusually low specific antigenic activity of the O-acetylserine sulfhydrylase-STA of the mutant in the complex and its greater increment on dissociation must result from an abnormal interaction between catalytically normal O-acetylserine sulfhydrylase and BB1 mutant serine transacetylase.

In all of the above studies, the inactivation of the O-acetylser-
ine sulfhydrylase activities by antiserum was incomplete (Fig. 5). The amount of residual activity, however, can be explained by the enzymic activity in the O-acetylserine sulfhydrylase-antibody complex.

The antiserum to O-acetylserine sulfhydrylase-A did not specific-
ally inhibit O-acetylserine sulfhydrylase-B activity.

Dissociation of Cysteine Synthetase Complexes of Wild Type (LT2) and Mutant (BB1)

The pooled, dialyzed fractions from the columns of Sephadex G-200-120 containing cysteine synthetase from LT2 and BB1 were concentrated 4-fold by addition of dry, coarse Sephadex G 25 and centrifugation of the slurry following swelling of the

<table>
<thead>
<tr>
<th>Source of STA</th>
<th>Source of OASS-STA</th>
<th>OASS activity before mixing with STA</th>
<th>OASS activity after mixing with STA</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT2</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BB1</td>
<td>LT2</td>
<td>0.64</td>
<td>2.06</td>
</tr>
<tr>
<td>LT2</td>
<td>LT2</td>
<td>0.31</td>
<td>2.06</td>
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<tr>
<td>BB1</td>
<td>LT2</td>
<td>0.22</td>
<td>2.88</td>
</tr>
<tr>
<td>LT2</td>
<td>BB1</td>
<td>0.59</td>
<td>2.03</td>
</tr>
<tr>
<td>BB1</td>
<td>BB1</td>
<td>0.29</td>
<td>2.03</td>
</tr>
<tr>
<td>LT2</td>
<td>BB1</td>
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<td>2.80</td>
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<tr>
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<td>2.82</td>
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</tr>
<tr>
<td>BB1</td>
<td>BB1</td>
<td>0.88</td>
<td>2.71</td>
</tr>
</tbody>
</table>

*The abbreviations used are: STA, serine transacetylase; OASS-STA, O-acetylserine sulfhydrylase-STA; OASS-A, O-acetylserine sulfhydrylase-A; OASS, O-acetylserine sulfhydrylase.

![Fig. 6. Resolution of LT2 (upper panel) and BB1 (lower panel) cysteine synthetases on Sephadex G-100-120.](image-url)
Heat Inactivation Studies

The heat inactivation rates at 70° of O-acetylserine sulfhydrylase-A activities from LT2 and BB1 are identical (see Fig. 7). However, the inactivation rates for O-acetylserine sulfhydrylase in the complexed form of cysteine synthetase are different. For both LT2 and BB1, the inactivation of O-acetylserine sulfhydrylase-STA in the complex proceeds more rapidly than that of O-acetylserine sulfhydrylase-A, but the inactivation of the bound sulfhydrylase from LT2 is more rapid than the inactivation of this fraction from BB1. Resolved O-acetylserine sulfhydrylase-STA from both sources is inactivated at the same rate as that of O-acetylserine sulfhydrylase-A (Fig. 7). Finally, binding of either sulfhydrylase fraction from BB1 to LT2 serine transacylase results in a heat inactivation pattern identical with that seen for wild type cysteine synthetase, while the reciprocal hybrid is inactivated at the same rate as the mutant cysteine synthetase (Fig. 7). These results show that the difference in heat inactivation between O-acetylserine sulfhydrylase-A and O-acetylserine sulfhydrylase-STA is dependent on the association of the latter with serine transacylase; moreover, the decreased heat sensitivity of the BB1 cysteine synthetase compared to LT2 cysteine synthetase resides in the mutant serine transacylase to which the O-acetylserine sulfhydrylase of the former is bound rather than to the BB1 O-acetylserine sulfhydrylase itself.

Discussion

The preceding experiments clearly establish that a particular point mutation (BB1) in the structural gene for serine transacylase produces two effects. An altered serine transacylase with diminished catalytic activity is produced; and the decrease in catalytic activity of structurally normal O-acetylserine sulfhydrylase is greater when this enzyme is bound to mutant serine transacylase in the cysteine synthetase complex. Despite the abnormal catalytic properties of O-acetylserine sulfhydrylase-STA in the mutant, there is no evidence of an abnormality in the
structure of O-acetylserine sulphydrylase itself. In addition, a hybrid cysteine synthetase, composed of wild type O-acetylserine sulphydrylase and mutant serine transacytase behaves in a manner identical with mutant cysteine synthetase both immunologically and with respect to heat stability. This provides strong evidence that the alteration in the sulphydrylase activity of the mutant results from the association of normal O-acetylserine sulphydrylase with abnormal serine transacytase. Furthermore, the wild type behavior of the reciprocal hybrid composed of mutant O-acetylserine sulphydrylase and wild type serine transacytase also supports this view.

Previous experiments have shown that the formation of the cysteine synthetase complex from wild type O-acetylserine sulphydrylase and wild type serine transacytase results in a 50% diminution in the specific activity of the sulphydrylase (1). The mechanism of this decrease in activity is unknown although it is likely that it results from a change in the conformation of the sulphydrylase incident to binding to serine transacytase. In the present case, wherein the association of normal O-acetylserine sulphydrylase and mutant serine transacytase results in a 67% diminution in catalytic activity of the sulphydrylase, it seems reasonable that either the conformation of O-acetylserine sulphydrylase induced by the mutant serine transacytase is different from that in the wild type complex or that the mutant serine transacytase somehow partially blocks the active sites of the sulphydrylase, thus lowering the latter's catalytic activity. Although not specifically ruled out by these experiments, it seems unlikely that our findings result from the binding of more sulphydrylase molecules to the mutant than to the wild type serine transacytase.

Several other cases have been described in which an enzymic activity is altered or regulated by interaction between that enzyme and another protein or proteins (14-18). In these cases, as in wild type cysteine synthetase, protein-protein interaction is a normal event and phenotypic aberrations result when mutation leads to a failure of such interaction. The alteration in O-acetylserine sulphydrylase activity resulting from the association of this protein with mutant serine transacytase from RR1 shows that a structural gene mutation which retains or enhances the activity is altered or regulated by interaction between that enzyme and another protein or proteins (14-18). In these cases, as in wild type cysteine synthetase, protein-protein interaction is a normal event and phenotypic aberrations result when mutation leads to a failure of such interaction. The alteration in O-acetylserine sulphydrylase activity resulting from the association of this protein with mutant serine transacytase from RR1 shows that a structural gene mutation which retains or enhances the activity is altered or regulated by interaction between that enzyme and another protein or proteins (14-18).

Another example of this type of interaction has been described in the case of the mitochondrial structural protein mutants mi-1 and mi-3 in Neurospora crassa (19, 20). These respiratory-deficient mutants exhibit a wide range of phenotypic abnormalities which can be related to structural mutations in mitochondrial structural protein which is required for the functional organization of the enzymes of electron transport in this organism. Of particular interest in the present context is the finding that the affinity of wild type malate dehydrogenase for malate is markedly diminished when the dehydrogenase is bound to mutant mitochondrial structural protein as compared to wild type mitochondrial structural protein. Both in our situation involving two enzymically active proteins and in the case of the Neurospora mutants, the resulting pleiotropy can be distinguished from simultaneous structural gene mutation or regulatory gene mutation only by extensive investigation of the proteins involved and of the macromolecular organization in the functions under study.

Several subsidiary findings in these experiments are worthy of comment. The identical results obtained when O-acetylserine sulphydrylase-A and resolved O-acetylserine sulphydrylase-STA were compared with regard to specific antigenic activity, heat stability, and the ability to form cysteine synthetase with wild type and mutant serine transacytases provide further confirmation of the identity of these sulphydrylase fractions previously suggested on the basis of chemical, physical, and kinetic studies (1). The significance of O-acetylserine sulphydrylase-B, a fraction of sulphydrylase not previously described, is unclear. Growth of the organism on L-cysteine, which represses O-acetylserine sulphydrylase-A synthesis (4) allowed the O-acetylserine sulphydrylase-B activity to be detected. Although the substrate specificity of this latter activity for the reaction is reminiscent of O-acetylserine sulphydrylase-A, the failure of O-acetylserine sulphydrylase-A antiserum to inactivate O-acetylserine sulphydrylase-B suggests that O-acetylserine sulphydrylase-B is structurally unrelated to O-acetylserine sulphydrylase-A. In addition, the fact that O-acetylserine sulphydrylase-B levels of activity are not altered significantly by growth on different sulfur sources (which have marked effects on the rates of formation of acetylserine sulphydrylase-A and cysteine synthetase) suggests that the enzyme showing O-acetylserine sulphydrylase-B activity is not involved in cysteine synthesis. On the other hand, the presence of O-acetylserine sulphydrylase-B in extracts of this organism might explain the difficulty encountered isolating O-acetylserine sulphydrylase-deficient mutants despite the use of various mutagenic and selection techniques.3

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