L-Cysteine Biosynthesis in *Escherichia coli*: Nucleotide Sequence and Expression of the Serine Acetyltransferase (cysE) Gene from the Wild-type and a Cysteine-excreting Mutant

By DAGMAR DENK AND AUGUST BÖCK*

Lehrstuhl für Mikrobiologie der Universität München, Maria-Ward-Straße 1a, D-8000 München 19, FRG

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Serine acetyltransferase (SAT) from *Escherichia coli* is subject to feedback inhibition by L-cysteine. A mutant was isolated which excretes L-cysteine because of a lesion in cysE, the structural gene for SAT, rendering the enzyme less feedback sensitive. To analyse the structural basis for this mutation the cysE genes both from wild-type *E. coli* and the mutant strain were cloned and their nucleotide sequences determined. The cysE gene contained an open reading frame consisting of 819 bp, equivalent to a protein of 273 amino acids. The mutant gene showed a single base change in position 767 resulting in a methionine to isoleucine substitution. A causal connection between this SAT sequence alteration, feedback insensitivity and L-cysteine excretion was demonstrated. The SAT from the wild-type strain was purified. It was composed of a single polypeptide chain migrating in SDS gels according to an *M*, of 34000. As in *Salmonella typhimurium*, the enzyme was associated in a bifunctional complex with O-acetylserine (thiol)-lyase.

INTRODUCTION

Conversion of L-serine to L-cysteine in *Escherichia coli* and *Salmonella typhimurium* is mediated by the sequential action of two enzymes. Serine acetyltransferase (SAT) (EC 2.3.1.30) catalyses the activation of L-serine by acetyl-CoA. The reaction product, O-acetyl-L-serine (OAS), is then converted to L-cysteine by O-acetyl-L-serine (thiol)-lyase (EC 4.2.99.8). In *S. typhimurium* these enzymes exist in a bifunctional complex of *M*, about 300000. In the presence of OAS the complex dissociates into the individual enzyme activities (Kredich *et al.*, 1969).

The synthesis of O-acetyl-L-serine (thiol)-lyase and of the enzymes involved in sulphate uptake and reduction is regulated by induction as well as by repression (Jones-Mortimer, 1968; Jones-Mortimer *et al.*, 1968; Kredich, 1971). The expression of cysE (the SAT structural gene), on the other hand, is constitutive whereas the catalytic activity of the gene product, SAT, is sensitive to feedback inhibition by L-cysteine (Kredich & Tomkins, 1966).

In the course of a project on developing a cysteine-excreting strain from *E. coli*, a mutant was isolated whose SAT was less sensitive to feedback inhibition by L-cysteine. To analyse the structural basis of cysteine excretion the cysE genes of mutant and wild-type were cloned and their nucleotide sequences were determined.

METHODS

*Strains and plasmids used.* Bacterial strains and plasmids used in this study are listed in Table 1.

*Media and growth conditions.* Luria broth (Miller, 1972) was used as rich medium and CM salt solution as minimal medium. CM medium consisted of (g l⁻¹): Na₂HPO₄, 7.0; K₂HPO₄, 3.0; (NH₄)₂SO₄, 2.0; MgSO₄ . 7H₂O, 0.2; CaCl₂ . 2H₂O, 0.011; 1 ml micronutrient solution (Neidhardt *et al.*, 1974) was added per 1 basal salt solution.

*Abbreviations:* OAS, O-acetyl-L-serine; SAT, serine acetyltransferase.
Table 1. *E. coli* strains and plasmids

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>JM240</td>
<td>Hfr proC47 cys-54 supE42</td>
<td>B. Bachmann</td>
</tr>
<tr>
<td>JM240/33</td>
<td>Hfr proC47 cysE* supE42 cys-54†</td>
<td>Cys* derivative of JM240</td>
</tr>
<tr>
<td>JM39</td>
<td>F* cysE51 tfr-8</td>
<td>B. Bachmann</td>
</tr>
<tr>
<td>JM39/2</td>
<td>F* cysE51</td>
<td>Pl sensitive derivative of JM39 (Goldberg et al., 1974)</td>
</tr>
<tr>
<td>JM39/4</td>
<td>F* cysE51 srl-300::Tn10 recA56</td>
<td>Mating: IC10240 × JM39/2</td>
</tr>
<tr>
<td>AC1</td>
<td>F* cysE52 argH1</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>KL19</td>
<td>Hfr</td>
<td>B. Bachmann</td>
</tr>
<tr>
<td>KL16</td>
<td>Hfr thi-1</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>KL16/8</td>
<td>Hfr thi-1 srl-300::Tn10 recA56</td>
<td>Mating: IC10240 × KL16</td>
</tr>
<tr>
<td>IC10240</td>
<td>Hfr thr-300 recA56 srl-300::Tn10 relA1 le-318 spOTI thi-1 rpsE2300</td>
<td>B. Bachmann</td>
</tr>
<tr>
<td>DS410</td>
<td>F*ara-l3 azi-8 tonA2 minA1 minB2 gal-6 xyl-7 mtl-2 thi-1 rpsL135</td>
<td>Dougan &amp; Sherratt (1977)</td>
</tr>
</tbody>
</table>

Plasmid

- pBR322: Amp*, Tet*
- pCys2: Amp*, cysE*
- pWT2: Amp*, cysE*

† There is no proof that cys-54 is present in JM240/33 in the same form as it is in the parental strain JM240.

The medium was supplemented with glucose (4 g l⁻¹), amino acids (40 µg ml⁻¹) and vitamins (5 µg ml⁻¹) at the indicated final concentrations. When required, ampicillin was added at 100 µg ml⁻¹ and tetracycline at 10 µg ml⁻¹.

The plates used for detection of cysteine excretion consisted of CM medium supplemented with glucose and the appropriate amino acids and seeded with the cysteine auxotrophic indicator strain AC1 at 5 × 10⁶ cells ml⁻¹.

**Genetic procedures.** Mutagenesis with ethyl methanesulphonate (EMS) and mating experiments were done as described by Miller (1972). The RecA phenotype of conjugants was tested by exposure to UV light as described by Maniatis et al. (1982).

**Recombinant DNA techniques.** Chromosomal DNA was prepared by following the method of Saito & Miura (1963), but, with an additional phenol extraction and a subsequent CsCl density gradient step for final purification. Small- and large-scale preparations of plasmids, as well as ligation, transformation, electrophoresis in agarose and recovery of DNA from low-melting agarose were done as described by Maniatis et al. (1982). Restriction endonuclease digestions were done under the conditions recommended by the manufacturers.

**DNA sequencing.** Nucleotide sequences of DNA were determined by the base-specific chemical cleavage method of Maxam & Gilbert (1980), but modifying the A + G reaction as suggested by Gray et al. (1978). DNA fragments were 3'-labelled as described by Maniatis et al. (1982) using [α-³²P]dATP and the Klenow fragment of *E. coli* RNA polymerase. The 5'-labelling was done with polynucleotide kinase and [γ-³²P]ATP according to Maxam & Gilbert (1980). Cleavage products were analysed by electrophoresis in 6, 8 and 20% (w/v) polyacrylamide gels containing 7 M-urea.

**Nuclease S1 mapping.** This was done as described by Wich et al. (1986).

**Minicell experiments.** Isolation and labelling of minicells from *E. coli* DS410 was done as described by Christen et al. (1983). The gradient was prepared by freezing and thawing M9 salt solution (Miller, 1972) containing 20% (w/v) sucrose. The minicell suspension was applied on the top of the gradient and centrifugation was done at 5000 r.p.m. for 20 min in a Sorvall SW 27 rotor. Minicells were recovered from the gradient by a Densi-flow (Buchler) and a peristaltic pump; they were collected by centrifugation (15 min, 8000 g), resuspended in M9 buffer (Christen et al., 1983) and subjected to filtration.

The determination of serine acetyltransferase (SAT) activity. CM medium containing the appropriate supplements was inoculated to an OD₆₀₀ of approximately 0.05. At an OD₆₀₀ of 0.8, cells were harvested by centrifugation, washed once with ice-cold Tris/HCl (50 mM, pH 7.5) and stored at −20°C until used. Cells were thawed, resuspended in 1-5 vols ice-cold Tris/HCl (50 mM, pH 7.5) containing 2 mM-dithiothreitol and DNAase I (5 µg ml⁻¹), and disrupted by a passage through a French press cell (Amicon) at 110-4 MPa. The homogenate was centrifuged for 30 min at 30000 g and the supernatant was used immediately to analyse SAT activity.

SAT activity was assayed spectrophotometrically in a Gilford 2600 spectrophotometer by following the decrease in A₂₃₂, which occurs as a result of the cleavage of the thioester bond of acetyl-CoA (Kredich & Tomkins, 1966). The assay was done at room temperature in cuvettes of 10 mm path length in a final volume of 1 ml which
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contained: Tris/HCl, pH 7.6 (50 μmol), L-serine (1 μmol), acetyl-CoA (0.1 μmol), and an appropriate amount of enzyme. Inhibition of SAT activity was tested in the same reaction mixture but in the presence of different amounts of L-cysteine. A blank lacking L-serine was run simultaneously and subtracted from the reaction rate obtained in the presence of L-serine. The molar absorption coefficient ε of acetyl-CoA was taken as 6.5 x 10³ l mol⁻¹ cm⁻¹.

Purification of SAT. A procedure for purification of SAT from S. typhimurium has been worked out by Kredich et al. (1969). A modification of this protocol was adopted for isolation of SAT from E. coli. Strain KL16/8 (pWT2) was grown in CM medium with the appropriate supplements in a Biostat UD50 fermenter (Braun–Melsungen) to an OD x 250 of 1.0. Cells were harvested by centrifugation, washed once with ice-cold Tris/HCl (50 mM, pH 7.5) and stored at -20°C. Frozen cells (90 g wet weight) were thawed and resuspended in 1.5 vols buffer A (Tris/HCl, 50 mM, pH 7.6; MgSO₄, 10 mM; NH₄Cl, 30 mM; dithiothreitol, 2 mM; phenylmethylsulphonyl fluoride, 0.5 mM; DNAase I, 5 μg ml⁻¹). All further steps were carried out at room temperature. Cells were disrupted by two passages through a French press cell (Amicon) at 82.8 MPa. The homogenate was centrifuged for 30 min at 30000 g. The supernatant (S30) was centrifuged again at 100000 g for 120 min. The resulting ribosome-free supernatant was subjected to ammonium sulphate fractionation by the addition of solid (NH₄)₂SO₄. The protein fraction that could be precipitated between 35 and 50% (NH₄)₂SO₄ saturation was dissolved in 15 ml buffer B (Tris/HCl, 110 mM, pH 7.6; L-cysteine, 20 μM; dithiothreitol, 2 mM; EDTA, 1 mM) and dialysed against 51 of the same buffer. The dialysed extract was applied to a column (22 cm x 7 cm³) of DEAE-Trisacryl M (LKB) equilibrated with buffer B. Elution occurred with a 11 linear gradient of NaCl in buffer B (0-0.5 M) at a flow rate of 30 ml h⁻¹. Fractions displaying SAT activity were pooled and the protein was precipitated with (NH₄)₂SO₄ (600 g l⁻¹). The precipitate was dissolved in a minimum amount of buffer C (Tris/HCl, 100 mM, pH 7.6; L-cysteine, 20 μM; dithiothreitol, 2 mM; EDTA, 1 mM), applied to a column (85 cm x 7 cm³) of Ultrogel AcA 34 (LKB) equilibrated with buffer C and eluted at a flow rate of 6 ml h⁻¹. The SAT-containing fractions were pooled and the protein was precipitated with (NH₄)₂SO₄ (600 g l⁻¹).

The precipitate was dissolved in a minimum of buffer D (sodium phosphate, 50 mM, pH 6.8; NaCl, 250 mM; EDTA, 1 mM; dithiothreitol, 2 mM; L-cysteine, 10 μM; OAS, 50 mM). The solution was applied onto a column (85 cm x 7 cm³) of Biogel P200 (Bio-Rad), equilibrated with buffer D, and eluted at a flow rate of 6 ml h⁻¹. The pooled and precipitated SAT-containing fractions were subjected to Biogel P200 size chromatography a second time.

Separation of proteins by SDS-PAGE. This was done as described by Laemmli (1970).

Determination of protein and of cysteine. Protein concentration was estimated by the Lowry method with BSA as a standard.

The amount of cysteine excreted in the culture medium was determined by the method of Gaitonde (1967) with the modifications suggested by Krauss (1984).

RESULTS

Isolation of mutants excreting L-cysteine

One of the approaches followed to isolate cysteine-excreting mutants consisted in the selection of Cys⁻ prototrophic derivatives of cysteine auxotrophs after EMS mutagenesis. Strains JM240, AC1 and JM39 were used; each strain gave rise to different numbers of revertants (or pseudorevertants) which were tested for cysteine excretion by cross-feeding assays employing strain AC1 as indicator. Amongst 300 prototrophs tested, 20 excreted L-cysteine both on plates and/or in liquid medium: concentrations measured ranged from 5 to 30 mg cysteine (l medium⁻¹). The highest amount was obtained in the case of a derivative of JM240, designated JM240/33; it was used in this study.

Determination of SAT activity

SAT activities were determined in order to locate the biochemical basis of cysteine excretion by strain JM240/33 (Table 2). In contrast to extracts from strains JM39 and AC1 which were devoid of SAT activity (not shown), those from JM240 displayed enzyme activity, even somewhat higher than that from a prototrophic reference strain. Sensitivity to inhibition by L-cysteine was comparable for the KL16 and the JM240 enzymes but was reduced about 10-fold for JM240/33. The results indicate (i) that JM240 carries a lesion outside the cysE structural gene rendering it cysteine auxotrophic, and (ii) that cysteine excretion by JM240/33 might be due to a lowered sensitivity of SAT to inhibition by cysteine.
Table 2. Inhibition by L-cysteine of wild-type and mutant SAT

<table>
<thead>
<tr>
<th>Strain</th>
<th>L-Cysteine concn (μM)</th>
<th>Specific activity [pkat (mg protein)^{-1}]^*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>KL16</td>
<td>130</td>
<td>13</td>
</tr>
<tr>
<td>JM240</td>
<td>270</td>
<td>16</td>
</tr>
<tr>
<td>JM240/33</td>
<td>250</td>
<td>170</td>
</tr>
</tbody>
</table>

* 1 kat = 1 mol substrate transformed s^{-1}.

Molecular cloning of the cysE structural genes from strains KL16 and JM240/33

To analyse the genetic basis of reduced feedback sensitivity of SAT from strain JM240/33 the cysE genes were cloned from strains KL16 and JM240/33. For this purpose a suitable recipient (JM39/5) was first constructed by the procedure outlined in Table 1. Its cysE marker displays a low reversion frequency and the strain itself can be easily transformed.

The genes for wild-type SAT (cysE) and feedback-desensitized SAT (cysE*) were cloned from strains KL16 and JM240/33, respectively. Genomic DNA from the two donor strains and DNA from the vector pBR322 were digested with one of the restriction endonucleases BamHI, EcoRI, SalI or PstI. After ligation of the different chromosomal DNA and vector DNA mixtures they were used to transform E. coli JM39/5 to cysteine prototrophy. In the case of the BamHI digestion and ligation experiment, transformants were obtained which besides being Cys^+ were ampicillin resistant and tetracycline sensitive. All these transformants (irrespective of whether the donor DNA was from strain KL16 or JM240/33) carried an 8.0 kb plasmid possessing a 3.7 kb BamHI insert. Plasmids pWT2, which contains insert DNA from KL16, and pCys2, which contains JM240/33 DNA, were chosen for further analysis. Southern hybridization experiments done with BamHI-digested genomic DNA of KL16 and JM240/33 and nick-translated pWT2 or pCys2 DNA revealed the existence of a single hybridizing fragment identical in size to that of the BamHI insert in the plasmids (results not shown).

Subcloning experiments

Subcloning experiments were done in order to permit localization of the cysE structural gene within the 3.7 kb BamHI insert of plasmids pWT2 and pCys2. First, the restriction map was established; it was identical for pWT2 and pCys2 (Fig. 1).

On the basis of the restriction map, subcloning experiments were done for plasmid pCys2 (Fig. 1). The fragments indicated were cloned into pBR322, resulting in plasmids pCys3, pCys4, pCys5 and pCys6. They were transformed into strain JM39/5 with selection for acquisition of antibiotic resistance. Complementation of the cysE marker of JM39/5 was observed with plasmids pCys3 and pCys4 but not with pCys5 and pCys6. The unique ClaI site within the BamHI insert, therefore, seems to inactivate expression of the putative cysE gene cloned. Accordingly, the smallest fragment displaying complementation is the 2.55 kb PvuII insert of plasmid pCys4.

Nucleotide sequences of wild-type and mutant SAT genes

The 2.55 kb PvuII fragments from pWT2 and pCys2 were sequenced following the strategy indicated in Fig. 1. It was found that pWT2 as well as pCys2 contained an open reading frame with a coding capacity of 819 bp within the sequenced region. The nucleotide sequences of the wild-type and mutant alleles of the cysE gene were completely identical except for a single base change at position 767. The transition of G (wild-type) to A (mutant) resulted in a putative substitution of methionine by isoleucine (Fig. 2).

To confirm that this base alteration is responsible for feedback inhibition insensitivity, a MluI/BstEII restriction fragment spanning this region was isolated from the wild-type allele of cysE and substituted for the corresponding DNA fragment of the mutant cysE* gene, and vice versa (Fig. 3). The recombinant plasmids of this DNA exchange experiments, pWT2' and pCys2', were used to transform JM39/5 to prototrophy.
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A cross-feeding experiment was done to compare the transformants JM39/5(pWT2'), JM39/5(pCys2'), JM39/5(pCys2) and JM39/5(pWT2) with regard to cysteine excretion. Zones of growth indicated a relatively strong cysteine excretion in the case of JM39/5(pCys2) and JM39/5(pWT2), while JM39/5(pWT2) and JM39/5(pWT2') showed only minimal feeding effects (data not shown). These results were confirmed by measuring the SAT activity in the crude extracts of the four different transformants. SAT activity from JM39/5(pWT2') was inhibited (up to 80%) by 10 μM-L-cysteine. This result is typical for the enzyme from JM39/5(pWT2). On the other hand, SAT activity from strains JM39/5(pCys2) and JM39/5(pCys2') showed the decreased sensitivity characteristic of the enzyme from JM240/33.

The MluI/BstEII fragment from pWT2' and pCys2' was sequenced and again showed the single basepair exchange at position 767. Altogether these data indicate that a single amino acid substitution in the E. coli cysE gene is responsible for reduction of feedback sensitivity of SAT and for excretion of L-cysteine.

**Mapping of 5'-ends of the cysE gene transcript**

Plasmid pWT2 was digested with restriction endonucleases BstEII and SauI and a 1.3 kb DNA fragment was recovered from low-melting agarose (Fig. 1). After further digestion with
Fig. 2. Nucleotide sequence (RNA-like strand) of the wild-type *E. coli* cysE gene plus flanking regions; the derived amino acid sequence is written above the nucleotide sequence. The *M* of this protein is 29260.8. The EMS-induced mutation which results in a feedback-desensitized enzyme is indicated (nucleotide 767). The amino acid substitution (Ile replaces Met) is boxed. The transcription start point is designated +1; upstream of this transcription start two putative transcription signals (−10, −35) are present. A putative ribosome-binding site (SD) is indicated by underlining. Because of the blocked N-terminus of SAT there is no direct proof that the chosen ATG initiation codon is actually used.
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Fig. 3. Mutual exchange of the 0.6 kb MluI/BstEII restriction fragment of plasmids pCys2 and pWT2 spanning the mutated region (indicated by a cross). —, pBR322; —, chromosomal DNA from E. coli; →, wild-type cysE gene; ↔, mutant cysE gene.

Table 3. Purification of SAT from E. coli KL16/8(pWT2)

<table>
<thead>
<tr>
<th>Total protein (mg)</th>
<th>Total activity (ukat)</th>
<th>Specific activity [nkat (mg protein)(^{-1})]</th>
<th>Yield (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S30 crude extract</td>
<td>15 750</td>
<td>25</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td>S100</td>
<td>10 850</td>
<td>22</td>
<td>2.0</td>
<td>86</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>747</td>
<td>14</td>
<td>19.3</td>
<td>57</td>
</tr>
<tr>
<td>DEAE-Trisacryl M</td>
<td>138</td>
<td>14</td>
<td>77.0</td>
<td>42</td>
</tr>
<tr>
<td>AcA 34 Ultrogel</td>
<td>28</td>
<td>4.9</td>
<td>169</td>
<td>19</td>
</tr>
<tr>
<td>Biogel P200*</td>
<td>3.2</td>
<td>2.2</td>
<td>697</td>
<td>9</td>
</tr>
</tbody>
</table>

* Elution in the presence of 50 mM-OAS.

Purification of SAT

SAT was purified in order to correlate protein structure with the cysE nucleotide sequence information described. Determination of SAT activity in crude extracts from E. coli KL18/8 (pWT2) showed that the plasmid-bearing strain was an excellent source for the enzyme. The specific activity of SAT in extracts of this strain was approximately 40 times higher than that of the plasmid-free strain. Details of the purification of SAT from E. coli KL16/8 (pWT2) are given in Table 3. The purification scheme used was similar to that employed for the purification of cysteine synthase from S. typhimurium (Kredich et al., 1969). The key steps were: preparation of S30 and S100 crude extracts, (NH₄)₂SO₄ precipitation, ion-exchange chromatography on DEAE-Trisacryl M, and gel filtration on Ultrogel AcA 34 and Biogel P200. During these purification steps an intensive yellow pigment co-purified with SAT activity. Kredich et al. (1969) observed a similar phenomenon when purifying cysteine synthase from S. typhimurium.
They demonstrated that the SAT activity is associated with the pyridoxalphosphate-containing O-acetyl-L-serine (thiol)-lyase in a bifunctional protein complex which they designated 'cysteine synthase' (Kredich et al., 1969).

In order to determine whether this is also the case for the *E. coli* enzyme we attempted to separate both enzymic activities by gel filtration in the presence of 50 mM-OAS which dissociates the *S. typhimurium* enzyme complex (Kredich et al., 1969). Separation of the SAT activity from the coenzyme-containing protein was indeed achieved by passage through a Biogel P200 column equilibrated with 50 mM-OAS (Fig. 5).

The course of purification depicted by SDS-PAGE is given in Fig. 5. Lane G exhibits the migration position of purified SAT; by size, a single subunit species of *M*<sub>r</sub> 34000 is resolved. Lane F reveals the polypeptide composition of the pooled fractions containing SAT activity after gel filtration in the absence of OAS. The band immediately above the SAT polypeptide is associated with the yellow pigment described above. This polypeptide separates from the SAT activity upon gel filtration in the presence of OAS.
Fig. 5. SDS-PAGE of protein fractions containing SAT activity. Lanes A and H, molecular mass standards; lane B, S30 (50 µg protein); lane C, S100 (50 µg protein); lane D, (NH₄)₂SO₄ precipitate (50 µg protein); lane E, DEAE-Trisacryl M (50 µg protein); lane F, AcA 34 Ultrogel (20 µg protein); lane G, Biogel P200/50 mM-OAS (6 µg protein).

Determination of cysE gene product in a minicell system

Plasmids pBR322 and pCys2 were transformed into the minicell-producing strain E. coli DS410. Minicells were isolated and used to follow the formation of plasmid encoded protein by L-[³⁵S]methionine incorporation (Fig. 6). In minicells containing the vector pBR322 the bands characteristic of β-lactamase (precursor, mature protein and degradation product) could be evidenced. Minicells containing pCys2 showed a less intensive incorporation into β-lactamase polypeptides, possibly due to the lower copy number of pCys2 compared to pBR322. An additional band (arrow) is resolved which migrates to the position of the constituent subunit of SAT.

DISCUSSION

There are several established procedures which can be used for the isolation of mutants with altered feedback inhibition properties of an allosteric enzyme: (i) selection for resistance to some analogue of the genuine effector; (ii) selection of an intragenic suppressor mutation relieving the effect of a primary mutation (e.g. a mutation conferring auxotrophy) and concomitantly introducing feedback resistance; and (iii) selection for extragenic suppression of a primary mutation (e.g. of a mutation limiting the flow of metabolites through a pathway).

It is the final aim of this project to follow a more synthetic approach. The idea is first to genetically localize an amino acid position within the structural gene involved in mediating inhibition by an allosteric effector and second, to permute this amino acid (or neighbouring ones) by localized mutagenesis. The results described in this communication show that a conversion of a methionine to an isoleucine residue at position 256 of the SAT constituent polypeptide decreases feedback sensitivity by L-cysteine about 10-fold. This alteration promotes
excretion of L-cysteine into the medium to about 30 mg l\(^{-1}\). Overproduction of the amino acid may well be limited by the remaining, albeit reduced, sensitivity to the effector. It will be interesting to follow whether substitution of the methionine residue at the SAT position 256 by any of the other 18 amino acids may lead to a completely desensitized enzyme.

The cysteine auxotroph of *E. coli* used in this study, JM240, was described as a putative cysE mutant (B. Bachmann, personal communication). The enzyme assays performed, however, demonstrate that it contains wild-type levels of SAT activity and that it displays full feedback sensitivity to L-cysteine. Experiments done to resolve the genetic lesion showed that supplementation with any of the following compounds stimulates growth of JM240: serine, OAS, sulphite, sulphide and L-cysteine. A possible explanation is that JM240 carries a lesion in the cysB regulatory gene which partially limits expression of those cysteine biosynthetic genes which are less sensitive to cysB-mediated derepression (see Kredich, 1983). In JM240/33, the prototrophic phenotype could be due to increased formation of OAS (the activator of the cysB gene product) via desensitized SAT. This enables the derepression of all genes of the cysteine regulon.

SAT from *E. coli* was purified to apparent electrophoretic homogeneity and demonstrated to migrate as a single band in SDS-PAGE. The N-terminal amino acid of SAT is blocked as was reported for the enzyme from *S. typhimurium* (Kredich *et al.*, 1969). This provides some evidence (but does not prove) that the enzyme is composed of a single polypeptide species. Circumstantial evidence for a single constituent polypeptide chain also comes from the 40-fold increased SAT activity in transformants carrying the cysE gene described on a multicopy plasmid.
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It was consistently observed that a polypeptide with a molecular mass of 36 kDa co-purified with SAT. Densitometric evaluation (not shown) pointed to a 1:1 stoichiometry. Size chromatography in the presence of OAS (under non-denaturing conditions) led to dissociation of the two polypeptides. Since the protein peak containing the 36 kDa polypeptide showed *O*-acetyl-L-serine (thiol)-lyase activity the conclusion is justified that in *E. coli*, as in *S. typhimurium*, SAT forms a multi-enzyme complex with *O*-acetyl-L-serine (thiol)-lyase which Kredich et al. (1969) termed 'cysteine synthase'.

SAT is a constitutively expressed enzyme (Jones-Mortimer et al., 1968; Kredich, 1971). S1 nuclease mapping experiments of the transcription start site showed that the *cysE* gene mRNA contains an unexpectedly long leader sequence of approximately 118 bases. At present, there is no information on the role of this leader sequence in expression.

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REFERENCES


