Purification and Characterization of Serine Acetyltransferase from Escherichia coli Partially Truncated at the C-Terminal Region

Koshiki MINO, Tsuyoshi YAMANOUE, Takaharu SAKIYAMA, Naoki EISAKI,*
Asahi MATSUYAMA,* and Kazuhiro NAKANISHI†

Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, 3-1-1 Tsushima-naka, Okayama 700-8530, Japan
*Research and Development Division, Kikkoman Corporation, 399 Noda, Chiba 278-0037, Japan
†Research Institute of Innovative Technology for the Earth (RITE), 2-8-11 Nishi-shinbashi, Minato-ku, Tokyo 105-0003, Japan

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Incubation of serine acetyltransferase (SAT) from Escherichia coli at 25°C in the absence of protease inhibitors yielded a truncated SAT. The truncated SAT was much less sensitive to feedback inhibition than the wild-type SAT. Analyses of the N- and C-terminal amino acid sequences found that the truncated SAT designated as SAT::ΔC20 was a resultant form of the wild-type SAT cleaved between Ser 253 and Met 254, deleting 20 amino acid residues from the C-terminus. Based on these findings, we constructed a plasmid containing an altered cysE gene encoding the truncated SAT. SAT::ΔC20 was produced using the cells of E. coli JM70 transformed with the plasmid and purified to be homogeneous on an SDS-polyacrylamide gel. Properties of the purified SAT::ΔC20 were investigated in comparison with those of the wild-type SAT and Met-256-Ile mutant SAT, which was isolated by Denk and Böck but not purified (J. Gen. Microbiol., 133, 515-525 (1987)). SAT::ΔC20 was composed of four identical subunits like the wild-type SAT and Met-256-Ile mutant SAT. Specific activity, optimum pH for reaction, thermal stability, and stability to reagents for SAT::ΔC20 were similar to those for the wild-type SAT and Met-256-Ile mutant SAT. However, SAT::ΔC20 did not form a complex with O-acetylserine sulfhydrylase-A (OASS-A), a counterpart of the cysteine synthetase and did not reduce OASS activity in contrast to the wild-type SAT and Met-256-Ile mutant SAT.

Key words: serine acetyltransferase; O-acetylserine sulfhydrylase-A; cysteine synthetase; truncated serine acetyltransferase; enzyme complex

Serine acetyltransferase (SAT) (EC 2.3.1.30) and O-acetylserine sulfhydrylase (OASS) (EC 4.2.99.8) are the key enzymes involved in the final step of sulfate assimilation in microorganisms and also in plants. SAT catalyzes the first reaction in the two-step enzymatic pathway of the biosynthesis of l-cysteine from l-serine, the formation of O-acetyl-l-serine (OAS) and CoA from l-serine and acetyl-CoA. The second reaction in the pathway is catalyzed by OASS, which converts OAS and sulfide to l-cysteine and acetate. Although there are two isozymes of OASS from bacteria, OASS-A and OASS-B, the former is expressed predominantly under aerobic conditions. SATs from bacteria such as Escherichia coli and Salmonella typhimurium were purified and their characteristics have been investigated. The structural genes of SAT (cysE) and OASS-A (cysK) were also analyzed. Kredich et al. showed that SAT and OASS exist as an enzyme complex, usually called cysteine synthetase, and that it is composed of one molecule of SAT and two molecules of OASS-A. The existence of the enzyme complex is also confirmed in some higher plants such as Allium tuberosum, Arabidopsis thaliana, Citrullus vulgaris, and spinach. Usually a formation of a multi-enzyme complex plays a role in regulation of the reaction in metabolic pathways. However, a role of the complex formation between SAT and OASS-A in l-cysteine biosynthesis is not known in spite of the efforts of several investigators.

Another important characteristic of SAT is that it is inhibited by l-cysteine in a feedback manner. The activity of the wild-type SAT from E. coli is decreased to one fifth in the presence of 4 μM of l-cysteine. Denk and Böck isolated a mutant strain of E. coli that produces SAT that is much less sensitive to inhibition by l-cysteine. The l-cysteine concentration that reduces the SAT activity to one-fifth is 100 μM. They showed from DNA sequence analysis that a single base change took place in position 767, equivalent to the 256th amino acid position, resulting in a Met to an Ile substitution. However, they did not purify the mutant enzyme (Met-256-Ile mutant SAT) and its characteristics have not yet been studied. There is also no information on the relationship between the inhibitory effect of l-cysteine and the formation of the enzyme complex.

We have recently started to discover the role of the complex formation between SAT and OASS-A in connection with the inhibitory effect and to understand the...
behavior of the \( \text{cysE} \) gene. During incubation at 25°C of the purified wild-type SAT from \( E. \ coli \) carrying a plasmid containing the \( \text{cysE} \) gene, the enzyme was partially truncated near the C-terminal region and showed much less sensitivity to inhibition by l-cysteine. On the basis of the N-and C-terminal amino acid sequences we constructed a plasmid that contained the altered \( \text{cysE} \) gene encoding the truncated SAT. The truncated SAT was then purified in a large quantity using \( E. \ coli \) JM70 transformed with the plasmid. We investigated the properties of the truncated SAT such as degree of inhibition by l-cysteine, optimum \( \text{pH} \), thermal stability, stability to some reagents, and behaviors for the complex formation with OASS-A in comparison with those for the purified wild-type SAT and Met-256-Ile mutant SAT.

Materials and Methods

**Materials.** Acetyl-CoA, l-serine, l-cysteine hydrochloride monohydrate, sodium sulfide nonahydrate, and EDTA disodium salt dihydrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sephacryl S-200HR, DEAE Sephadex A-50, Blue Sepharose CL-6B, Phenyl Sepharose CL-4B, Octyl Sepharose CL-4B, Superose 6HR, and Superose 12HR were products of Pharmacia Biotech. (Uppsala, Sweden). OAS hydrochloride and PLP were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Streptomycin sulfate was obtained from Life Technologies (Gaithersburg, MD, USA). *Achromobacter* protease I (lysl endopeptidase) and TPCK-treated trypsin were obtained from Wako Pure Chemical Industries and Sigma Chemical Co., respectively. The enzymes used for DNA manipulations were obtained from Takara Shuzo Co. (Kyoto, Japan). All other reagents were of reagent grade.

**Bacteria and plasmids.** A \( \text{cysE} \) deficient strain of \( E. \ coli \) JM70 was a generous gift of the National Institute of Genetics (Mishima, Japan). \( E. \ coli \) NK3 lacking both the \( \text{cysK} \) gene and the \( \text{cysM} \) gene, which encodes OASS-B, was kindly donated by Dr. N. M. Kredich, Duke University Medical Center (Durham, NC, USA).

The expression plasmids designated as pOHE100, pOHE100T, pPOHK100, and pPOHC100ΔC20, which express a wild-type SAT, Met-256-Ile mutant SAT, OASS-A, and both SAT deleting 20 amino acid residues from a C-terminus (SATΔC20) and OASS-A under the lactose promoter were constructed by the following procedures.

A plasmid, pOHE100, was constructed as follows: First, two polymerase chain reaction (PCR) primers (sense, \( 5'\)-ATGTCGTGAGAAGACTGGAA-3'; antisense, \( 5'\)-ACATTAGATCCCATCCCACT-3') were synthesized on the basis of the nucleotide sequence of the \( \text{cysE} \) gene analyzed by Denk and Bock. 29 Genomic DNA was prepared from \( E. \ coli \) 1100 (\( F^-\), thi1, endA1). 30 A 8-kb portion of the genomic DNA was added as a template to a solution containing 10 \( \mu \)l of 10 \( \times \) PCR buffer (Takara Shuzo Co., Gene Amp DNA Amplification Reagent Kit), 16 \( \mu \)l of a mixture of dATP, dCTP, dGTP, and dTTP each at 1.25 mM, 10 \( \mu \)l of a mixture of the two primers each at 3 \( \mu \)M, 2.5 U of Taq DNA polymerase, and distilled water to bring the volume to 0.1 ml. PCR was done with a DNA thermal cycler (Perkin-Elmer Cetus Instruments, Model PJ2000, Norwalk, CT, USA). In the first step, one cycle (incubation at 94°C for 3 min, at 55°C for 1.5 min, and at 72°C for 2.5 min) was done. In the second step, the first cycle was done at 94°C for 1.5 min, at 55°C for 2 min, and at 72°C for 2 min, from the second cycle the time for incubation at 72°C was increased by 2 sec per cycle, and this procedure was repeated 24 times. The resultant fragment was ligated to the \( \text{HpaI} \) site of pUTE100K to construct pOHE100. The pUTE100K' was prepared by substituting the NdeI cloning site in pUTE500K 20 with a \( \text{HpaI} \) site.

Site directed mutagenesis was done to alter the Met 256 codon to Ile codon of wild-type SAT in pOHE100 by the Kunkel method 21, 22 with an antisense mutagenic oligonucleotide, \( 5'\)-AAATGCTGGTCAATATCCATTGG-3'\) (the underlining indicates the location of mutation).

The resulting plasmid with the altered \( \text{cysE} \) gene with the Met-256-Ile mutation was designated pOHE100T. A plasmid pOHK100 was constructed by the same method as that used for the pOHE100. The sense primer, \( 5'\)-ATAGAGTTAGATTATTGAAGA-3'\) and the antisense primer, \( 5'\)-AACACTGTCATTACTGGTTG-3'\) were used for cloning the \( \text{cysK} \) gene.

A plasmid, pOHC100ΔC20, was constructed as shown in Fig. 1. The pOHE100 was digested with SpeI and Nhel. Then, a region containing both the lactose promoter and \( \text{cysE} \) gene was recovered and ligated into the SpeI site of pOHK100. The resultant plasmid was designated as pOHC100. PCR was done with the plasmid pOHC100 as a template using two primers: The sense and antisense primers were \( 5'\)-TAAACTAACAAGTATGATCCTGTCAC-3'\) and \( 5'\)-GGATGGCTTATACTGGCTTCTGTTT-3'\) respectively. The resultant fragment was ligated to prepare pOHC100ΔC20.

**Cultivation of cells.** Plasmids, pOHE100, pOHE100T, and pOHC100ΔC20 were introduced into \( E. \ coli \) JM70 and pOHK100 was into \( E. \ coli \) NK3, by electroporation using gene transfer equipment (Shimadzu, GTE-10, Kyoto). Recombinant cells of \( E. \ coli \) JM70 were grown at 37°C in 2.4 liters of modified Luria-Bertani (LB) medium, containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.2% glucose, and 100 \( \mu \)g/ml ampicillin (\( \text{pH} \) 7.0) with reciprocal shaking at 120 strokes/min. In cultivation of the recombinant \( E. \ coli \) NK3, thiamine was added at 0.002%. In every culture, IPTG was added to the medium at 1 mM after 5 h of shaking from the start of cultivation. Shaking was continued for a further 6 h and cells were recovered by centrifugation (3,000 \( \times \) g) for 15 min at 4°C.

**Enzyme assays.** SAT activity was assayed at 25°C either by measuring the absorbance of TNB due to the reaction of CoA with DTNB (Method I) 9 by following the cleavage of thioester bond of acetyl-CoA (Method 2) 9 as follows. Reactions were done either in 50 mM potassium phosphate buffer, pH 7.5, containing
Purification of wild-type SAT. The wild-type SAT was purified at 4°C by combining the method adopted by Denk and Böck and that by Wigley et al. During purification SAT activity was measured by Method 1. About 14 g of the wet cells of E. coli JM70 carrying pOHE100 were washed with 10 mM potassium phosphate buffer, pH 7.5 and resuspended in 120 ml of buffer B, containing 1 mM PMSF, EDTA and PMSF were added to protect SAT from degradation by endogenous proteases. Cells were sonicated disrupted using a homogenizer (Branson Ultrasonics Corp., Model 250 Sonifier, Danbury, CT) at 100 W for 5 min. Cell debris were removed by centrifugation at 18,000 × g for 30 min. The supernatant fluid was regarded as a crude extract. Streptomycin sulfate was added at 2% into the crude extract to remove nucleic acids. After standing for 1 h with gentle stirring, the suspension was centrifuged at 18,000 × g for 20 min to separate the precipitate. The supernatant layer, which contained SAT activity, was fractionated with ammonium sulfate. The precipitate formed at 50% (NH₄)₂SO₄ saturation was recovered by centrifugation at 18,000 × g for 20 min, and was dissolved in 5 ml of buffer B. This solution was dialyzed against buffer B, and the dialyzer was put on a Sephacryl S-200HR column (2.2 × 90 cm). Elution was done with buffer B at a flow rate of 0.25 ml/min. Fractions showing SAT activity were collected and dialyzed against buffer B, containing 0.1 M NaCl. This enzyme solution was put on a DEAE Sephadex A-50 column (1.6 × 13 cm) and was eluted by a linear increase of NaCl concentration in buffer B from 0.1 to 0.5 M at a flow rate of 0.25 ml/min. Fractions displaying SAT activity were collected and dialyzed against buffer B, and then OAS was added at 50 mM to dissociate a trace amount of

5.2 mM l-serine in buffer A. The cleavage of the thioester bond of acetyl-CoA was followed at 232 nm. The differential extinction coefficient between acetyl-CoA and CoA was 3.2 mm⁻¹ cm⁻¹.

OASS activity was measured at 25°C by a modification of the method adopted by Droux et al. Fresh solution of OAS was prepared just before experiments by dissolving it in 2 mM potassium phosphate buffer, pH 6.0, to a final concentration of 200 mM to avoid the rapid O- to N-acetyl shift. Sodium sulfide was dissolved in 50 mM NaOH at 20 mM. A 0.03 ml portion of the 200 mM OAS was added to 0.27 ml of a reaction mixture, containing 0.11 mM PLP, 2.2 mM sodium sulfide, and an appropriate amount of enzyme in buffer B. The final pH of the reaction mixture was 7.5. After incubation for 5 min, the reaction was stopped by adding 0.15 ml of 20% (w/v) trichloroacetic acid, 0.3 ml of concentrated acetic acid, and 0.6 ml of ninhydrin reagent (1 g of ninhydrin dissolved in 40 ml of a mixture of concentrated acetic acid and concentrated HCl (60:40, v/v)). The reaction mixture was boiled for 10 min and cooled rapidly before the addition of 1.8 ml of cold ethanol. The concentration of l-cysteine was measured by the absorbance at 560 nm. One unit of OASS activity was defined as the amount of the enzyme producing 1 μmol of l-cysteine in one minute at 25°C at pH 7.5.

In Method 2, a 0.1-ml portion of the enzyme solution of an appropriate concentration was added to a reaction mixture (3.2 ml) composed of 0.103 mM acetyl-CoA and 1 mM EDTA (buffer A) or buffer A, containing 5 mM 2-mercaptoethanol (buffer B).

In Method 1, a 0.1-ml portion of the enzyme solution diluted appropriately with buffer A was added to a reaction mixture (3.2 ml), containing 0.103 mM acetyl-CoA, 5.2 mM l-serine, and 0.52 mM DTNB in buffer A. The initial rate of the increase in absorbance at 412 nm was measured and that obtained for a solution containing all the materials except l-serine (a blank) was subtracted. The reaction rate was calculated using an extinction coefficient for TNB of 13,600 M⁻¹ cm⁻¹. One unit of SAT activity was defined as the amount of the enzyme that can produce 1 μmol of CoA in one minute at 25°C at pH 7.5.

In Method 2, a 0.1-ml portion of the enzyme solution of an appropriate concentration was added to a reaction mixture (3.2 ml) composed of 0.103 mM acetyl-CoA and
OASS-A that had formed a complex with SAT. This solution was affinity chromatographed using a Blue Sepharose CL-6B column (1.6 × 15 cm) equilibrated with buffer B, containing 50 mM OAS adjusted to pH 7.0 with 5 mM NaOH. The wild-type SAT was eluted using buffer B, containing 200 µM L-cysteine at a flow rate of 0.18 ml/min. The SAT fractions eluted were collected, concentrated, and dialyzed against buffer B. The wild-type SAT thus prepared was found to be homogeneous by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The specific activity of the purified SAT measured by Method 1 was around 150 U/mg with a purification yield of 24%. Wigley et al. obtained 72 U/mg for the purified enzyme from E. coli using 0.1 mM acetyl-CoA and 1 mM L-serine as the substrates. The specific activity for the wild-type SAT purified in this study was higher than the value reported by Wigley et al., probably because we used a higher concentration of L-serine (5 mM).

**Purification of truncated SAT.** We purified the wild-type SAT at 4°C to homogeneity, using about 14 g of the wet cells of E. coli JM70 carrying pOHK100 without addition of EDTA and PMSF by the method described above. Then 5 ml of the purified enzyme solution (about 6 mg/ml) was incubated for about 110 h at 25°C. During the incubation, SAT underwent gradual degradation and sensitivity to L-cysteine inhibition became lower as compared to the wild-type SAT. After 110 h of incubation the enzyme solution was dialyzed against buffer B at 4°C. The major component of the truncated enzyme fractions was purified as follows: The dialyze was put on a Blue Sepharose CL-6B column (1.6 × 7.5 cm) equilibrated with buffer B. Elution was done first by increasing the L-cysteine concentration in buffer B linearly up to 200 mM and then with 1 mM acetyl-CoA in buffer B at a flow rate of 0.18 ml/min. SAT activity was eluted both with 200 µM L-cysteine and 1 mM acetyl-CoA. The SAT activity eluted with 1 mM acetyl-CoA was collected, concentrated, and dialyzed against buffer B.

**Purification of SATΔC20.** SATΔC20 was purified at 4°C, using about 10 g of the wet cells of E. coli JM70 carrying plasmid pOHK100ΔC20. The purification procedures were similar to those used for the wild-type SAT except for Blue Sepharose CL-6B affinity chromatography at the final step. Here, 1 mM acetyl-CoA dissolved in buffer B was used to elute the enzyme in place of 200 µM L-cysteine for the wild-type SAT and this chromatography was done repeatedly.

**Purification of Met-256-Ile mutant SAT.** Purification of the Met-256-Ile mutant SAT was done, using about 14 g of the wet cells of E. coli JM70 carrying plasmid pOHK100T. The purification procedures were similar to those used for SATΔC20 except that Blue Sepharose CL-6B affinity chromatography was done only once at the final step.

**Purification of OASS-A.** Purification of OASS-A was done at 4°C by a method similar to that adopted by Hara et al., using about 24 g of the wet cells of E. coli NK3 carrying plasmid pOHK100. OASS-A was purified from the crude extract by the purification procedures, including removal of nucleic acids with streptomycin sulfate, ammonium sulfate fractionation, Sephacryl S-200HR gel filtration chromatography, and DEAE Sephadex A-50 ion-exchange chromatography, followed by hydrophobic chromatographies using an octyl-Sepharose CL-6B column and phenyl-Sepharose CL-4B column. The operating conditions in the purification steps from cell disruption to DEAE Sephadex A-50 ion-exchange chromatography were the same as those for the purification of wild-type SAT. The OASS activities eluted from a DEAE Sephadex A-50 column were recovered and dialyzed against buffer B, containing 1.7 mM ammonium sulfate adjusted to pH 7.5 with 5 mM NaOH (buffer C). The dialyze was put on an octyl-Sepharose CL-4B column (1.6 × 16 cm) equilibrated with buffer C, and was eluted by linearly decreasing the ammonium sulfate concentration in buffer B to zero mM at a flow rate of 0.25 ml/min. The OASS activities were eluted at around 1.1 mM of ammonium sulfate. The active fractions were recovered, and dialyzed against buffer C. The dialyze was finally put on a phenyl-Sepharose CL-4B column (1.6 × 16 cm) equilibrated with buffer C. Elution was first done by linearly thus decreasing the ammonium sulfate concentration as in the octyl-Sepharose CL-4B chromatography, and then by isocratic elution with buffer B at a flow rate of 0.25 ml/min. The active fractions eluted with buffer B were collected and dialyzed against buffer B, containing 0.1 mM PLP. OASS-A thus purified was found to be homogeneous by SDS-PAGE and showed a specific activity of 750 U/mg. The purification yield was 47%. The ratio of the absorbance at 280 nm to that at 412 nm of the dialyzed enzyme solution was 4.1, which was similar to that reported for OASS-A from E. coli and S. typhimurium, indicating that the purified OASS-A was of the holo enzyme type. The purified OASS-A was confirmed to be a dimer on the basis of the molecular mass (64 kDa) by gel chromatographic analysis and subunit molecular mass (34,358 Da) as reported previously for E. coli OASS-A.

**Analysis of N- and C-terminal amino acid sequences.** For analysis of N-terminal amino acid sequences, about 1 µg of the purified wild-type SAT and truncated SAT were developed by SDS-PAGE on a 5–20% gradient gel (Bio-Rad Laboratories, Mini-Protein II Ready Gels, Hercules, CA, USA) and then blotted electroetically onto a PVDF membrane (Atto Corp., Clear Blot Membrane, Tokyo) using a blotting apparatus (Atto Corp., AE-6755) with blotting buffer (0.1 M Tris, 0.192 M glycine, and 5% methanol) at 2 mA per cm² of a membrane for 90 min. The bands stained with Coomassie Brilliant Blue R-250 (CBB R-250, Sigma Chemical Co.) on the membrane were put into a protein sequencer (Applied Biosystems, Model 491, Foster, CA).

The amino acid sequence of the C-terminal peptide fragment of the truncated SAT was analyzed as follows, using the wild-type SAT as a control. About 0.8 mg of
the purified wild-type SAT and truncated SAT were first treated with 0.5% iodoacetic acid to carboxymethylate their cysteine residues by the procedure of Hir's. The S-carboxymethylated enzymes were digested using lysyl endopeptidase at a final concentration of 1.5 μM at 37°C for 12 h in 0.2 ml of 0.02 M Tris-HCl buffer, pH 9.2, containing 10% (v/v) acetonitrile. Then, a 0.1-mM portion of the solution was put on a YM-10 ODS-A column (6 × 150 mm) (YMC Co., Kyoto) connected to a HPLC system (Waters, 600E, Milford, MT, USA). Elution was done by a linear increase in the acetonitrile concentration from 1 to 54% (v/v) in 0.1% HCl solution at a flow rate of 0.8 ml/min monitored at 210 nm with an UV detector (Waters, 484). The C-terminal peptides of the truncated SAT and wild-type SAT were eluted at the acetonitrile concentrations of 23% and 28%, respectively. Each peptide was collected and freeze-dried using a centrifugal concentrator (Tomiy, CC-105, Tokyo) connected to a freeze drier (Tokyo Rikakikai Co., Eyela FD-5N, Tokyo). The dried preparations were dissolved in 0.11 ml of 0.02 M HEPES-NaOH buffer, pH 7.0, containing 0.02 mM CaCl2 and again digested using TPCK-trypsin at a final concentration of 0.18 μM at 37°C for 3 h. Finally, about 0.09 ml of each peptide solution was put on HPLC and eluted as described above to prepare the C-terminal peptide fragment: The C-terminal fragments of the truncated SAT and wild-type SAT appeared at 9% and 25% acetonitrile concentrations, respectively. The total amino acid sequence of the C-terminal peptide fragment of the truncated SAT was analyzed by the protein sequencer.

Inhibition of SAT by l-cysteine. The activity for the wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 was measured by Method 2 in a reaction mixture composed of 0.15 mM acetyl-CoA, 5 mM l-serine, 0-200 μM l-cysteine, and an appropriate amount of enzyme.

Molecular mass measurement by gel chromatography. The molecular masses of the purified wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 were estimated by gel chromatography on a Superose 6HR column (1 × 30 cm) and Superose 12HR column (1 × 30 cm) connected in series equilibrated in buffer B, containing 0.15 M KCl at room temperature. As marker proteins, human IgG (Jackson Immuno Research Laboratories, Inc., West Grove, PA, USA), and BSA (monomer, dimer, and trimer) and ovalbumin (Pharmacia Biotech., Gel Filtration Calibration Kit) were used. A 0.1-mM portion of the SAT and marker protein solution (0.3 mg/ml for both the solutions) was injected onto the column and eluted at a flow rate of 0.2 ml/min with the same buffer used for equilibration. The purified OASS-A was also eluted using buffer B, containing 0.15 M KCl and 0.1 mM PLP. The molecular mass was predicted from a plot of log molecular mass against the elution volume.

pH dependence of SAT activity. Fifty mM potassium phosphate buffer and 50 mM sodium carbonate buffer, containing 1 mM EDTA were used for the pH range of the reaction mixture from 5.3 to 8.5 and for that from 8.3 to 11.1, respectively. The activity for the wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 was measured by Method 2.

Thermal stability of SAT. The thermal stability of the wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 was studied at 4, 20, 30, 40, 50, and 60°C. A 0.4-mM portion of each enzyme solution (27 μg/ml) was incubated for 12 h at prescribed temperatures. In the experiments at 20-50°C, the enzyme was first incubated for 1 h to reactivate it, since it was reversibly inactivated during storage at 4°C. After incubation 50 μl of the enzyme solution was diluted with 950 μl of buffer A and the relative remaining activity was measured by Method 1. The activity just after the first incubation was set to 100%.

Effects of reagents on the stability of SAT. The purified wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 were dialyzed against 50 mM Tris-HCl buffer, pH 7.5, to protect against aggregation of potassium phosphate buffer with metal ion. A 0.1-mM portion of the enzyme solution (20 μg/ml) was added to 0.1 ml of 2 mM reagent solution (EDTA, (NH4)2MoO4, ZnCl2, MgSO4, FeSO4, CuSO4, AgNO3, PCMB, iodoacetamide, and dithiothreitol) or 0.1 ml of 10 mM 2-mercaptoethanol, dissolved in 50 mM Tris-HCl buffer, pH 7.5, and incubated at 25°C for 10 min. As a control, 0.1 ml of 50 mM Tris-HCl buffer, pH 7.5 was used instead of the reagent solution. The remaining activity was assayed either by Method 2 in the case of dithiothreitol and 2-mercaptopethanol or by Method 1 for the other reagents.

Gel chromatography of a mixture of SAT and OASS-A. A 0.1-mM portion of a mixture, containing SAT (0.24 mg/ml) and OASS-A (0.57 mg/ml) with a molar ratio of around 4.0 (OASS-A/SAT) was put onto a Superose 6HR column (1 × 30 cm) and Superose 12HR column (1 × 30 cm) connected in series and was eluted with buffer B, containing 0.15 M KCl and 0.1 mM PLP at a flow rate of 0.2 ml/min at room temperature. The eluate was monitored at 280 nm and fractions of 0.2 ml were collected using a fraction collector to assay for SAT activity by Method 1 and OASS-A activity. A 0.1-mM portion of the wild-type SAT, Met-256-Ile mutant SAT, SATΔC20, and OASS-A was also eluted separately at the same flow rate for comparison. Some peak fractions were collected, dialyzed in distilled water, and then the dialyzates were lyophilized. The proteins were developed on an SDS-polyacrylamide gel at 35 mA for 2 h. The protein bands were stained with CBB R-250. For densitometric analysis, the stained gels were digitized and scanned using a densitometer (Molecular Dynamics, Inc., Model 300A-T, Sunnyvale, CA) with a computer system loaded with Image Quant software ver.3.0 (Molecular Dynamics Inc.). The stoichiometry of subunits of SAT and OASS-A was calculated from the ratio of density for the two enzymes, using authentic enzyme samples of which the weight was known.
OASS activity in the presence of SAT. OASS activity in the presence of the wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 was measured by the method described previously. The concentration of SAT was varied from 0.029 μg/ml to 3.2 μg/ml at 0.19 μg/ml of OASS-A in the reaction mixture.

Protein measurement and SDS-PAGE. The concentration of protein was measured either by the method of Lowry,30 using BSA (Sigma Chemical Co.) as a standard protein or by using an amino acid analyzer (Hitachi, L-8500A, Tokyo). The molar ratio of OASS-A to SAT was calculated on the basis of the amino acid analysis.

SDS-PAGE for examining the purity was done using a 12% gel by the method of Laemmli.31 The protein bands were stained with CBB R-250. As marker proteins for SDS-PAGE, phosphorylase b, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin (Pharmacia Biotech., LMW Electrophoresis Calibration Kit) were used.

Results

Isolation and purification of truncated SAT

Wild-type SAT was purified even without the addition of EDTA and PMSF, the protease inhibitors, indicating that the SAT was sufficiently stable during purification at 4°C. However, we found that the purified enzyme underwent gradual degradation when it was incubated at 25°C. The solution containing degraded enzymes showed much less sensitivity to l-cysteine inhibition when compared to the wild-type SAT. Sensitivity became lowest at 110 h of incubation (data not shown).

We purified a major fraction of the degraded enzymes prepared after 110 h of incubation by the method described in “Materials and Methods”. The enzyme fraction eluted with 1 mM acetyl-CoA was nearly homogeneous on an SDS-polyacrylamide gel and the specific activity was around 160 U/mg. The sensitivity of the purified truncated SAT to inhibition by l-cysteine was much less than that of the wild-type SAT as shown in Fig. 2.

N- and C-Terminal amino acid sequences of truncated SAT

In Table 1, the N- and C-terminal amino acid sequences of the truncated SAT thus purified are shown. The N-terminal amino acid sequence was identical with that of the wild-type SAT. In both the wild-type SAT and truncated SAT, the first residue of Met was missing, probably due to the action of methionine aminopeptidase in E. coli.32 On the other hand, a C-terminal amino acid sequence from Met 254 to Ile 273 (C-terminus) of the wild-type SAT was deleted in the truncated SAT. These results indicate that the truncated SAT was a resultant form of the wild-type SAT cleaved at a position between Ser 253 and Met 254, deleting 20 amino acid residues from the C-terminus.

Purification of SATΔC20

Since it was difficult to purify a sufficient quantity of the truncated SAT deleting 20 amino acids from a C-terminus (designated as SATΔC20) by degradation of the purified wild-type SAT, we constructed a plasmid, pOHC100ΔC20, containing both the genes for SATΔC20 and OASS-A to express the enzyme. Construction of a plasmid containing a gene only for SATΔC20 was not successful although the reason is not known. SATΔC20 was purified to be homogeneous on an SDS-polyacrylamide gel with a specific activity of about 176 U/mg and a yield of 12%. In Table 2, a summary of the purification steps of SATΔC20 is shown.

Figure 1. Inhibitory Effects of L-Cysteine on SAT Activity at 25°C. SAT activity was measured by varying l-cysteine concentrations at 5 mM l-serine and 0.15 mM acetyl-CoA. The activity in the absence of l-cysteine was set to 100%. Symbols: ○, wild-type SAT; △, Met-256-Ile mutant SAT; ◇, SATΔC20; ■, truncated SAT.

Table 1. Composition of N- and C-Terminal Amino Acid Sequences of the Wild-type SAT and Truncated SAT

<table>
<thead>
<tr>
<th>N-Terminal</th>
<th>C-Terminal</th>
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<tr>
<td>Deduced from the cDNA sequencea</td>
<td>241VGGKPSDKPSMDMDQHQHNGINHTFEYGDGI253</td>
</tr>
<tr>
<td>Wild-type SAT</td>
<td>5S EEEILVWN11</td>
</tr>
<tr>
<td>Truncated SAT</td>
<td>241VGGKPSDKPS255</td>
</tr>
<tr>
<td></td>
<td>nd</td>
</tr>
</tbody>
</table>

a Cited from ref. 8.
b Not determined.
Table 2. Purification Summary of SATΔC20

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total activity (units)</th>
<th>Total protein (mg)</th>
<th>Specific activity (U/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>3490</td>
<td>300</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Streptomycin sulfate</td>
<td>3270</td>
<td>295</td>
<td>11</td>
<td>94</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>1860</td>
<td>162</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>Sephacryl S-200HR</td>
<td>1570</td>
<td>146</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>DEAE Sephadex A-50</td>
<td>1090</td>
<td>19.5</td>
<td>56</td>
<td>31</td>
</tr>
<tr>
<td>1st Blue Sepharose CL-6B</td>
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<td>5.2</td>
<td>112</td>
<td>17</td>
</tr>
<tr>
<td>2nd Blue Sepharose CL-6B</td>
<td>404</td>
<td>2.3</td>
<td>176</td>
<td>12</td>
</tr>
</tbody>
</table>

* About 10 g of the wet cells of E. coli JM70 transformed with plasmid pOHC100 JC20 were used.

Table 3. Purification Summary of Met-256-Ile Mutant SAT

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total activity (units)</th>
<th>Total protein (mg)</th>
<th>Specific activity (U/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>10500</td>
<td>612</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Streptomycin sulfate</td>
<td>12000</td>
<td>610</td>
<td>20</td>
<td>114</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>13700</td>
<td>544</td>
<td>25</td>
<td>130</td>
</tr>
<tr>
<td>Sephacryl S-200HR</td>
<td>12000</td>
<td>267</td>
<td>45</td>
<td>114</td>
</tr>
<tr>
<td>DEAE Sephadex A-50</td>
<td>6000</td>
<td>75</td>
<td>80</td>
<td>57</td>
</tr>
<tr>
<td>Blue Sepharose CL-6B</td>
<td>1800</td>
<td>9</td>
<td>200</td>
<td>17</td>
</tr>
</tbody>
</table>

* About 14 g of the wet cells of E. coli JM70 transformed with plasmid pOHE100T were used.

Purification of Met-256-Ile mutant SAT

Met-256-Ile mutant SAT was purified to be homogeneous on the SDS-polyacrylamide gel with a yield of 17% as shown in Table 3. The purified Met-256-Ile mutant SAT showed a specific activity of around 200 U/mg, which was similar to that for the wild-type SAT (150 U/mg) and SATΔC20 (176 U/mg).

Properties of SATΔC20 and Met-256-Ile mutant SAT

Some properties of SATΔC20 and Met-256-Ile mutant SAT were investigated in comparison with those of the wild-type SAT. Figure 2 shows the inhibitory effects of L-cysteine on the activity of SATΔC20 and Met-256-Ile mutant SAT in comparison with that of wild-type SAT. SATΔC20 was much less sensitive to inhibition by L-cysteine than the wild-type SAT and showed a tendency similar to the Met-256-Ile mutant SAT. The result for SATΔC20 was coincident with that for the truncated SAT, as expected.

The molecular mass of SATΔC20 was around 120 kDa (Fig. 3) from gel chromatographic analysis. Judging from the subunit molecular mass calculated from the DNA sequence (26,862 Da), SATΔC20 was considered to be a homotetramer or homopentamer. The molecular mass of the Met-256-Ile mutant SAT was estimated to be 140 kDa from Fig. 3. From its subunit molecular mass (29,167 Da) the Met-256-Ile mutant SAT may also be considered to be a homotetramer or homopentamer. However, Wigley et al. showed that the wild-type SAT from E. coli exists as a homotetramer on the basis of a preliminary crystallographic analysis, although it had the same molecular mass from our gel chromatographic analysis (Fig. 3) as the Met-256-Ile mutant SAT. Thus, according to the same argument, SATΔC20 and the Met-256-Ile mutant SAT are probably composed of four identical subunits although further study is required. Deletion of 20 amino acid residues from the C-terminus of the wild-type SAT did not change the subunit stoichiometry of the enzyme.

The optimum pHs for the reaction of both SATΔC20 and Met-256-Ile mutant SAT were in the range of 7.5 and 8.5 in a way similar to that for the wild-type SAT as shown in Fig. 4, indicating no appreciable change in the state of dissociable groups at the catalytic site.

As shown in Fig. 5, thermal stability was also similar in the SATΔC20, wild-type SAT, and Met-256-Ile mutant SAT. All the SATs were quite stable from 20 to 40°C. There were no remaining activities in all enzymes at 60°C. However, the enzymes were slightly labile at 4°C.

In Table 4, the effects of some reagents on SAT activity are shown. There were no appreciable differences in the remaining activity among the three SATs except in the case of silver nitrate. It should be noted that SAT activity was completely lost in the presence of 1 mM PCMB while iodoacetamide had little effect.

Complex formation between SAT and OASS-A

We investigated the complex formation between the wild-type SAT, Met-256-Ile mutant SAT or SATΔC20 and OASS-A by gel chromatography. An experiment using the wild-type SAT was done as a control, since it is known to form a complex with OASS-A. Figure 6 illustrates the elution profiles of a mixture containing...
**Table 4.** Effect of Reagents on the Stability of SATs

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Concen. (mM)</th>
<th>Wild-type SAT</th>
<th>Met-256-Ile Mutant SAT</th>
<th>SAT.JC20</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂MoO₄</td>
<td>1</td>
<td>104</td>
<td>100</td>
<td>107</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>1</td>
<td>27</td>
<td>29</td>
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<tr>
<td>MgSO₄</td>
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<td>104</td>
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<td>FeSO₄</td>
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<td>89</td>
<td>80</td>
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<td>29</td>
<td>20</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>1</td>
<td>98</td>
<td>100</td>
<td>61</td>
</tr>
<tr>
<td>PCMB</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>1</td>
<td>103</td>
<td>105</td>
<td>104</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>5</td>
<td>89</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>1</td>
<td>89</td>
<td>106</td>
<td>103</td>
</tr>
</tbody>
</table>

*Concentration at first incubation.*

Fig. 4. pH Dependency of SAT Activity at 25°C.
Fifty mM potassium phosphate buffer (a) and 50 mM sodium carbonate buffer (b) were used in the pH range from 5.3 to 8.5 and that from 8.3 to 11.1, respectively. Symbols: ○, wild-type SAT; △, Met-256-Ile mutant SAT; ■, SAT.JC20. Open symbols show the results in (a) and closed symbols in (b).

Fig. 5. Relative Remaining Activity of SAT after 12 h of Incubation.
Symbols: ○, wild-type SAT; △, Met-256-Ile mutant SAT; ■, SAT.JC20.

either one of the three SATs and OASS-A, expressed by SAT and OASS activities. The elution profiles detected at 280 nm showed tendencies similar to those of the activity (data not shown). When the mixture containing the wild-type SAT or Met-256-Ile mutant SAT and OASS-A was put on the column, a peak showing both activities appeared at the elution time of around 114 min (peaks 1 and 2 in Fig. 6). The wild-type SAT and Met-256-Ile mutant SAT were both eluted at 128 min in the absence of OASS-A. The major parts of peaks 1 and 2 were collected and developed on an SDS-polyacrylamide gel. As shown in Fig. 7, both the subunit bands of the SAT and OASS-A were detected in the fractions of peak 1 (lane 6) and peak 2 (lane 7). A densitometric analysis of lanes 6 and 7 (Fig. 7) revealed a nearly 1:1 stoichiometry for the mass ratio of the subunit of SAT to OASS-A. Thus, the complex of the wild-type SAT or Met-256-Ile mutant SAT with OASS-A must be formed from one molecule of each SAT and two molecules of OASS-A, since SAT and OASS-A are composed of four and two identical subunits, respectively. However, in the case of a mixture containing SAT.JC20 and OASS-A, no peak showing both activities was seen (Fig. 6(c)). Peaks 3 and 4 in Fig. 6 show only the SAT.JC20 and OASS activities, respectively. The median retention time of peak 3 was 131 min and was in agreement with that of SAT.JC20 in the absence of OASS-A. The SDS-PAGE also supported the idea that peaks 3 and 4 contained only SAT.JC20 and OASS-A (lanes 8 and 9 in Fig. 7), respectively. Thus, by removing the 20 amino acid residues from the C-terminus, SAT lost the ability to form a complex with OASS-A.

**OASS activity in the presence of SAT**

Figure 8 shows OASS activity in the presence of the wild-type SAT, Met-256-Ile mutant SAT, and SAT.JC20, varying the molar ratio of SAT to OASS-A. The OASS activity in the presence of the wild-type SAT or Met-256-Ile mutant SAT decreased rapidly and then approached a constant when the molar ratio of the enzyme increased. At the molar ratio of SAT to OASS-A of 5 or higher, the relative activity was only 5%. However, the relative activity was around 30% at the molar ratio of 0.5 which corresponds to the composition of SAT and OASS-A of the enzyme complex eluted on the gel chromatography as shown previously. On the other hand, OASS activity was not reduced in the
Fig. 6. Gel Chromatographic Profiles for the Mixtures of SAT and OASS-A.

A 0.1-mL portion of a mixture of each purified SAT (0.24 mg/mL) and OASS-A (0.57 mg/mL) was put onto a column (see “Materials and Methods”) and was eluted at a flow rate of 0.2 mL/min with buffer B, containing 0.15 M KCl and 0.1 mM PLP. (a), Wild-type SAT and OASS-A; (b), Met-256-Ile mutant SAT and OASS-A; (c), SAT.C20 and OASS-A. Symbols: ○, SAT activity; △, OASS activity.

Fig. 7. SDS-PAGE of the Protein Fractions Eluted on Gel Chromatography.

Peaks 1-4 in Fig. 6 were developed by SDS-PAGE. As marker proteins, phosphorylase b (94 kDa), BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa) were used. Lane 1, marker proteins (4.3 µg of each protein); lane 2, wild-type SAT (1 µg); lane 3, Met-256-Ile mutant SAT (1 µg); lane 4, SAT.C20 (1 µg); lane 5, OASS-A (1 µg); lane 6, peak 1 (1.8 µg); lane 7, peak 2 (3 µg); lane 8, peak 3 (0.5 µg); lane 9, peak 4 (1.5 µg).

Fig. 8. Effect of the Molar Ratio of SAT/OASS-A on OASS Activity.

OASS activity was measured by varying the molar ratio of SAT/OASS-A at a constant OASS-A concentration of 2.6 nm. The OASS activity in the absence of SAT measured under the same conditions was set to 100%. Symbols: ○, wild-type SAT; △, Met-256-Ile mutant SAT; ○, SAT.C20.
presence of SATΔC20.

Discussion

There are many enzymes in cells which form a multi-enzyme complex and are concerned particularly with sequential reactions in metabolic pathways. In many cases, by forming a multi-enzyme complex, the reaction proceeds efficiently through minimization of competition with other pathways by keeping an intermediate in a limited microenvironment, increasing the local concentration of the intermediate product to the active site of the next enzyme in the sequence, and so on.\(^{18,23}\)

Cysteine synthetase is present as an enzyme complex composed of SAT and OASS-A, which catalyzes L-cysteine synthesis from L-serine with OAS as an intermediate compound. Many attempts have been made to discover such a complex, not only in bacteria\(^{5,6,8}\) but also in higher plants, with its localization.\(^{13-17}\) However, in the L-cysteine synthesis a role for the complex formation is not known and little information on the structural region of interaction is available. Only some qualitative findings are reported on the properties of the complex of the enzymes from *E. coli* and *S. typhimurium*. Cook et al. supposed that cysteine synthetase does not channel the intermediate product, OAS between the active sites of the two enzymes. The cysteine synthetase first releases OAS in solution, then it reassociates with the active site on OASS-A and is finally converted to L-cysteine.\(^{18}\)

Thus, in the L-cysteine synthesis, a role of the complex formation may not be an increase in the reaction efficiency. Kredich et al. have shown that OASS activity is repressed by forming a complex with SAT.\(^{6}\)

In this study, we purified three SATs, i.e., the wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 from *E. coli*, and compared their characteristics particularly with respect to the complex formation with OASS-A and the sensitivity to L-cysteine inhibition. Met-256-Ile mutant SAT was first isolated by Denk and Böck but it has not yet been purified. SATΔC20, deleting 20 amino acid residues from the C-terminus, was isolated while incubating the purified wild-type SAT at 25°C. This deletion may be caused by the action of endogenous proteases arising from the cells since the enzyme did not suffer from truncation during incubation at 4°C in the presence of protease inhibitors. Wild-type SAT was also truncated at the C-terminal region by treatment with thermolysin, a neutral metal protease, although in a slightly different manner. Thus, the C-terminal region of the wild-type SAT probably takes a loose structure to undergo proteolysis. We showed that the Met-256-Ile mutant SAT kept the ability to form a complex in the same manner as the wild-type SAT on the basis of a chromatographic analysis although its sensitivity to inhibition by L-cysteine is greatly reduced. On the other hand, SATΔC20 did not form a complex with OASS-A and showed sensitivity similar to that for the Met-256-Ile mutant SAT. Furthermore, the sensitivity to L-cysteine inhibition for the wild-type SAT and Met-256-Ile mutant SAT did not change in the presence of OASS-A (data not shown). Thus, there seems to be no relationship between the complex formation and sensitivity to inhibition by L-cysteine.

The activity of OASS-A was repressed in the same manner when the reaction was done in the presence of the Met-256-Ile mutant SAT as well as with wild-type SAT. This decrease is probably due to a complex formation, since gel chromatographic analyses showed that the wild-type SAT and Met-256-Ile mutant SAT form a complex with OASS-A in the presence of the substrate composed of 20 mM OAS and 2 mM sulfide (data not shown) as well as in the absence of the substrate (Fig. 6). OASS activity rapidly decreased when the molar ratio of SAT to OASS-A was increased and approached to around 5% at the ratios of around 5 or higher (Fig. 8). However, at a ratio of SAT to OASS-A of 0.5, which corresponds to an enzyme composition of the cysteine synthetase presumed from the gel chromatographic analysis (OASS-A/SAT = 2), the relative activity decreased to only 30%. These findings indicate that complex formation was not complete in the reaction medium when the molar ratio of SAT to OASS-A was 0.5, probably because of an extremely low concentration level of enzyme (on the order of nanomolar). It was estimated that around 75% of OASS-A forms the complex with SAT in this reaction medium. On the other hand, OASS activity was not reduced in the presence of SATΔC20, indicating no interaction between the two enzymes in coincidence with the result of gel chromatographic analysis. The activity of the wild-type SAT and Met-256-Ile mutant SAT was not influenced by the complex formation (data not shown). Thus, the complex formation may be concerned with control of the OASS activity although this should be studied further.

Some other properties such as pH optimum for reaction, thermal stability, and stability to reagents for the wild-type SAT, Met-256-Ile mutant SAT, and SATΔC20 were similar except for the sensitivity to silver nitrate. Thus, kinetic studies using SATΔC20 in the presence of OASS-A under various conditions in comparison with those using the wild-type SAT and Met-256-Ile mutant SAT would not only clarify the behavior of L-cysteine synthesis but also provide information on the role of a complex formation that has not yet been identified.

Recently, Bogdanova and Hell showed that C-terminal regions of SAT from bacteria and higher plants are well conserved.\(^{40}\) They briefly predicted that a region on cystolic SAT-B in *A. thaliana* responsible for interaction with cystolic OASS is localized at the C-terminal part, starting between amino acids 130 and 213 and stretching to the C-terminus, by using the yeast two-hybrid system.\(^{40}\) However, they did not identify the detailed region of interaction between SAT and OASS. In the case of SAT from bacteria, no experimental results on the region interacting with OASS are available. In this study, we showed that a region on the SAT protein from *E. coli* responsible for binding with OASS-A is a C-terminal peptide having 20 amino acid residues or fewer. Although further studies are necessary, the findings obtained in this study provide an important key to specify the binding sites.

Secondary structures of the C-terminal region of the
wild-type SAT, Met-256-Ile mutant SAT, and SAT·IC20 were analyzed by the method of Chou and Fasman. The analysis predicted that in the wild-type SAT from E. coli the region from Met 254 to Gly 262 forms an α-helix and the region from Ile 263 to Tyr 269 a β-sheet. In the Met-256-Ile mutant SAT, the α-helix region of the wild-type SAT was changed into a β-sheet. In the case of SAT·IC20, both regions were deleted without yielding any change in the secondary structure of the other peptide regions. These findings indicate that a change in the secondary structure of the C-terminal region from Met 254 to Gly 262 may play a role in reduction of the l-cysteine inhibition but has no effect on the complex formation.

The findings obtained in this study might be helpful in clarification of the behaviors of l-cysteine synthesis particularly with respect to a role of a complex formation and inhibitory effect by l-cysteine.

Acknowledgments

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References

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