Increase in the Stability of Serine Acetyltransferase from Escherichia coli against Cold Inactivation and Proteolysis by Forming a Bienzyme Complex

Koshiki Mino, Koreyoshi İmamura, 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

Received October 2, 2000; Accepted December 18, 2000

Cysteine synthetase from Escherichia coli is a bi-enzyme complex composed of serine acetyltransferase (SAT) and O-acetylserine sulfhydrylase-A (OASS). The effects of the complex formation on the stability of SAT against cold inactivation and proteolysis were investigated. SAT was reversibly inactivated on cooling to 0°C. Ultracentrifugal analysis showed that SAT (a hexamer) was dissociated mostly into two trimers on cooling to 0°C in the absence of OASS, while in the presence of OASS one trimer of the SAT subunits formed a complex with one dimer of OASS subunits. In the presence of OASS, not only the cold inactivation rate was reduced but also the reactivation rate was increased. Furthermore, SAT became stable against proteolytic attack by α-chymotrypsin and V8 protease by forming the complex with OASS. On the other hand, SAT was degraded by trypsin in the same manner both in the presence and in the absence of OASS. The different tendency in the stability against proteolysis with the different proteases was discussed with respect to the substrate specificity of the proteases and amino acid sequence of the C-terminal region of SAT that interacts with OASS.

Key words: serine acetyltransferase; cysteine synthetase; enzyme complex; cold inactivation; proteolysis

Cysteine synthetase composed of serine acetyltransferase (SAT) (EC 2.3.1.30) and O-acetylserine sulfhydrylase (OASS) (EC 2.2.1.130) participates in the final step of sulfate assimilation in microorganisms and plants. SAT catalyzes the formation of O-acetyl-L-serine (OAS) and CoA from L-serine and acetyl-CoA, while OASS (OAS is predominantly expressed under aerobic conditions) is involved in the L-cysteine synthesis from OAS and sulfide with PLP as a cofactor. SAT and OASS from E. coli and S. typhimurium were purified and their characteristics have been investigated. Hindson et al. have recently shown, using equilibrium sedimentation and quasi-elastic light scattering methods, that SAT is a hexamer composed of two trimers with the subunit molecular mass of 29,316 Da. On the other hand, OASS from the same strain is a dimer with the subunit molecular mass of 34,358 Da.

SATs from higher plants as well as from bacteria are particularly characterized by their formation with OASS to form a bi-enzyme complex called cysteine synthetase. The cysteine synthetase is composed of one molecule of SAT and two molecules of OASS. In a previous study, we investigated the effects of complex formation on the kinetics and some properties of the wild-type SAT and OASS from E. coli and showed preliminary that the wild-type SAT seemed to be stabilized against cold inactivation by forming a complex with OASS. We also showed that SAT tends to suffer from proteolytic attack, particularly at the C-terminal region. Furthermore, the C-terminal peptide having 20 amino acid residues or fewer is responsible for interaction with OASS. In this study, we investigated in detail the effects of the complex formation on the cold inactivation and proteolytic degradation of the wild-type SAT (designated as SAT) and discussed on the importance of the C-terminal region that interacts with OASS.

1 To whom correspondence should be addressed: Kazuhiro Nakanishi, Fax: +81-86-251-8264; E-mail: kazuhiro@biotech.okayama-u.ac.jp

Abbreviations: SAT, serine acetyltransferase; OASS, O-acetylserine sulfhydrylase-A; OAS, O-acetyl-L-serine; DTNB, 5,5'-dithio-bis(2-nitrobenzoic acid); PLP, pyridoxal 5-phosphate; TPCK, N-tosyl-L-phenylalanlanyl chloromethyl ketone; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluorid
Materials and Methods

Materials. Acetyl-CoA trilithium salt, CoA trilithium salt, L-serine, L-cysteine hydrochloride monohydrate, sodium sulfide nonahydrate, and DTNB were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). OAS hydrochloride and PLP were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Superose 6HR and Superose 12HR were products of Pharmacia Biotech. (Uppsala, Sweden). α-Chymotrypsin and TPCK-treated trypsin were products of Sigma Chemical Co. V8 Protease was obtained from Wako Pure Chemical Industries. TPCK and a soybean trypsin inhibitor were purchased from Nacalai Tesque Co. (Kyoto) and Wako Pure Chemical Industries, respectively. All other reagents were of reagent grade.

Preparation of enzymes. SAT was prepared from E. coli JM70 carrying a plasmid, pOH100, and OASS was from E. coli NK3 harboring pOHK100.19) SAT and OASS were purified to be homogeneous on an SDS-PAGE using the same procedures as those reported previously.19) Purified SAT was dissolved in 50 mM potassium phosphate buffer, pH 7.5, and 1 mM EDTA (abbreviated as the assay buffer), containing 5 mM 2-mercaptoethanol and stored at 4.7°C until use. OASS was dissolved in the assay buffer, containing 5 mM 2-mercaptoethanol and 0.1 mM PLP and then stored at −80°C. The molar enzyme concentration was evaluated from the protein concentration19) measured by the Lowry’s method.20) In the following experiments, PLP was added usually at 0.1 mM when the reaction mixture contained OASS.

Enzyme assay for SAT. SAT activity was assayed at 25°C at pH 7.5 by measuring continuously the increase in absorbance at 412 nm in a 1-cm path length cuvette using 5 mM L-serine, 0.1 mM acetyl-CoA, and 0.5 mM DTNB dissolved in the assay buffer as the substrate.19) One unit of SAT activity was defined as the amount of enzyme producing 1 μmol of CoA in 1 min at 25°C at pH 7.5.

Enzyme assay for OASS. OASS activity was assayed at 25°C at pH 7.5 by measuring the amount of L-cysteine formed, using 20 mM OAS and 2 mM sodium sulfide dissolved in the assay buffer as the substrate. L-Cysteine was assayed by the method of Gaitonde.20) One unit of OASS activity was defined as the amount of enzyme producing 1 μmol of L-cysteine in 1 min at 25°C at pH 7.5.

Cold inactivation and reactivation experiments. SAT was dissolved in the assay buffer usually either at 0.013 μM or 0.2 μM and incubated at 0°C for 30 min. Then, the enzyme solution was incubated in a range of 10 to 50°C (mostly at 25°C) for 60 min to reactivate and then incubated again at 0°C. At different times during the reactivation and second incubation at 0°C, a 0.1-ml portion of the enzyme solution was withdrawn. For the enzyme concentration of 0.013 μM, the solution was mixed with 3.2 ml of the substrate solution incubated at 25°C to assay for SAT activity by the method described above. The activity was calculated from the slope of the linear part of the course of reaction following a short lag-phase period. For the 0.2 μM enzyme concentration, the sample solution was diluted 15 times with the assay buffer incubated at 25°C and then immediately the activity was measured in a way similar to that for 0.013 μM. In some cases, the effect of the addition of OASS was examined. Namely, the SAT solution was first incubated at 0°C for 30 min and then OASS was added at molar ratios (OASS/SAT) of 7.5, 15, and 30.

Effects of temperature on SAT activity. SAT was dissolved in the assay buffer at 0.2 μM in the absence and in the presence of OASS (1.5 μM) and then incubated at 40°C for 20 min to reactivate the enzyme. At 40°C, SAT activity was assayed without previous incubation by the method described above. In other cases, the enzyme solution was first incubated at 0–25°C for 60 min and assayed at the same temperature as that for the incubation.

Gel chromatography. SAT was dissolved in the assay buffer at 1.8 μM in the absence and in the presence of OASS (13.5 μM) and reactivated for 20 min at 40°C, followed by incubation either at 0°C or 25°C for 3 hr. OASS (2.7 μM) was also used as a control. A 0.1-ml portion of the enzyme solution that had been incubated at 0°C or 25°C was injected onto a Superose 6HR column (1 × 30 cm) and Superose 12HR column (1 × 30 cm) connected in series and eluted at a flow rate of 0.2 ml/min with the assay buffer at 0°C or 25°C. The eluate was monitored at 280 nm. Fractions collected were incubated at 40°C for around 10 min for reactivation and assayed for either SAT or OASS activity or both activities. A 0.1-ml portion of 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) was also eluted under the same conditions as those used for the enzyme solution.

Sedimentation equilibrium. A Beckman Optima XL-A (Beckman Coulter, Inc., Fullerton, CA, USA) analytical ultracentrifuge was used to obtain molecular masses for SAT and cysteine synthetase at 0°C. The cysteine synthetase was prepared as follows: At first SAT and OASS dissolved in the assay buffer containing 5 mM 2-mercaptoethanol and 0.1 mM PLP, were mixed to the final concentrations of 10 μM and
50 μM, respectively. Then, the mixture was injected onto the same Superose columns as described above and eluted at a flow rate of 0.2 ml/min with the buffer. The cysteine synthetase fraction eluted at around 115 min was collected and concentrated to 2.1 μM. Proteins were dialyzed against the assay buffer containing 5 mM 2-mercaptoethanol and 0.05 mM PLP. Analyses were done using an Amicon Ti rotor with 12-mm Epon-Chaclor filled double sector cells, containing 0.17 ml of the buffer, 0.066 ml of silicone oil (Dow Corning Toray Silicone Co., Tokyo), and 0.1 ml of SAT (6.9 μM) or the cysteine synthetase (1.05 μM and 2.1 μM).

The first sedimentation was done at a rotor speed of 5,000 rpm at 0°C. The redistribution of solute was monitored using its absorbance at 280 nm. Initial scans were taken at 42 h where the sedimentation equilibrium was confirmed to be reached and then the rotor speed was changed to 13,000 rpm for the second analysis after further 24 h. The same enzyme solutions as used for analysis at 0°C were heated to 20°C. Then, scans were done at a rotor speed of 6,000 rpm after 15 h for the cysteine synthetase and at 10,000 rpm after 19 h for SAT. Equilibrium centrifugation data were analyzed using the Beckman XL-A program (Ideal1 and Asso4). The partial specific volumes for SAT, OASS, and the cysteine synthetase were estimated to be 0.74, 0.7469, and 0.7428 ml/g from the amino acid compositions of the enzymes, respectively.

**Stability experiments against proteolysis.** SAT and OASS were respectively dialyzed against 50 mM potassium phosphate buffer, pH 7.5 and that containing 0.1 mM PLP to remove EDTA. SAT was dissolved in the phosphate buffer at 2 μM in the absence and in the presence of 15 μM OASS. The enzyme solution was incubated at 40°C for 20 min for reactivation. OASS was dissolved in the phosphate buffer containing 0.1 mM PLP at 6 μM and incubated at 25°C for 30 min. A 0.3-mM portion of the enzyme solution was mixed with 3 μl of 30 μM protease solution (α-chymotrypsin, V8 protease, and trypsin) and incubated for 2 h at 25°C. For proteolysis using α-chymotrypsin and trypsin a 40-μl portion of the enzyme solution was withdrawn at different times and mixed with TPCK (a final concentration of 0.1 mg/ml) and soybean trypsin inhibitor (0.1 mg/ml), respectively and then immediately SAT activity was measured. For V8 protease, SAT activity was measured immediately after incubation. All the enzyme solutions incubated for 2 h were put through an SDS-PAGE using a 12% gel for N-terminal amino acid sequences and densitometric analyses.

**SDS-PAGE.** SDS-PAGE was done by the method of Laemmli. Protein bands were stained with Coomassie Brilliant Blue R-250 (CBB R-250, Sigma Chemical Co.). For quantitative analysis, the stained gels were digitized using as Gel Print 2000i/VGA (Genome Solutions, MI, USA) and analyzed using an Intelligent Quantifier (Genome Solutions). As marker proteins, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor (Pharmacia Biotech., LMW Electrophoresis Calibration Kit) were used. The molecular masses of SATs truncated by a protease treatment were estimated from a calibration curve.

**Separation of peptides by reverse phase-HPLC.** Peptides formed by proteolytic digestion of SAT in the absence of OASS were separated by reverse phase-HPLC chromatography. Proteolysis was done by the method described above, using 0.16 ml of 50 μM potassium phosphate buffer, pH 7.5, containing SAT and protease solution (α-chymotrypsin, V8 protease, and trypsin) at a final concentration of 5 μM and 0.5 μM, respectively. The reaction was stopped by adding 1 μl of HCl. Then, a 0.08-ml portion of the solution was put on to a YMC-Pack ODS-A column (6 × 150 mm) (YMC Co., Kyoto) connected to an HPLC system (Waters, 600, Milford, MT, USA). Elution was done by a linear increase in the acetonitrile concentration from 1 to 40% (v/v) in 0.1% HCl solution at a flow rate of 0.8 ml/min monitored at 210 nm with a UV detector (Waters, 484). Major peaks were collected and analyzed for the first three residues of their N-terminal amino acid sequences to identify the sites cleaved by proteolytic digestions.

**N-Terminal amino acid sequence analysis.** Degraded SATs developed on the SDS-polyacrylamide gel were blotted electrodically onto a PVDF membrane (ATTO Corp., Clear Blot membrane-P, Tokyo) by the method described previously and stained with CBB R-250. Peptide solutions isolated by HPLC were adsorbed onto a PVDF membrane. The membrane prepared was put through a protein sequencer (Applied Biosystems, Model 491, Foster, CA) for analysis of the N-terminal amino acid sequence.

**Results**

**Cold inactivation and reactivation in the absence and in the presence of OASS.**

Figure 1 shows the courses of reactivation of SAT (a final concentration of 0.013 μM) that had been first incubated at 0°C for 30 min, at different temperatures and those for inactivation during the second incubation at 0°C. The reactivation rate became higher with increasing incubation temperatures and the activity after 60 min became nearly constant in the incubation at 25°C or higher. In Fig. 1, the course of reactivation of SAT (0.013 μM) at 25°C following the second inactivation at 0°C in the presence of 0.1 μM OASS is also shown. By adding OASS, the reactiva-
The rate of SAT was increased and the inactivation rate was appreciably reduced.

Figures 2(a) and (b) show the courses of reactivation and inactivation for SAT at the final concentration of 0.2 μM in the absence and in the presence of OASS (1.5, 3, and 6 μM), respectively. As shown in Fig. 2(a), in the presence of OASS, SAT recovered almost its full activity quite rapidly at 25°C, but in the absence of OASS the SAT activity was still lower than the initial activity even after 60 min of incubation. Figure 2(b) also indicates that addition of OASS suppressed the inactivation rate of SAT on cooling to 0°C. Furthermore, the cold inactivation rate of SAT was decreased with increases in the OASS concentration.

At the 0.2 μM SAT concentration, a lag time was observed during the measurement of SAT activity in the absence of OASS as shown in Fig. 3, but in the presence of OASS the lag time was much reduced. The lag time became shorter with decreases in the SAT concentration. That is, at the enzyme concentration of 0.013 μM or lower, the lag time was less than several seconds (data not shown) as compared with several dozen seconds at 0.2 μM (Fig. 3). The existence of the time lag in the absence of OASS may reflect a slow-rate reactivation process at high enzyme concentrations and addition of OASS seems to induce the reactivation.

OASS was quite stable even without complex formation with SAT.

**Effects of temperature on SAT activity**

Figure 4 shows Arrhenius plots for the activities of SAT (0.2 μM) in the absence and in the presence of 1.5 μM OASS. In the presence of OASS, an almost linear relationship was obtained in the range of 10°C to 40°C with an activation energy of around 51.3 kJ·mol⁻¹·K⁻¹. The activity at 0°C was only slightly lower than that deduced from the straight line (broken line). On the other hand, in the absence of OASS the SAT activities at 0°C and 10°C were much lower than those extrapolated from the straight line obtained from the values at 20°C to 40°C with an activation energy of 67.5 kJ·mol⁻¹·K⁻¹. The SAT activity in the absence of OASS was about 1/10 at 0°C that in the presence of OASS. These results support the findings obtained above that cold inactivation was reduced in the presence of OASS.

**Gel chromatographic analysis of the SAT structural change during cold inactivation**

We investigated the structural changes of SAT in
Fig. 3. Changes of Absorbance during Measurement of the Remaining SAT Activity at 25°C.

Changes of the absorbance were measured, using SAT incubated at 0°C (0.2 μM) for 30 min in the absence of OASS (1), SAT incubated at 25°C for 10 min following incubation at 0°C in the absence of OASS (2), and SAT incubated at 25°C for 10 min, following incubation at 0°C in the presence of 0.6 μM (3) and 1.5 μM (4) OASS. The final SAT concentration in all the assay mixtures was 0.4 nM.

Fig. 4. Arrhenius Plots of SAT Activities.

Assays were done at the temperature range of 0°C to 40°C using 0.2 μM SAT in the absence (○) and in the presence of 1.5 μM OASS (●) (see text for details).

the absence and in the presence of OASS upon cooling to 0°C by gel chromatography (Fig. 5). An experiment using OASS was also done as a control

Fig. 5. Gel Chromatographic Profiles for SAT, OASS, and Mixture of SAT and OASS.

A 0.1-ml portion of the sample solution was put on a column and was eluted with the assay buffer at a flow rate of 0.2 ml/min. SAT and OASS activities were measured for the fractions eluted at 0°C (● ▲) and 25°C (○ ▼). (a) SAT eluted at 0°C and 25°C; (b), OASS eluted at 0°C and 25°C; (c) a mixture of SAT and OASS eluted at 25°C; (d), a mixture of SAT and OASS eluted at 0°C. Dotted and broken lines indicate the absorbance at 280 nm for 0°C and 25°C, respectively. Peaks 1, 2, 3, and 4 respectively show SAT, SAT dissociated, cysteine synthetase, and a complex of the dissociated SAT and OASS (see text for details).
Sedimentation equilibrium analyses of SAT and cysteine synthetase

The molecular masses of the SAT and cysteine synthetase at 0°C were measured by equilibrium centrifugation. Figure 6(a) shows equilibrium centrifugation data for SAT at 0°C at 13,000 rpm. The data were fitted well to a model assuming a trimer-hexamer equilibrium of the SAT subunit with a molar ratio (trimer/hexamer) of 95% or slightly higher. As a control we put SAT through a sedimentation equilibrium experiment at 20°C. The equilibrium centrifugation data for SAT at 20°C at 10,000 rpm were fitted to a model assuming a unique species with molecular mass of 165,820 ± 1,182 Da, which was nearly the same to the estimated molecular mass of 175,110 Da for the hexameric form of the SAT subunit, coincident with the experimental results obtained previously (160-180 kDa).5,6

Equilibrium centrifugation for the cysteine synthetase was done at 20°C as a control. Assuming a unique species for the cysteine synthetase, the best fit was obtained with molecular masses of 283,220 ± 1,515 Da (at the rotor speed of 6,000 rpm) and 293,470 ± 1,494 Da (13,000 rpm) (data not shown). These values were similar to the deduced molecular mass of 312,542 Da for the cysteine synthetase composed of one hexamer of SAT subunits and two dimers of OASS subunits, and the experimental results reported previously (309 kDa).6

Figure 6(b) shows the equilibrium centrifugation...
data for the cysteine synthetase at 0°C at 5,000 rpm. The experimental data were fitted to a model assuming an equilibrium between a pentamer (one molecule of trimer of the SAT subunits bound to one dimer of OASS subunits) and decamer (the intact cysteine synthetase) with a molar ratio (pentamer/decamer) of 85% or slightly higher.

Effects of proteolysis on degradation of SAT

Figures 7(a)–(c) respectively show the courses of the remaining SAT activity at 25°C during treatment with α-chymotrypsin, V8 protease, and trypsin in the absence and in the presence of OASS. In the treatment with α-chymotrypsin, the decrease of the SAT activity was reduced in the presence of OASS (Fig. 7(a)). In the case of V8 protease, the SAT activity was stable both in the absence and in the presence of OASS except a slight increase at the initial stage of incubation (Fig. 7(b)). In the case of trypsin, the SAT activity was gradually decreased both in the absence and in the presence of OASS (Fig. 7(c)).

Figure 8 shows photos of the SDS-PAGE for SAT and OASS treated with α-chymotrypsin (Fig. 8(a)), V8 protease (Fig. 8(b)), and trypsin (Fig. 8(c)) for 2 h. In the absence of OASS, most of the SAT was degraded to lower masses than that for the intact SAT (lane 2) except for trypsin (lane 4 in Fig. 8(c)). Arrows in Fig. 8 (lane 4) indicate the degraded SAT subunits. Analyses of N-terminal amino acid sequences of these degraded SAT subunits showed that their N-terminal amino acid sequences were identical to that of the intact SAT, indicating that degradation of SAT occurred in the C-terminal region.

On the other hand, OASS was degraded to species with slightly lower molecular masses by treatment with V8 protease and trypsin (lane 7 in Fig. 8). OASS was not degraded by α-chymotrypsin. OASS activity was not changed in all the cases. In the presence of OASS, degradation of SAT was reduced by treatment with α-chymotrypsin and V8 protease, while it seemed to be slightly accelerated by trypsin (lane 5 in Fig. 8).

N-Terminal amino acid sequences of the peptides obtained by proteolytic digestions of SAT in the absence of OASS were analyzed. By the α-chymotrypsin digestion, two major peptides were obtained. Their first three amino acid sequences from the N-terminus were Asn-Gly-Ile and Glu-Tyr-Gly, respectively. These results indicated that cleaved sites of SAT by α-chymotrypsin were between Phe 260 and Asn 261, and Phe 267 and Glu 268, which yielded respectively SATs deleting 13 and 6 amino acid residues from the C-terminus (abbreviated as SATΔC13 and SATΔC6, respectively). Peptides formed by the V8 protease digestion of SAT showed one major peptide of the first three amino acid sequence of Tyr-Gly-Asp with the cleaved site between Glu 268 and Tyr 269, yielding SATΔC5 (SAT deleting 5 amino acid residues from the C-terminus). When SAT was treated with trypsin, one major peptide was isolated with the first three N-terminal amino acid sequence of Ile-Val-Gly. Thus, the cleaved site was between Arg 242 and Ile 243, yielding SAT deleted by 31 amino acid residues from the C-terminus (abbreviated as SATΔC31). Differences between the subunit molecular masses of the all degraded SATs measured above and that of the intact SAT coincided with those predicted from the gel (Fig. 8). Thus, arrows (1–4) in Fig. 8 (lane 4)
respectively indicate SATΔC6, SATΔC13, SATΔC5, and SATΔC31.

The relative band volumes measured from the SDS-PAGE are summarized in Table 1. When SAT was treated with α-chymotrypsin, the remaining amount of the intact SAT was appreciably higher in the presence of OASS than that in the absence of OASS, as shown in Table 1. In particular, the amount of SATΔC6 was much reduced in the presence of OASS in comparison with that in the absence of OASS. Although most of the intact SAT was degraded to SATΔC5 in the absence of OASS by treatment with V8 protease, its amount was appreciably increased in the presence of OASS (Table 1). On the other hand, the remaining amount of the intact SAT was similar both in the absence and in the presence of OASS by treatment with trypsin (Table 1).

Discussion

Although the fact that SATs from some higher plants as well as from bacteria such as E. coli and S. typhimurium form a bienzyme complex with OASS is well established,6,12-17 the roles of the complex formation are not fully understood.

In this study, we investigated the roles of the complex formation from the viewpoint of stabilities of SAT against cold inactivation and proteolytic attack. Gel chromatographic and ultracentrifugal analyses showed that SAT (a hexamer) was likely dissociated into two trimers of the subunit on cooling to 0°C accompanied with the decrease in the activity. The enzyme activity was recovered by increasing the incubation temperature, indicating that the cold inactivation of SAT is a reversible process. Cold inactivation is observed for a number of enzymes, as reviewed by Privalov,25 and is usually thought to result from the weakening of hydrophobic interactions with decreasing temperature.25 Enzymes such as fatty acid synthetase and threonine deaminase are inactivated by dissociating their quaternary structure respectively into subunit monomers and dimers upon cooling to 0°C in a way similar to the cysteine synthetase.26,27 In the case of SAT, its reactivation was repressed at a high enzyme concentration (0.2 μM) (Fig. 2) and a lag period appeared during a reactivating process (Fig. 3). Smith and Abraham28 have reported that a definite lag period is observed for fatty acid synthetase from rat mammary gland when the incuba-

![Fig. 8. SDS-PAGE for SAT Treated with α-Chymotrypsin (a), V8 Protease (b), and Trypsin (c) in the Absence and in the Presence of OASS.](image)

Lane 1, marker proteins (4.3 μg for each protein); lane 2, SAT (1.8 μg); lane 3, a mixture of SAT (1.8 μg) and OASS (10.3 μg); lane 4, SAT treated for 2 h; lane 5, a mixture of SAT and OASS treated for 2 h; lane 6, OASS (4.1 μg); lane 7, OASS treated for 2 h. As marker proteins, ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) were used. Arrows 1, 2, 3, and 4 in the photos indicate SATs, deleting 6, 13, 5, and 31 amino acid residues from the C-terminus, respectively.

Table 1. Degradation of SAT by a Treatment with Different Proteases in the Absence and in the Presence of OASS

<table>
<thead>
<tr>
<th>Protease</th>
<th>Site specificity</th>
<th>Intact SAT (%)</th>
<th>Partially truncated SAT (%)</th>
<th>Degraded SAT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Chymotrypsin</td>
<td>Tyr, Phe, Trp,</td>
<td>0 (50)</td>
<td>ΔC6 26 (6)</td>
<td>36 (22)</td>
</tr>
<tr>
<td></td>
<td>Leu, Met(^{1})X</td>
<td></td>
<td>ΔC13 38 (22)</td>
<td></td>
</tr>
<tr>
<td>V8 Protease</td>
<td>Glu, Asp(^{1})X</td>
<td>0 (67)</td>
<td>ΔC5 84 (13)</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Lys, Arg(^{1})X</td>
<td>62 (51)</td>
<td>ΔC31 25 (48)</td>
<td>13 (1)</td>
</tr>
</tbody>
</table>

* The numbers show the relative band area after protease treatment in the absence of OASS to the initial value. Those in the parenthesis show the result in the presence of OASS (see text for details).

The C-terminal amino acid sequence of SAT (238-273) is: 28GVPARYGKPSKPSMDMDQHFGINHTFEYGDG[27]
Stability of Cysteine Synthetase from E. coli

Acknowledgments

We would like to thank Miyo Sakai and Yuji Goto, Osaka University for ultracentrifugal analyses. This work was supported in part by the New Energy Industrial Technology Development Organization (NEDO).

References


