Serine Acetyltransferase from Escherichia coli Is a Dimer of Trimmers

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Equilibrium sedimentation studies show that the serine acetyltransferase (SAT) of Escherichia coli is a hexamer. The results of velocity sedimentation and quasi-elastic light scattering experiments suggest that the identical subunits are loosely packed and/or arranged in an ellipsoidal fashion. Chemical cross-linking studies indicate that the fundamental unit of quaternary structure is a trimer. The likelihood, therefore, is that in solution SAT exists as an open arrangement of paired trimers. Crystals of SAT have 32 symmetry, consistent with such an arrangement, and the cell density function that is expected for a hexamer. Electron microscopy with negative staining provides further evidence that SAT has an ellipsoidal subunit organization, the dimensions of the particles consistent with the proposed paired trimeric subunit arrangement. A bead model analysis supports the view that SAT has a low packing density and, furthermore, indicates that the monomers may have an ellipsoidal shape. Such a view is in keeping with the ellipsoidal subunit shape of trimeric LpxA, an acetyltransferase with which SAT shares contiguous repeats of a hexapeptide motif.

In bacteria l-serine is converted to cysteine by a two-step process (1, 2) described as “sulfur fixation,” which is also observed in fungi (3) and higher plants (4, 5). In Salmonella typhimurium and Escherichia coli the first reaction is catalyzed by serine O-acetyltransferase (SAT); EC 2.3.1.30, the cysE gene product, whereas the second reaction is catalyzed by O-acetylserine (thiol)-lyase A, the product of cysK. Previous studies have shown that SAT from the above bacteria is reversibly associated with about 5% of the total cellular O-acetylserine (thiol)-lyase, a much more abundant protein, in the cytochrome synthase multienzyme complex (2). However, data on the quaternary structures of SAT and cysteine synthase have not been consistent (6–8).

The cysE gene was cloned, and its nucleotide sequence was determined by Denk and Böck (9). The deduced molecular weight of CysE was calculated to be 29,260 daltons. Sequence alignments (10) show CysE to share striking homology with the lacA gene product of E. coli, a galactoside acetyltransferase (11), and the nodL gene product of Rhizobia, an oligosaccharide acetyltransferase (12, 13). Moreover, each protein has been shown to share the same basic reaction mechanism, one that involves the formation of a productive ternary complex (11).2 However, whereas structural studies have revealed that LacA and NodL also share a trimeric subunit arrangement, the quaternary structure of SAT has remained uncertain.

SAT has been identified as a member of an extended family of microbial O-acetyltransferases (16). One such member, the lpxA gene product, has been studied by x-ray crystallography and shown to be a trimeric protein with a left-handed parallel -helix fold (17). Moreover, this fold arises from a hexapeptide repeat, which occurs, albeit to varying degrees, in all members of this protein family, including SAT. More recently the structure of another acetyltransferase of the same family has been determined by x-ray diffraction (18). The present study was undertaken to determine whether uncomplexed SAT would prove to show the same homotrimeric quaternary structure as NodL, LacA, and LpxA. The structural studies reported here revealed SAT to be composed of homotrimers, but unlike all of the above enzymes, the overall structure is that of a dimer of trimers.

MATERIALS AND METHODS

Overexpression and Purification of SAT—SAT was overexpressed and purified from E. coli strain E1053 harboring plSAT3, which contains genes for ampicillin resistance and cysE (9). Growth conditions were as described by Wigley et al. (7). These authors’ purification protocol was adopted with various modifications. Cells were harvested by centrifugation and resuspended in 20 mM Tris-HCl buffer, pH 7.5, containing EDTA (5 mM) and 3,4-dichloroisocoumarin (0.25 mM) in dimethyl sulfoxide at a final concentration of 0.5% (v/v). Cells were disrupted by sonication, and a cell-free extract was collected by centrifugation at 4 °C. SAT was precipitated by the stepwise addition of ammonium sulfate (45%) at 4 °C. Precipitated protein was collected by centrifugation, dissolved in 20 mM Tris-HCl buffer, pH 7.5, containing EDTA (0.1 mM) and applied to a Cibacron Blue-Sepharose column equilibrated in 20 mM Tris-HCl buffer, pH 7.5, containing NaCl (0.4 M). The column was washed first with 20 mM Tris-HCl buffer, pH 7.5, and then with TSE buffer (50 mM Tris-HCl, pH 7.5, containing 0.1 M NaCl and 0.1 mM EDTA) until the absorbance of the column eluent was less than 0.05 at 280 nm. SAT was eluted with TSE buffer containing 0.8 mM cysteine. Fractions with an absorbance greater than 0.15 at 280 nm were pooled and applied to a MonoQ × 10 anion exchange column (equilibrated in 20 mM Tris-HCl buffer, pH 7.5) run on a Pharmacia fast protein liquid chromatography system (Amersham Pharmacia Biotech) at 25 °C. SAT was eluted by a linear NaCl concentration gradient (0.15–0.7 M) containing 20 mM Tris-HCl buffer, pH 7.5. Fractions containing SAT activity were dialyzed against 50 mM Tris-HCl, pH 7.5, containing NaCl (0.4 M) at 4 °C, concentrated to approximately 5 mg/ml by ultrafiltration, and stored at −70 °C. An extinction coefficient for purified SAT was calculated by amino acid composition (ε280nm = 0.97) and determined spectrophotometrically (ε280nm = 0.87) under denaturing conditions (19). A mean value (ε280nm = 0.92) was used to calculate the concentration of purified SAT.

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1 The abbreviations used are: SAT, serine acetyltransferase; DMS, dimethyl suberimidate.

2 V. J. Hindson and W. V. Shaw, submitted for publication.
Sedimentation Equilibrium—A Beckman Optima XL-A Ultracentrifuge was employed to obtain a concentration versus radial distance distribution of the control protein. A control trimeric protein, chloramphenicol acetyltransferase (monomer $M_r = 24,965$), was dialyzed against 20 mM Tris-HCl, pH 7.5, containing NaCl (0.35 M). The loading concentrations of SAT were 0.096, 0.73, and 3.54 mg/ml and that of chloramphenicol acetyltransferase was 0.5 mg/ml. Sedimentation was performed at a rotor speed of 8,000 rpm in 1.6-mm cells at 5 °C. The redistribution of solute was monitored using its absorbance at 280 nm. Initial scans were taken at 10 h and then every 3 h until equilibrium was attained (typically within 24 h). The analysis of the equilibrium solute distribution was performed using the Beckman XL-A program IDEAL 1 encoded in MicroCal Origin (single species fit, Beckman Coulter, UK Ltd) to produce an absorption versus radial displacement plot in the solution region from which the absolute molecular weight was derived. The (fairly small) correction for thermodynamic nonideality was performed following the basic principles of physical chemistry, from which we know that

$$M_r(\text{apparent}) = M_r(\text{ideal}) \times (1 + BMc)^{-1}$$

(Eq. 1)

where $B$ is the second virial coefficient ($= 2kT$ from osmotic pressure), $c$ is the mass concentration. In the absence of significant charge effects, compact perfect spheres have a BM of $8 \pi r^3$/v arising from their excluded volume. The approximation used to give a value for BM in mgl terms uses the frictional ratio ($f/f_0$) as a simple measure of the total correction made to the excluded volume by surface rugosity, entrained and bound solvent, and particle asymmetry. The frictional ratio is the numerical factor that relates the experimental friction $(f)$ found when a particle is translated in solution to that which would hypothetically be experienced by a compact sphere of the same mass. Thus,

$$f = f_0 \times \frac{6 \pi r (3n/4 \pi N)}{M} \times (1 + k_c c)^{-1}$$

(Eq. 2)

where $O$ is the solvent viscosity, $r$ is the partial specific volume, and $N$ is Avogadro’s number. The partial specific volume of SAT was estimated from the derived amino acid sequence (21).

Sedimentation Velocity—Sedimentation velocity was performed for three concentrations of SAT (0.21, 1.06, and 3.17 mg/ml) in 20 mM Tris-HCl, pH 7.5, containing NaCl (0.55 M) at a rotor speed of 40,000 rpm at 5 °C, and concentration distributions were followed by scanning at 280 nm. Initial scans of the sedimenting solutes were first taken at 30 min and then every 30 min. The run duration was 14 h 15 min. For the highest concentration of SAT employed (3.17 mg/ml) the concentration was followed qualitatively, since the measured absorption was no longer linearly proportional to the loaded solute concentration. The data were analyzed using the Beckman XL-A program IDEAL 1 encoded in MicroCal Origin (Beckman Coulter). The concentration dependence of the sedimentation coefficient was determined by means of Equation 3 (21),

$$s = \frac{s_o}{(1 + k_c c)}$$

(Eq. 3)

where $s$ is the sedimentation coefficient, $s_o$ is the limiting (ideal) sedimentation coefficient, $c$ is the concentration, corrected for radial dilution at which $s$ was determined (usually the mean plateau concentration for the experiment), and $k_c$ is the concentration dependence coefficient.

The frictional ratio of SAT was derived in the conventional manner from the experimentally determined sedimentation coefficient, the molecular weight, and the partial specific volume. An estimate of the packing density of SAT relative to that of a typical globular protein ($V_r$) was derived on the assumption that SAT is spherical by means of Equation 4 (21),

$$V_r = \left(\frac{M_r}{n \nu}\right)^{1/3}$$

(Eq. 4)

where $f$ is the frictional ratio of SAT, and $f_0$ is the frictional ratio of a typical globular protein, taken to be 1.15.

Quasi-elastic Light Scattering—Translational diffusion coefficients were measured at a mean temperature of 23.1 °C using a DynoPro-801 detector. SAT (3.16 mg/ml) in 20 mM Tris-HCl, pH 7.5, containing NaCl (0.55 M) was injected into the cell, and 30 successive estimated values were logged after the gross photon count had been checked for stability. Measurements with a polydispersity greater than 2.5 were omitted from the calculation of the mean diffusion coefficient. A temperature correction was made (22).

Chemical Cross-linking with Dimethyl Suberimidate (DMS) and Glutaraldehyde—SAT (0.8 mg/ml) was treated with DMS (final concentration 10 mM) at 25 °C in 0.1 triethanolamine buffer, pH 8.0, containing NaCl (0.1 M) and dithiothreitol (0.4 mM). To correct for any decrease in the concentration of DMS due to hydrolysis, a further addition of DMS (final concentration 5 mM) was made at 40-min intervals. Reactions were quenched by the addition in each case of a 10-fold molar excess of glycine over total DMS.

Cross-linking reactions with glutaraldehyde were performed over a range of concentrations of protein (0.3 to 3 mg/ml) and glutaraldehyde (10 to 80 mM) in 20 mM sodium phosphate buffer, pH 8.0, containing NaCl (0.1 M). Reactions were quenched in each case with a 30-fold excess of hydrazine over glutaraldehyde. The detection of cross-linked protein was carried out by SDS-polyacrylamide gel electrophoresis using either a continuous phosphate-buffered system (23) or a discontinuous Tris-HCl-buffered system (24).

Cryostalization—Crystals were grown by the hanging drop method (25) at 15 °C from 15 to 20% polyethylene glycol 2000 containing 0.1 mM Tris-HCl, pH 8.5, and cysteine (2 mM) from a solution of SAT (8.3 mg/ml). Data were collected from crystals using a Raxis IIE image plate system (Rigaku Molecular Structure Corp.), mounted on a Rigaku RU200HB rotating anode generator with a copper anode, a nominal focus of 0.3 × 3.0 mm, and powered at 50 kV, 100 mA. Molecular Structure Corp./Yale mirrors and nickel foil were used to focus and separate the CuKα (1.5418 Å) radiation. The primitive cell type was assigned by subjecting data to the auto indexing routines in DENZO (26). Space group and cell dimensions were determined from the 180° data at 5 Å resolution. Calculation of the self-rotation function was performed according to the method of Rossmann and Blow (27) as implemented in POLARFFN (29).

Electron Microscopy—For negative staining, a solution of SAT was adsorbed onto carbon grids and after vapor fixation negatively stained using 2% uranyl acetate. SAT (3 mg/ml) was diluted in 25 mM sodium phosphate buffer, pH 7.5, containing NaCl (100 mM) to a final concentration of 0.18 mg/ml. Carbon films were prepared by depositing carbon onto Pioloform-coated grids in a vacuum evaporator at a pressure of 10−6 torr. The carbon-coated Pioloform grids were then subjected to a gelatinization treatment in order to minimize the protein solution. After vapor fixation over glutaraldehyde for 2 min and washing with double-distilled water, a droplet of 2% uranyl acetate was applied, excess uranyl acetate was removed with filter paper, and the sample was left to dry.

For preparation of shadowed replicas, SAT (0.15 mg/ml) in 50% glycerol, 50–100 mM sodium phosphate buffer, pH 7.5, containing NaCl (50–100 mM) was sprayed onto freshly cleaved mica using a modified artist’s airbrush (14). Samples were then placed in the vacuum coating unit directly beneath the carbon evaporation source and at a distance of 130 mm and an angle of 7.5° from the platinum evaporation source. The unit was then evacuated to a pressure of the order of 1 × 10−6 torr, and samples were left for 10–20 min to dry. Samples (rotating if necessary) were shadowed by careful evaporation of the platinum source and then carbon-backed by evaporation of the carbon source. After carbon backing the vacuum was released, and replicas were floated onto the surface of double-distilled water and then lifted onto the surface of cleaned 300-mesh copper electron microscope grids. All samples were observed at 80 kV with a Siemens S102 electron microscope. Images were recorded on Agfa Scientia EM film (23D56 P3AH).

Gel-exclusion Chromatography—A 100-ml aliquot of SAT (0.2 mg/ml) in 20 mM Tris-HCl, pH 7.5, containing 0.1 mM NaCl was applied to a calibrated Superose 6 HR 10/30 column connected to a Pharmacia fast protein liquid chromatography system. The column was run at a flow rate of 0.2 ml/min, and fractions were monitored by absorbance at 280 nm. The void (6.75 ml) and total volumes (21.4 ml) were determined with blue dextran and thymidine, respectively. The proteins used for column calibration are given in the legend to Fig. 3.

RESULTS AND DISCUSSION

Purification of SAT—Harvesting routinely took place when cell yield was in the range of 7 to 10 g/liter of medium (35 to 105 mg of SAT/liter). Although preliminary experiments revealed the effect of C-terminal proteolysis of SAT (not shown), protease activity was substantially reduced by utilizing a Lorn strain of E. coli (E1035) and by performing the complete purification protocol within 16 h. Further reductions in proteolysis were achieved by the inclusion of protease inhibitors in the purification buffer. The results of a typical purification of SAT are summarized in Table I. SAT eluted from the MonoQ column
between 0.4 and 0.5 mM NaCl. The purified enzyme showed no O-acetylsereine (thiol)-lyase activity and migrated as a homogeneous band upon SDS-polyacrylamide gel electrophoresis. The deduced molecular weight was 33.8 as compared with the sequence-derived value of 29.3 kDa (9). Modifications of the purification protocol of Wigly and co-workers (7), including the incorporation of a protease inhibitor mixture, gave rise to both a significant improvement in yield and a 10-fold increase in the final specific activity of purified SAT. Moreover, the specific activity of SAT purified by this protocol of 719 units/mg is significantly greater than published values for the *S. typhimurium* variant of 97 units/mg (8) and 70 units/mg (28), the latter at nonsaturating concentrations of substrates.

**Sedimentation Equilibrium Analysis of SAT**—Equilibrium centrifugation data at 8,000 rpm (Table II) for three different concentrations of SAT reveal that the protein is hexameric. An observed slight correlation between loading concentration and apparent molecular weight might possibly be consistent with a small degree of self-association (Table II), but the random distribution of residuals (Fig. 1) suggests that the protein forms no higher order oligomers. The reliability of the data is shown by the accurate determination of the molecular weight of a control protein, trimeric chloramphenicol acetyltransferase (15).

**Velocity Sedimentation**—Velocity sedimentation data for SAT at three different concentrations also indicates that the protein does not self-associate (Fig. 2). A least squares fit to the data gives a value for the limiting (ideal) sedimentation coefficient ($s_{20,w}$) of 7.08 ± 0.09 S, from which the frictional ratio of SAT was calculated to be 1.53, a value significantly greater than that of a typical globular protein of about 1.15 and more in keeping with an ellipsoid with an axial ratio of 10 or a sphere with one-third the packing density of a typical globular protein (Equation 4). Furthermore, the concentration dependence ($k_v$) was derived to be 16 ± 0.86 S.E. of the estimate, again a value significantly greater than that expected of a typical globular protein (−5) and more in keeping with an open and/or extended subunit organization.

**Translational Diffusion Coefficient**—A precise time averaged translational diffusion coefficient for SAT (3.16 mg/ml) of 3.6 ± 0.05 × 10⁻⁷ cm²/s was measured by quasi-elastic light scattering (data not shown). However, at lower concentrations of protein, the signal was unstable, preventing an estimation of the ideal diffusion coefficient.

A second value for the molecular weight of SAT ($M$, 200), calculated according to Svedberg from the ideal sedimentation coefficient and the diffusion coefficient at a protein concentration of 3.16 mg/ml, was slightly higher than the values obtained by equilibrium sedimentation (Table II). However, since the ideal diffusion coefficient was not utilized in the calculation, error due to nonideality cannot be ruled out, prompting greater confidence to the molecular weight derived from the equilibrium data.

Equilibrium sedimentation was also employed to determine whether serine or cysteine, the latter a feedback inhibitor of SAT (1, 2), have effects on the aggregation state of uncomplexed SAT, since both amino acids have been reported to be regulators of the aggregation state of the cysteine synthase complex from *S. typhimurium* (6). Neither subsaturating (0.4 mM) nor saturating (40 mM) concentrations of serine nor cysteine at 4 mM promoted the self-association of SAT (data not shown). Together, the observations suggest that it is only the aggregation state of SAT in the cysteine synthase complex that is regulated by these effectors. Moreover, the quaternary structure of SAT (1.0 mg/ml solution in 50 mM Tris-HCl buffer) was unperturbed by variations in ionic strength (corresponding to NaCl at 0.1, 0.3, and 1.0 M) or pH (7.3, 8.3, and 9.3), which are

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**Table I**

| Purification step | Volume ml | Protein mg/ml | SAT units | Yield % | Specific activity units/mg | Purification factor |
|------------------|-----------|---------------|-----------|---------|---------------------------|-------------------|
| Crude extract    | 16        | 1,295         | 74,400    | 100     | 57                        | 1                 |
| (NH₄)₂SO₄ precipitate | 130     | 585           | 69,000    | 93      | 119                       | 2.1               |
| Cibicon Blue     | 90        | 69            | 45,000    | 60      | 650                       | 11.4              |
| Sepharose        | 61        | 60            | 43,000    | 58      | 719                       | 12.6              |

* 1 unit = 1 μmol/min.

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**Table II**

| Enzyme        | Loading concentration mg/ml | Molecular weight | Apparent quaternary structure |
|---------------|-----------------------------|------------------|-------------------------------|
| SAT           | 0.10                        | 169,700 ± 1,060  | 5.8                           |
| SAT           | 0.73                        | 177,600 ± 1,060  | 6.1                           |
| SAT           | 3.60                        | 184,300 ± 1,060  | 6.3                           |
| CAT           | 0.50                        | 75,100 ± 740     | 3.0                           |

* Ratio of observed molecular weight to that of the subunit (29,260 for SAT and 24,965 for CAT).

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**Figure 1.** Equilibrium centrifugation data for SAT at 8,000 rpm. The lower panel shows the plot of absorbance versus radial distance for SAT (loading concentration of 0.73 mg/ml). The upper panel shows the distribution of the residuals about the best fit curve.
expected to influence ionic attractive forces (data not shown), suggesting that hydrophobic interactions play a significant role in maintaining the quaternary structure of SAT.

**Gel Exclusion Chromatography**—SAT activity was equally distributed between two peaks, the elution volumes of which corresponded to (a) a molecular weight of 140 (40 kDa less than that calculated from the sedimentation data) and (b) a much higher molecular weight aggregate, which had no counterpart in the sedimentation studies and lay outside the range of the molecular weight markers utilized (Fig. 3). Furthermore, the data are in agreement with the findings of Baecker and Wedding (8), which showed SAT from S. typhimurium to form higher-order aggregates upon gel exclusion chromatography. The discrepancy between the gel exclusion chromatography and equilibrium sedimentation measurements of molecular weight in the present study was almost certainly due to the high $f_{ro}$ of SAT, since gel exclusion chromatography provides a measure of molecular weight that is sensitive to this property (21).

**Chemical Cross-linking**—The quaternary structure of SAT was further investigated by chemical cross-linking with glutaraldehyde and dimethylsuberimidate. Three major species were observed (results not shown), and their $M_v$ values corresponded in each case (± 5 to 10%) to those calculated for the monomer, dimer, and trimer of SAT. Although trace amounts of higher order oligomers were discerned, subsequent experiments suggested that such species probably arise from crosslinking of trimers, since their presence was dependent upon the concentration of SAT. As such, the data indicate that the fundamental unit of quaternary structure is a "compact" trimer. Furthermore, since ultracentrifugation data show that SAT is hexameric, the likelihood is that SAT has a paired trimeric subunit arrangement in which the packing density between trimers might be low.

**Crystallization**—Crystals of SAT typically grew to 0.1 mm in cross-section in 7 days, and diffraction to beyond 2.9 Å was observed in a 3-h oscillation exposure (not shown). 180° of data were collected to 5 Å, which allowed the cell dimensions of the primitive orthorhombic cell to be refined to $a = 105.15$, $b = 120.4$, and $c = 123.4$ Å. Examination of the distribution of intensities on the principle axes clearly shows the space group to be $P2_12_12_1$.

The self-rotation function shows peaks consistent with a 32 symmetry (see Fig. 4 for the section at $\kappa = 180^\circ$). Such data are consistent with either a trimeric or hexameric (dimer of trimers) quaternary structure and are in agreement with the findings of hydrodynamic and chemical cross-linking studies. Furthermore, a hexamer in the asymmetric unit yields a $V_m$ value of 2.4 Da/Å³ and an estimated solvent content of 45–50%, satisfying the constraints of crystal packing.

**Electron Microscopy**—Negative staining and rotary and unidirectional shadowing studies were undertaken to determine the molecular shape and dimensions of SAT. Fig. 5 shows a typical field of negatively stained SAT at 246,000-fold magnification. Although it is apparent that SAT molecules have a roughly ellipsoidal shape, the subunit arrangement could not be discerned with confidence because of the small size of the protein and its tendency to aggregate. Nonetheless, the average length and width of clearly resolved ellipsoidal particles was established to be 10.9 and 7.00 nm, respectively, with the average diameter of the circular particles being 6.8 nm (Table III). As such, the data are in accord with the predictions of velocity sedimentation and chemical cross-linking studies, which point to SAT being an elongated assembly of paired trimers.

Rotary shadowing revealed individual monomers of SAT to be loosely packed; however, their organization was unclear (Fig. 6). Nonetheless, the dimensions of such particles were of the same order as those visualized by negative staining.

Unidirectional shadowing was employed to determine the
The linear shadowing angle was assigned from the shadow cast by a tobacco mosaic virus control, which resided within the droplet, to be $7.15^\circ$. A typical electron micrograph is presented in Fig. 6. Again there is evidence that SAT associates, and the dimensions of what appeared to be aggregates were omitted from the estimation of the mean particle height and width (Table III). Although some smaller particles were observed, these were also omitted from the calculation of particle dimensions. Finally, since it was uncertain whether individual molecules were standing on their “circular” or “ellipsoidal” faces, the measured dimensions are necessarily an average of the height and width of both. From an examination of such micrographs a height of 4.1 nm and a width of 7.2 nm were estimated, suggesting that the majority of molecules are lying on their ellipsoidal side. Nevertheless, the overall size of the molecule is significantly smaller than estimated by negative staining (after a correction was made for a 3-nm shadow cap). The height estimate is especially low and suggests that the sample may have become flattened during preparation. As such more confidence is placed in the data obtained by negative staining.

The method of Garcia de la Torre and Bloomfield (14) was utilized to establish whether the dimensions of SAT measured by negative staining reflect the shape of the molecule in aqueous solution as measured by hydrodynamics (Table IV). For these studies SAT monomers were modeled as hydrated spheres (0.3 g of H$_2$O/g of protein) with a radius of 2.05 nm (calculated from the partial specific volume and molecular weight). For example, arranging such spheres as a pair of tightly bound trimers separated by a 2.7-nm gap yields a model with an ellipsoidal length of 10.9 nm and a minimum height of 7.8 nm, in good agreement with the dimensions of negatively stained SAT. However, the sedimentation coefficient for such a model (8.43 S) is significantly greater than the experimentally determined value of 7.1 S. The likelihood therefore is that SAT is less densely packed. Table IV shows that a very low subunit packing density is necessary for the sedimentation coefficient to resemble the experimentally determined value, suggesting that either the dimensions, as derived by negative staining, are nonphysiological or that it is not appropriate to model SAT monomers as spheres. The latter notion is in keeping with the ellipsoidal subunit shape of LpxA, a trimeric O-acyltransferase with which SAT shares contiguous repeats of a hexameric motif.

**Conclusions**—The hydrodynamic and quasi-elastic light scattering studies presented are consistent with a quaternary structure for SAT that can be conveniently described as that of an “open” hexamer. Chemical cross-linking studies revealed the fundamental building block to be a trimer, indicating that

| Preparation       | Ellipsoidal Diameter | Circular Diameter | Height |
|-------------------|----------------------|------------------|--------|
|                   | Width (nm) | Length (nm) | Width (nm) | Height (nm) |
| Negative stain    | 7.00 ± 0.5  | 10.9 ± 1.1  | 6.8 ± 0.65  |             |
| Unidirectional shadow | 7.2 ± 1.1°  | 4.1 ± 0.5°  |             |             |

*Corrected for 3-nm shadow cap.

**TABLE IV**

The sedimentation coefficient of bead models of hexameric SAT, illustrated in three orientations

| xy  | yz  | zx  | s (S) |
|-----|-----|-----|-------|
| ![Model](image1.png) | ![Model](image2.png) | ![Model](image3.png) | 8.43  |
| ![Model](image4.png) | ![Model](image5.png) | ![Model](image6.png) | 7.64  |
| ![Model](image7.png) | ![Model](image8.png) | ![Model](image9.png) | 7.14  |

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| ![Model](image7.png) | ![Model](image8.png) | ![Model](image9.png) | 7.14  |
SAT is arranged as a pair of loosely stacked trimers. Furthermore, negative staining electron microscopy shows SAT to have an ellipsoidal shape, the dimensions of which are consistent with a dimer of trimers. The self-rotation function, as determined by x-ray diffraction, is in accord with these findings.

In view of such data and the observation that SAT shares a hexapeptide repeat with an extended family of trimeric acyltransferases that includes the lacA gene product of E. coli (galactoside acetyltransferase) and the nodL-encoded protein of Rhizobium (an oligosaccharide acetyltransferase), SAT could in principle have arisen simply by the stacking of an “ancestral” trimer. Moreover, such an evolutionary transition may have facilitated the formation of a larger and possibly more stable cysteine synthase multienzyme complex in which the SAT and O-acetylserine (thiol)-lyase activities could be coupled and regulated efficiently.

Finally, in light of the experimentally determined quaternary structure for SAT (this work) and the reported molecular weight of the cysteine synthase complex (2), the subunit structure of the latter assembly is likely to be that of a SAT hexamer plus two dimers of O-acetylserine (thiol)-lyase. Furthermore, in view of the “stacked trimer” model for SAT and from considerations of symmetry, the deduced quaternary structure for cysteine synthase seems most likely to be one described by \((A_3)_2(B_2)_2\) (where A and B are monomers of SAT and O-acetylserine (thiol)-lyase respectively). Such a model leads to an attractive quaternary geometry for cysteine synthase, one in which the two dimers of O-acetylserine (thiol)-lyase are located at opposite ends of the proposed stacked trimers.

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