# Seryl Transfer Ribonucleic Acid Synthetase of *Escherichia coli* B. Purification, Subunit Structure, and Behavior in the Acylation Reaction†

Elizabeth A. Boeker,‡ Arthur P. Hays,§ and Giulio L. Cantoni*

ABSTRACT: Seryl-tRNA synthetase has been purified from *Escherichia coli* B; the preparation is essentially homogeneous by the criteria of acrylamide gel electrophoresis and high speed equilibrium ultracentrifugation. The Michaelis constants for serine and ATP in the tRNA acylation reaction are 0.09 and 0.15 mM, respectively. The pH optimum is 7.4 and the maximum velocity is 8 μmol/min per mg. The enzyme has a mol wt of 103,000 and a sedimentation coefficient, $s^0_{20,w}$, of 5.4 S when examined as the free enzyme, the ATP–enzyme complex, or the seryl adenylate–enzyme complex. The results of acrylamide gel electrophoresis in sodium dodecyl sulfate and isoelectric focusing in 6 M urea indicate that it is composed of two subunits, with a mol wt of 53,000, which are identical within the limits of the techniques.

Seryl-tRNA synthetase was first isolated and crystallized from baker's yeast by Makman and Cantoni (1965, 1966); their preparation had a mol wt of 89,000 and Michaelis constants for ATP, serine, and tRNA of 0.5 mM, 0.01 mM, and 0.24 μM, respectively, and was not inhibited by tRNA lacking the 3′-terminal adenosine. Bluestein *et al.* (1968) isolated by gel filtration the seryl adenylate complex formed by this enzyme in the absence of tRNA; the observed stoichiometry was 1 mol/89,000 g.

Katze and Konigsberg (1970) purified seryl-tRNA synthetase from *Escherichia coli* K12; this preparation displayed a mol wt which increased with protein concentration to a maximum value of 95,000. Studies in denaturing solvents indicated that this enzyme has two identical subunits of mol wt 50,000. It acylates all of the five chromatographically distinct species of seryl-tRNA (Roy and Soll, 1970) and forms a specific complex with *E. coli* K12 seryl-tRNA (Knowles *et al.*, 1970). Complex formation was abolished by 0.5 M NaCl or 10 mM $P_i$, and markedly reduced or abolished by 0.5 M ATP. The tRNA:enzyme ratio was 0.8:1.0, based on tRNA and enzyme molecular weights of 25,000 and 100,000, respectively.

Seryl-tRNA synthetase thus appears to consist of two identical subunits, yet the stoichiometry of both seryl adenylate and tRNA complex formation is one per dimer, suggesting that the two subunits form a single active site, a situation which would be unusual and especially interesting. The mode of addition of ATP and serine is not known. $Mg^{2+}$ is required for activity (Makman and Cantoni, 1965, 1966; Bluestein *et al.*, 1968; Katze and Konigsberg, 1970) but its role in the reaction is only superficially understood. The possibility that serine, ATP, or seryl adenylate influence the quaternary structure of the enzyme has not been investigated.

To answer these questions, we have purified the seryl-tRNA synthetase of *E. coli* B to homogeneity, characterized its subunit structure and behavior in the acylation reaction, carried out a kinetic analysis of the [$^{32}$P]$PP_i$–ATP exchange reaction, and measured ATP, serine, and seryl adenylate binding under equilibrium or steady-state conditions (Boeker and Cantoni, 1973).

## Materials and Methods

*Materials.* L-[U-$^{14}$C]Serine was obtained from Schwarz, ATP (disodium salt) from Sigma, DNase, β-galactosidase, catalase, ovalbumin, and chymotrypsinogen from Worthington, bovine serum albumin and hemoglobin from Pentex, *E. coli* B cell paste and average tRNA from General Biochemicals, and Hyflo Super-cel from Fisher Scientific.

Superbrite glass beads (3M Co.) were boiled in 50% $HNO_3$, washed until neutral, and discarded after each use. tRNA was extracted three times with equal volumes of phenol and ether and dialyzed exhaustively against 0.1 M Tris-Cl buffer, pH 7.4. Hydroxylapatite was prepared by Mr. David L. Rogerson, Jr., of the National Institute of Arthritis and Metabolic Diseases according to the procedure of Tiselius *et al.* (1956). DEAE-cellulose (Whatman DE-52) and DEAE-Sephadex (Pharmacia A-50) were cycled twice through 0.5 N HCl and 0.5 N NaOH and carefully equilibrated with 0.05 M Tris-Cl–0.05 M KCl (pH 7.4) or 0.02 M potassium phosphate–0.2 M KCl (pH 6.5), respectively.

*Enzyme Assays and Kinetics.* During purification procedures, seryl-tRNA synthetase was routinely assayed for 15 min at 37° using the conventional Millipore filter method. The reaction mixture, in 0.2 ml of 0.05 Tris-Cl, pH 7.4 (37°), contained 2.5 mM ATP, 0.0375 mM L-[1-$^{14}$C]serine (*ca.* 15 μCi/μmol), 0.08 mM each of the other 19 amino acids, 10 $A_{260}$ units of *E. coli* average tRNA, 15 mM $MgCl_2$, and 0.2 mg/ml of bovine serum albumin. The assay is linear with time and protein concentration if less than 0.2 nmol (*ca.* 7000 cpm) of tRNA is acylated, but the concentration of serine is less than half its apparent Michaelis constant; the rate of tRNA acylation is not maximal.

In order to measure this maximum rate and to obtain a standard system for kinetic measurements, a second assay using 1.0 mM serine was developed. All other amino acids were omitted, with no change in the rate of tRNA acylation by pure preparations, and serum albumin, which binds small molecules readily, was replaced by 0.5 mg/ml of dialyzed

† From the Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, Bethesda, Maryland 20014. *Received October 4, 1972.*

‡ Present address: Department of Biochemistry, University of Washington, Seattle, Wash. 98195.

§ Present address: Department of Pathology, Columbia University, New York, N. Y. 10032.

gelatin. Millipore filters gave large, variable background values under these conditions and were replaced by Schleicher and Schuell Bac-T-Flex membrane filters, type B-6, which gave background values of about 400 cpm; the incubation time was reduced to 10 min. This assay is also linear with time and protein concentration; it gives a rate of tRNA acylation that is about four times that of the routine assay and at least 90% of the extrapolated maximum rate.

For kinetic measurements, the specific activity of the serine was adjusted appropriately and 0.15-ml aliquots were removed from a 1.0-ml total volume at intervals for 5 min. To ensure that measured rates were in fact initial rates, the reaction was limited to 15% or less of the concentration of the variable substrate.

*Protein Determinations.* An extinction coefficient, $A_{280}^{1\%} = 7.7$, was determined refractometrically in a Beckman Model E analytical ultracentrifuge using a synthetic boundary cell, interference optics, and a specific refractive index increment of 40.5 fringes for a 1.0% protein solution (calculated from Perlmann and Longsworth, 1948). All protein concentrations were based on this value except for those determined routinely during purification, which are based on the method of Warburg and Christian (1942).

*Acrylamide Gel Electrophoresis and Isoelectric Focusing.* Electrophoresis of native seryl-tRNA synthetase was carried out in 5 × 50 cm gels of 5% acrylamide using the system described by Davis (1964). The sample was applied in 25% glycerol; gels were stained for 1 hr in 1% Amido Black and destained electrophoretically. The system of Shapiro *et al.* (1967) was used for acrylamide gel electrophoresis in sodium dodecyl sulfate.

For isoelectric focusing (Wrigley, 1968), 5 × 100 mm gels were photopolymerized from 2 ml of a solution containing 5% acrylamide, 0.2% methylenebisacrylamide, 0.067% tetramethylethylenediamine, 0.001% riboflavine, 6 M urea, 0.2% Triton X-100, 1.0% LKB pH 3–10 ampholytes, and 20 μg/ml of seryl-tRNA synthetase. Samples were run at 1 mA/gel or 120 V, whichever was greater, for *ca.* 7 hr; the cathode solution was 0.4% ethanolamine and the anode solution was 0.2% $H_2SO_4$. Gels were stained in 0.2% Bromophenol Blue in 50% ethanol and 5% acetic acid for 1 hr and destained by diffusion into 30% ethanol and 5% acetic acid. Approximate pH gradients were obtained by slicing an unstained gel into 5 mm sections and soaking each section in 2 ml of distilled water for 1 hr.

*Analytical Ultracentrifugation.* Sedimentation coefficients were determined at 20° using four protein concentrations. For the first four measurements, the enzyme was dialyzed against 0.1 M Tris-Cl at pH 7.4; for the second set, 2.5 mM ATP and $MgCl_2$ were added; and for the third set, additional 2.5 mM L-serine was added. Samples at 0.8 mg/ml were sedimented at 52,000 rpm in 30-mm single sector cells; all others were sedimented at 60,000 rpm in 12 mm double sector cells. Photographs were taken at 8-min intervals for 72 min.

Molecular weights were determined by equilibrium ultracentrifugation (Yphantis, 1964) under three sets of conditions: in 0.01 M Tris, pH 7.4, and 0.01 M 2-mercaptoethanol containing 0.1 M NaCl alone, NaCl plus 2.5 mM ATP and $MgCl_2$, or NaCl, ATP, $MgCl_2$, and 2.5 mM L-serine. Samples were sedimented at 22,000 rpm and 20° for at least 40 hr; concentration gradients were recorded with Rayleigh interference optics. A $\bar{V}$ of 0.743 (Katze and Konigsberg, 1970) was used to calculate molecular weights.

*Purification of Enzyme.* All phosphate buffers were made up at the indicated pH and phosphate concentration from $K_2HPO_4$ and $KH_2PO_4$ according to Green and Hughes (1955), and also contained 1.0 mM $MgCl_2$, 0.1% 2-mercaptoethanol, and 10% glycerol. The standard Tris buffer contained 0.05 M Tris, pH 7.4, 1 mM EDTA, 0.1% 2-mercaptoethanol, 10% glycerol, and other components where indicated. Each large column was equilibrated with the sample buffer until the conductivity of the eluent matched that of the buffer. The small DEAE-cellulose column was equilibrated with 0.01 M phosphate buffer, pH 6.5, the Sephadex G-25 column with 0.2 M KCl in 0.02 M phosphate buffer, pH 6.5, and the small hydroxylapatite column with 0.02 M phosphate buffer, pH 6.5. The large hydroxylapatite column can be used three times if it is stripped with 100 ml of 1.0 M phosphate buffer, pH 6.8. Unless otherwise specified, all steps were carried out at 4–8°.

The activity of the crude extract is variable; preparations which had more than 700 units after step 2 were divided and each half processed separately.

*Step 1: Preparation of Cell Extract.* Frozen *E. coli* B cell paste (500 g) was thawed overnight at 4–8°, mixed with 500 ml of 0.02 M phosphate buffer, pH 7.6, and 1 kg of acid-washed glass beads, and blended for 20 min in a Waring Blendor (Model CB5) at a temperature of 15° or less (maintained by circulating 60% ethanol at −30° through an internal cooling coil), and an additional 1 l. of buffer was added. After 10 min, the solution was decanted, the beads were washed with 500 ml of buffer, and the combined supernatant solutions centrifuged at 13,000*g* for 45 min. The supernatant solution was mixed with 200 g of Hyflo Super-cel, the suspension filtered under pressure through Whatman No. 1, and the filtrate incubated with 4 mg of DNase/100 g of protein at 37° for 3 hr.

*Step 2: Ammonium Sulfate Fractionation.* Ammonium sulfate (23.3 g/100 ml of original solution, 33%) was added slowly, with stirring, to the extract from step 1. After 30 min, the suspension was centrifuged at 13,000*g* for 30 min and the pellet discarded. Additional ammonium sulfate (15.3 g/100 ml of original solution, 55%) was added and the suspension centrifuged; the precipitate was then back extracted with successive 400-ml portions of Tris buffer containing 60, 52, and 36% ammonium sulfate, centrifuging as before. The final supernatant solution was dialyzed against 20 l. of 0.05 M KCl in Tris buffer for 36 hr on a continuous flow rocking dialysis unit. The fine precipitate which formed was removed be centrifugation at 13,000*g* for 30 min.

*Step 3: DEAE-Cellulose Chromatography.* The enzyme solution from step 2 was applied to a 2.5 × 100 cm column of DEAE-cellulose, washed with 800 ml of 0.05 M KCl in Tris buffer, and eluted with a 3-l. linear gradient from 0.05 to 0.2 M KCl; the flow rate was 3 ml/min and the fractions were 10 ml. Ammonium sulfate (50 g/100 ml of original solution, 70%) was added to the pooled active fractions, the suspension centrifuged at 13,000*g* for 30 min, and the supernatant solution discarded. The precipitate was dissolved in a minimum volume (about 25 ml) of 0.005 M phosphate buffer, pH 6.8, dialyzed in three steps against a total of 6 l. of this buffer, and centrifuged at 27,000*g* for 30 min; the resulting precipitate was discarded.

*Step 4: Hydroxylapatite Chromatography.* The enzyme solution from step 3 was applied to a 2.5 × 100 cm column of hydroxylapatite and eluted with a 3-l. linear gradient from 0.02–0.06 M phosphate buffer, pH 6.5, at a flow rate of 20 ml/hr; faster flow rates impaired resolution. Fractions of 10 ml were collected, assayed, and pooled appropriately. This very dilute solution of enzyme was applied to a 1.2 × 5 cm column of DEAE-cellulose and eluted with *ca.* 30 ml of 0.4 M KCl in 0.01 M phosphate buffer, pH 6.5. The active fractions were

TABLE I: Purification of Seryl-tRNA Synthetase from *Escherichia coli* B.[a]

| Purification Step | Vol (ml) | Total Protein (mg) | Total Act. (μmol/min) | Sp Act. (μmol/min per mg) | Yield (%) |
|---|---|---|---|---|---|
| 1. Cell extract | 1700 | 44,000 | 130 | 0.0030 | (100) |
| 2. $(NH_4)_2SO_4$ fractionation | 580 | 13,000 | 100 | 0.0073 | 75 |
| 3. DEAE-cellulose chromatography[b] | 55 | 970 | 73 | 0.073 | 55 |
| 4. Hydroxylapatite chromatography | 18 | 41 | 64 | 1.5 | 48 |
| 5. DEAE-Sephadex chromatography | 7.5 | 12 | 33 | 2.7 | 25 |

[a] The numbering corresponds to the description in the text. The routine activity assay and the Warburg and Christian (1942) method for determining protein concentration were used throughout. The final preparation contained 17 mg of protein, measured at 280 nm, and had a true specific activity of 8.1 μmol/min per mg, measured at maximum velocity. [b] The preparation was divided before chromatography and each half processed separately. Combined totals are given.

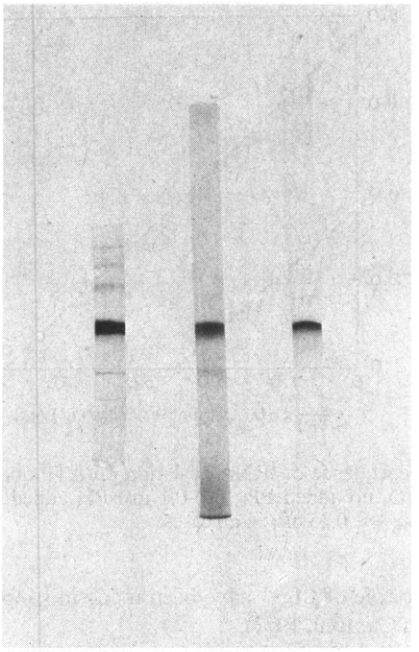

FIGURE 1: Seryl-tRNA synthetase on acrylamide gels. Left gel: Electrophoresis of 60 μg of enzyme under standard conditions; stained with Amido Black. Center gel: Electrophoresis of 10 μg of enzyme in 0.1% sodium dodecyl sulfate; stained with Coomassie Blue. Right gel: Isoelectric focusing of 40 μg of enzyme in 6 M urea and 0.2% Triton X-100; stained with Bromophenol Blue. Details are given in Materials and Methods.

further concentrated by adding ammonium sulfate (5 g/10 ml of original solution, 70%) and centrifuging at 27,000*g* for 30 min. The precipitate was dissolved in a minimum volume (1–3 ml) of 0.2 M KCl in 0.02 M phosphate buffer, pH 6.5, and desalted on a 1.0 × 15 cm column of Sephadex G-25 using approximately 20 ml of the same buffer.

*Step 5: DEAE-Sephadex Chromatography*. The filtrate from step 4 was applied to a 2.5 × 100 cm column of DEAE-Sephadex A-50. Elution was carried out with a 2-l. linear gradient of 0.225–0.4 M KCl in 0.02 M phosphate buffer, pH 6.5, at a flow rate of 20 ml/hr. Fractions of 1 ml were collected, assayed, pooled, and concentrated on a 1.2 × 5 cm column of hydroxylapatite which was stripped with approximately 20 ml of 0.1 M phosphate buffer, pH 7.4. After final assays were performed, the preparation was diluted with an equal volume of glycerol and stored at −10°.

## Results and Discussion

*Purification*. The procedure is summarized in Table I. It was worked out independently (Hays and Cantoni, unpublished data) of that used by Katze and Konigsberg (1970) for purification of this enzyme from *E. coli* K12, and resembles their procedure only superficially. Since Katze and Konigsberg (1970) used the method of Lowry *et al.* (1951) to determine protein, and assayed activity only at 0.02 mM serine, while we have used absorbance at 280 nm and assayed at 0.0375 or 1.0 mM serine, direct comparison of the two procedures is difficult. The one presented here is apparently more efficient; it requires one less chromatographic separation and no preparative electrophoresis. The final product is essentially pure, as judged by analytical gel electrophoresis and isoelectric focusing (Figure 1).

The maximum velocity of tRNA acylation at pH 7.4 (Table I, Figures 2 and 3) is 8 μmol/min per mg; Katze and Konigsberg (1970) report a value of 1.0 μmol/min per mg for the *E. coli* K12 enzyme. Although these numbers do not appear to correspond, the *E. coli* K12 value was obtained at pH 8.0 and 0.02 mM serine, which is *ca.* $^1/_4K_m$ (see below); the true value would then be about 5 μmol/min per mg. The specific activities of both *E. coli* enzymes are considerably higher than the value for the yeast enzyme of 0.56, which can be calculated from its turnover number (Makman and Cantoni, 1965).

The absorbance ratio, $A_{280}/A_{260}$, of the purified enzyme is 1.8 as compared to a ratio of 1.48 for the yeast enzyme (Makman and Cantoni, 1965). Tyrosine and tryptophan determinations (Bencze and Schmid, 1957) gave 22.6 and 8.7 residues/100,000 g, respectively. The tyrosine value is identical with that obtained for the enzyme from *E. coli* K12 by amino acid analysis (Katze and Konigsberg, 1970).

*tRNA Acylation*. When assayed at saturating concentrations of serine (1.0 mM), the *E. coli* B seryl-tRNA synthetase is maximally active between pH 7.1 and 7.7 (pH was measured at 37°); 75% of the activity remains at pH 6.5 or 8.3. This contrasts with the *E. coli* K12 enzyme which, measured at 0.02 mM serine, attains optimal activity only at pH 8 (Katze and Konigsberg, 1970). This may reflect the difference in substrate concentration; alternately, since both measurements were made in Tris buffer, which has a pH of 8.0 if measured at 20° and 7.4 at 37°, it may only reflect the temperature at which the pH was measured.

If tRNA acylation is measured at 1.0 mM serine, 2.5 mM ATP, 0.08 mM (0.2%) total tRNA, and variable $MgCl_2$, the rate begins to increase rapidly at 3 mM $MgCl_2$, plateaus at 5 mM, and remains constant at least to 15 mM. At 5 mM $MgCl_2$, both ATP (Martell and Schwarzenbach, 1956) and tRNA (Willick and Kay, 1971) will be present as magnesium complexes; small amounts of free $Mg^{2+}$ will also be present. Clearly, at least one of these three species is required; which, or how many, cannot be determined. Further attempts to

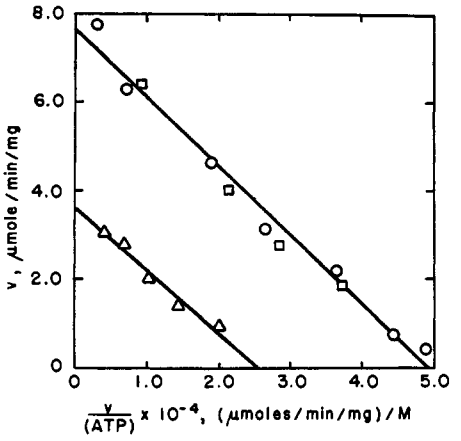


FIGURE 2: Dependence of tRNA acylation on ATP under standard conditions: (○) no added $PP_i$; (□) 0.1 mM $PP_i$ added; (△) 1.0 mM $PP_i$ added. $K_{ATP}$ is 0.15 mM in all cases.

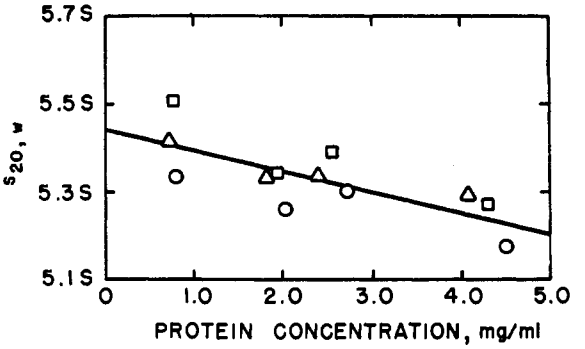


FIGURE 4: Sedimentation coefficient of seryl-tRNA synthetase. Sedimentation velocity experiments were carried out first in 0.1 M Tris-Cl, pH 7.4 (○), second, after addition of 2.5 mM ATP and $MgCl_2$ (□), and third after the further addition of 2.5 mM serine (△). Details are given in Materials and Methods. The line shown is the least-squares regression line for all of the data.

evaluate the role of $Mg^{2+}$ have been made in simpler systems (Boeker and Cantoni, 1973).

The dependence of tRNA acylation on ATP and serine is shown in Figures 2 and 3. Since the observed value of $K_{Ser}$ is 0.091 mM, the serine concentration used in the routine assay (0.0375 mM) is not saturating, while that used to measure maximum rates (1.0 mM) is adequate. $K_{Ser}$ is somewhat higher than the value of 0.03–0.07 mM obtained for the *E. coli* K12 enzyme (Katze and Konigsberg, 1970) and considerably higher than the value of 0.01 mM obtained for the yeast enzyme (Makman and Cantoni, 1965). $K_{ATP}$ is 0.15 mM; the yeast enzyme is reported to have a value between 0.5 and 1.0 mM (Makman and Cantoni, 1965).

Inhibition of tRNA acylation by $PP_i$ is also shown in Figures 2 and 3; the effects for ATP and serine are quite different. $K_{ATP}$ is not significantly altered by 0.1 or 1.0 mM $PP_i$, while 0.1 mM $PP_i$ cause a more than twofold increase in $K_{Ser}$.

Such behavior is consistent only with an ordered addition of ATP and serine in the reaction mechanism. Since this synthetase catalyzes the [$^{32}$P]$PP_i$–ATP exchange reaction in the absence of tRNA, $PP_i$ release, and therefore the addition of ATP and serine, must precede the addition of tRNA. Random addition of serine and ATP will produce hyperbolic kinetics as in Figures 2 and 3 only if the rapid equilibrium assumption is justified; *i.e.*, if the breakdown of the enzyme–ATP–serine complex is sufficiently slow that it equilibrates with ATP and serine. Rate equations for such mechanisms (for example, see Cleland, 1963a) are symmetrical with respect to ATP and serine; the kinetic constants should show parallel behavior in the presence of $PP_i$. Since this is not the case for *E. coli* B seryl-tRNA synthetase, the addition of ATP and serine must be ordered.

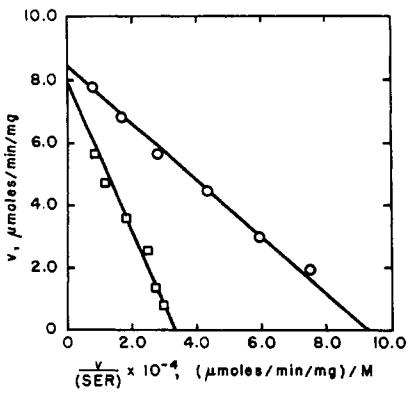


FIGURE 3: Dependence of tRNA acylation on serine under standard conditions: (○) no $PP_i$; (□) 0.1 mM $PP_i$ added. $K_{Ser}$ is 0.091 mM in the absence of $PP_i$ and 0.23 mM in the presence of 0.1 mM $PP_i$.

The order of ATP and serine addition could be determined by varying each at fixed levels of the other in the presence of $PP_i$ (Cleland, 1963b,c); this requires large quantities of high specific activity serine and is both technically difficult and expensive. On the other hand, the order of addition can be very easily determined by kinetic analysis of the [$^{32}$P]$PP_i$–ATP exchange reaction (Boeker and Cantoni, 1973).

*Subunit Structure.* The subunit molecular weight of the *E. coli* B enzyme was determined by acrylamide gel electrophoresis in sodium dodecyl sulfate. Six standards (mol wt 15,000–130,000) produced a logarithmic relationship between mol wt and migration; six samples of synthetase gave an average mol wt of 53,000 (total scatter, ±3000). This is probably not significantly different from the value of 50,000 obtained for the *E. coli* K12 enzyme (Katze and Konigsberg, 1970). One gel is shown in Figure 1 (center); none showed any indication of subunit heterogeneity.

The subunits were further examined by isoelectric focusing, in 6 M urea and 0.2% Triton X-100, on acrylamide gels. The gradients extended from pH 3.7 to 9.2 and were slightly sigmoid rather than linear. Seryl-tRNA synthetase gave a single band (Figure 1, right) with an observed isoelectric point of approximately 6.1–6.2.

The resolving power of this procedure is one to two charged residues per subunit, as a consequence of the following argument. Assuming that the amino acid compositions of the two *E. coli* seryl-tRNA synthetases are similar, there are ten histidyl residues per subunit (Katze and Konigsberg, 1970). Taking the $pK_a$ of the histidyl residue as 6.1, the loss or gain of one charged residue other than histidine will change the isoelectric point 0.17 pH unit, while loss or gain of a histidyl residue will change it 0.09 pH unit. For the pH gradients and gel lengths used here, these changes would produce center-to-center band separations of 3.0 and 1.5 mm, respectively; the first at least should produce an easily distinguishable doublet.

Seryl-tRNA synthetase was examined in the ultracentrifuge as the native enzyme, the ATP–enzyme complex, and the seryl adenylate–enzyme complex. When appropriate, the concentrations of $MgCl_2$, ATP, and serine were each 2.5 mM; complex formation should be more than 98% complete (Boeker

TABLE II: Weight Average Molecular Weight of Seryl-tRNA Synthetase.

| Additions[a] | Initial Protein Concn (mg/ml) | | |
|---|---|---|---|
| | 0.12 | 0.4 | 1.2 |
| None | 104,000 | 97,000[b] | 101,000 |
| ATP, $MgCl_2$ | 102,000 | 106,000 | 104,000 |
| ATP, $MgCl_2$, serine | 100,000 | 105,000 | 103,000 |

[a] All determinations were carried out in 0.1 M NaCl, 0.01 M β-mercaptoethanol, and 0.01 M Tris, pH 7.4; the added components were 2.5 mM each. Details are given in Materials and Methods. [b] The base-line correction for this determination was unusually large; the result is not reliable.

and Cantoni, 1973). The enzyme showed a single symmetrical peak in all sedimentation velocity experiments; sedimentation coefficients are shown in Figure 4. The extrapolated value of $s^0_{20,w}$, obtained by least-squares analysis of all the data, is 5.44 S. Individually, the values for the free enzyme, the ATP, and the seryl adenylate complexes are 5.43, 5.52, and 5.37 S, respectively. Since each determination was carried out independently, in order to conserve enzyme, these differences are probably not significant.

The molecular weights of the native enzyme and both complexes were determined at three protein concentrations using the meniscus depletion method of Yphantis (1964). No evidence of dissociation or heterogeneity was observed under any of the conditions employed, even at displacements as low as 0.1 fringe. From the results presented in Table II the average mol wt is 103,000, slightly higher than the value of 95,000 obtained for the *E. coli* K12 enzyme, which, unlike the *E. coli* B enzyme, may dissociate at low concentrations (Katze and Konigsberg, 1970).

The native mol wt of 103,000 and the subunit mol wt of 53,000 clearly indicate that seryl-tRNA synthetase is a dimer; it has little tendency to dissociate and there are no readily detectable differences between the free enzyme and the two enzyme–substrate complexes. It will be shown in the following paper (Boeker and Cantoni, 1973) that the dimer has two binding sites for ATP or seryl adenylate; there is no discrepancy between the subunit structure and the number of active sites.

Acknowledgment

We thank Mr. Henry Richards and Mr. Elmer Dixon for their invaluable help in purifying the enzyme.

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