Molecular Weight of *Escherichia coli* Glutaminyl Transfer Ribonucleic Acid Synthetase, and Isolation of Its Complex with Glutamine Transfer Ribonucleic Acid*

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ABSTRACT: Gln-tRNA synthetase from *Escherichia coli* has been purified to near homogeneity. The molecular weight of the native enzyme is 69,300. The enzyme probably consists of a single polypeptide chain. Its molecular weight under reducing and denaturing conditions is the same as that of the native enzyme. A specific complex between Gln-tRNA synthetase and $tRNA^{Gln}$ has been isolated by sucrose gradient sedimentation.

The relative change in sedimentation coefficient, and the amount of active enzyme and $tRNA^{Gln}$ found in the complex, indicate that there is one binding site for $tRNA^{Gln}$ per enzyme molecule of 69,300.

Aminoacyl-tRNA synthetases have been studied intensively for well over a decade; one of the more interesting goals of this research has been to understand how these enzymes recognize distinct tRNA species, or in a broader sense, what factors are important in specific protein–nucleic acid interactions.

Work in this laboratory has approached these problems by using purified enzyme preparations and their cognate tRNAs (Baldwin and Berg, 1966; Yarus and Berg, 1967). It was recognized early that mutationally altered enzymes and tRNAs could provide important information on how the structures of both contribute to their specificity (Carbon and Curry, 1968; Folk and Berg, 1970). Recently, Soll and Berg (1969) reported the isolation of a new nonsense suppressor in *Escherichia coli* which inserts glutamine in response to the codon UAG. This suppressor is a tRNA (unpublished data of Cordes and Primakoff, and Folk and Yaniv) which is probably derived from a $tRNA^{Gln}$. In preliminary experiments, I observed that esterification of glutamine to the suppressor tRNA was much more difficult than to wild-type $tRNA^{Gln}$. A preparation of Gln-tRNA synthetase free of other proteins became an important requirement for further studies. To this end, the Gln-tRNA synthetase from *E. coli* has been purified; additionally, its molecular weight has been determined and the complex that it forms with $tRNA^{Gln}$ isolated.

Materials and Methods

Reagents. Isotopically labeled compounds were obtained from New England Nuclear Corp. Whatman DE-52 DEAE was from Reeve Angel, Bio-Gel HTP hydroxylapatite from Bio-Rad Laboratories, and benzoylated DEAE-cellulose was from Schwarz Bio-Research. All other reagents were of the highest purity commercially available.

Unfractionated tRNA used for routine assays was prepared by a modification of the procedure of Zubay (1962). Highly purified $tRNA^{Gln}$ species and $tRNA_1^{Val}$ from *E. coli* (prepared by chromatography in two steps on benzoylated DEAE-cellulose according to the procedure of Gillam *et al.* (1967) and Tener *et al.* (1967) was kindly provided by Dr. M. Yaniv.

Assays. Gln-tRNA-synthetase activity was assayed by measuring the rate of formation of [^{14}C]Gln-tRNA by the procedure of Calendar and Berg (1966). The reaction mixture contained 100 mM sodium cacodylate buffer (pH 7.0), 1 mM ATP, 10 mM $MgCl_2$, 10 mM KCl, 4 mM reduced glutathione, 100 μg of bovine plasma albumin, 0.5 mM [^{14}C]-glutamine (uniformly labeled, 3000 cpm/nmole) (in early stages of enzyme purification, [^{12}C]glutamic acid, at 2 mM, was included), 20–25 A_{260} of tRNA, and 0.05 to 0.5 unit of Gln-tRNA synthetase. (One unit of enzyme activity is equivalent to the formation of 1 nmole of Gln-tRNA in 10 min at 37°.) The reaction mixture was incubated for 10 min at 37°, and processed as described by Calendar and Berg (1966). Occasionally, the reaction mixture was scaled down to 0.1 ml. Then, less tRNA and higher specific activity [^{14}C]glutamine were used.

tRNA acceptor capacity was assayed under the same conditions as Gln-tRNA synthetase activity, except that the reaction volume was 0.1 ml, [^{14}C]Gln specific activity was 400,000 cpm/nmole, and excess enzyme was used. Incubation was usually for 20 min at 37° and yeast RNA was added as a carrier prior to precipitation with 2 N HCl.

Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine plasma albumin as a standard.

Purification of Gln-tRNA Synthetase. Frozen cells (*E. coli* K12 strain W3110) (300 g) were suspended in 850 ml of 20 mM potassium phosphate buffer (pH 7.2) containing 10 mM β-mercaptoethanol and 1 mM EDTA at room temperature. The suspension was passed twice through a Manton Gaulin homogenizer (6000 psi, 4°) and centrifuged for 60 min at 24,000*g*. The supernatant was collected and adjusted to 10 mg/ml of protein with additional buffer. Its specific activity was 34 units/mg of protein.

A one-tenth volume of a 10% solution of streptomycin sulfate was added dropwise, and the solution was stirred for 10 min at 4°. The precipitated nucleic acids were removed by centrifugation for 20 min at 10,000*g*. Solid ammonium sulfate (310 g/l.) was added slowly to the supernatant; after 15 min

* From the Department of Biochemistry, Stanford University School of Medicine, Stanford California 94305. *Received September 14, 1970.* This research was supported by Grant PHS GM 13235 05, National Institutes of Health.

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at 4°, the precipitated proteins were removed by centrifugation at 24,000*g* for 30 min. Additional ammonium sulfate (77 g/l. of streptomycin sulfate supernatant) was added to the supernatant, and the solution was stirred for 10 min at 4°, then centrifuged at 24,000*g* for 60 min. The pellet, which contained 75% of the initial Gln-tRNA synthetase activity, and 20% of the initial protein, was dissolved in 20 mM potassium phosphate (pH 7.0), containing 2 mM β-mercaptoethanol and 1 mM EDTA to a concentration of 20 mg/ml, and dialyzed against the same buffer. Its specific activity was 130 units/mg of protein.

The dialyzed sample was diluted 1:1 with buffer and applied to a column (6 × 30 cm) containing DEAE equilibrated with 20 mM potassium phosphate (pH 7.0), 2 mM β-mercaptoethanol, and 1 mM EDTA. The column was washed with buffer until the A_{280} was less than 0.1, then a linear gradient from 20 mM potassium phosphate (5 l.) to 300 mM potassium phosphate (5 l.) (both at pH 7.0 with 2 mM β-mercaptoethanol and 1 mM EDTA) was used to elute the adsorbed proteins; Gln-tRNA synthetase activity was eluted after 3 l. had passed through the column. The peak fractions were pooled and concentrated (with concomitant ultrafiltration) in an Amicon ultrafiltration apparatus (Amicon Corp., Lexington, Mass.), using an XM-50 membrane. At this point, the specific activity of the Gln-tRNA synthetase was 2400 units/mg of protein.

The protein, in 10 mM potassium phosphate buffer (pH 7.0) containing 1 mM DTT,[1] was then applied to a hydroxylapatite column (3.5 × 45 cm) equilibrated with 20 mM potassium phosphate containing 1 mM DTT. The column was washed with 500 ml of initial buffer, then the adsorbed proteins were eluted with a linear salt gradient from 20 mM potassium phosphate (2 l.) to 300 mM potassium phosphate (2 l.) (both at pH 7.0 with 1 mM DTT). The Gln-tRNA synthetase activity was eluted with approximately 100 mM potassium phosphate. The peak fractions were pooled and dialyzed against 40 mM potassium phosphate (pH 7.0) containing 1 mM DTT. At this point, the protein had a specific activity of 15,400 units/mg of protein. The yield through this step was 30%. Polyacrylamide disc gel electrophoresis of a sample of the protein showed only two major bands.

As a final step, the protein was passed through a Sephadex G-200 column (0.9 × 50 cm) equilibrated with 40 mM potassium phosphate (pH 7.0) containing 1 mM DTT. This separates the Gln-tRNA synthetase activity from the single major contaminating protein whose molecular weight is approximately twice that of the Gln-tRNA synthetase. The peak fractions of Gln-tRNA synthetase activity were pooled and concentrated by vacuum dialysis in a Sartorius collodion membrane. The protein was placed in 40% glycerol and stored at −20°.

The specific activity of the purified preparation under the standard assay conditions was 3.4×10^4 units/mg. This is an increase of 1000-fold over the activity measured in crude extracts. The final yield of Gln-tRNA synthetase activity was 15%.

Amino Acid Analyses. Protein (0.10 mg) was dialyzed against 10 mM potassium phosphate (pH 7.0), and then hydrolyzed *in vacuo* with 6 N HCl for 24 or 72 hr at 110°, with a crystal of phenol to protect tyrosine from oxidation. Half-cystine residues were determined as cysteic acid and methionine residues as methionine sulfone after performic acid oxidation according to Hirs (1967). The resultant hydrolysates were analyzed on a Beckman Model 120B amino acid analyzer. Values for all amino acids except isoleucine, valine, and leucine are based on the 24-hr hydrolysate; values for the latter amino acids are based on the 72-hr hydrolysate.

Disc Gel Electrophoresis. SDS polyacrylamide gel electrophoresis was carried out essentially as described by Weber and Osborn (1969). Prior to electrophoresis, proteins were treated with 1% SDS and 1% β-mercaptoethanol at 37° for 200 min. (I am indebted to Dr. Irving Zabin for the gift of the β-galactosidase used as a molecular weight standard.) Protein was detected by staining with coumassie blue.

Ultracentrifugation. The molecular weight determination by sedimentation to equilibrium (method of Yphantis, 1964) was performed in a Beckman Model E ultracentrifuge, equipped with temperature control and Rayleigh interference optics, using a titanium rotor. A six channel Yphantis cell was used. Molecular weight determinations were made at two protein concentrations (0.1 and 0.5 mg per ml); each sample was dialyzed against 10 mM potassium phosphate (pH 6.9) containing 150 mM KCl and 10 mM DTT, and the dialysates were used in the reference chambers. The temperature was maintained at 25° and the rotor speed was 26,000 rpm. Equilibrium was considered to have been reached when there was no change in the fringe pattern over a 3-hr period.

Sucrose gradient centrifugation was carried out essentially as described by Martin and Ames (1961). Gradients (3.8 ml) of 5–20% sucrose buffered by 10 mM sodium cacodylate with 1 mM DTT, 10 mM $MgCl_2$, and 50 μg/ml of bovine plasma albumin (pH and other components are stated in Results) were centrifuged at 56,000 rpm in a SW-56 titanium rotor in a Beckman Model L2-65 B ultracentrifuge. Temperature was maintained at 4°.

Results

Purity of Gln-tRNA Synthetase. Polyacrylamide gel electrophoresis under nondenaturing conditions (Tris–Tricine, pH 8.1) of the protein from the Sephadex G-200 column yielded only one band. Gel electrophoresis under denaturing conditions (1% SDS with 1% β-mercaptoethanol) yielded one major band, and two minor bands (Figure 1). By visual inspection, I estimate that each of the minor bands is present in less than 5% the amount of the major band.

Sedimentation to equilibrium in a nondenaturing solvent gave a linear plot of the logarithm of the upward fringe displacement (ln Δy) *vs.* the square of the radial displacement, indicating molecular weight homogeneity in the protein preparation.

Of 17 different amino acids tested, only glutamine was esterified to unfractionated tRNA when incubated with the protein, indicating the absence of most other AA-tRNA synthetase activities in the preparation.

Amino Acid Composition. Amino acid analysis of an acid hydrolysate of the protein gave the composition presented in Table I. Tryptoptan was not determined. A molecular weight of 69,300 was assumed in calculating the moles of amino acid residues per mole of protein.

Molecular Weight. Three independent determinations of molecular weight were obtained. The least accurate measurement was made by determining the sedimentation coefficient of the Gln-tRNA synthetase activity in a sucrose gradient. Using tRNA and Ile-tRNA synthetase as markers (4 and 5.3 S, respectively (Baldwin and Berg, 1966)), Gln-tRNA synthetase was found to have a sedimentation coefficient of 4.1 S in a 5–20% sucrose gradient (pH 7.0, 4°). Assuming that

[1] Abbreviations used are: DTT, dithiothreitol; SDS, sodium dodecyl sulfate.

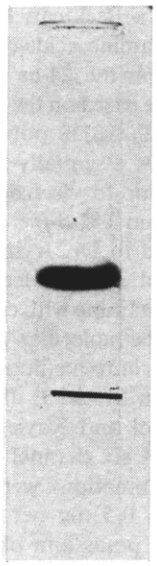

FIGURE 1: SDS polyacrylamide gel of purified Gln-tRNA synthetase. Protein (40 μg) was applied to the gel, and electrophoresis was carried out so that the protein migrated from top to bottom (anode). Dark line below the protein bands indicates the position of brom phenol blue, a tracking dye applied with the sample.

Gln-tRNA synthetase is a globular protein, an estimate of 78,000 ± 15,000 can be made for its molecular weight, using the procedure of Martin and Ames (1961).

The second measurement was made by determining the elution position of the Gln-tRNA synthetase activity from Sephadex G-200 (Andrews, 1965). Using five other proteins (lysozyme, chymotrypsinogen, hemoglobin, Ile-tRNA synthetase, and glycerol 3-phosphate dehydrogenase) as molecular weight markers, a linear relationship was obtained between the elution volume of a protein and the logarithm of its molecular weight. Gln-tRNA synthetase was found to have a molecular weight of 76,000 ± 10,000.

The third molecular weight determination was made by sedimenting the protein to equilibrium in an ultracentrifuge, using the meniscus depletion method described by Yphantis (1964). A plot of the upward fringe displacement versus the square of the radial displacement gave a straight line whose slope is related to M_w, the weight average molecular weight (Yphantis, 1964). Using $\bar{\gamma}$ calculated from the amino acid composition (McMeekin and Marshall, 1952), M_w was determined to be 69,300 ± 3000. This figure agrees well with the values determined above.

A measurement of the subunit molecular weight of the Gln-tRNA synthetase was made by SDS polyacrylamide disc gel electrophoresis (Shapiro *et al.*, 1967; Weber and Osborn, 1969). This method relies upon the observation that the relative mobility of a protein reduced with β-mercaptoethanol and denatured with SDS, during electrophoresis in SDS polyacrylamide gels, is proportional to the logarithm of its molecular weight. When the mobility of Gln-tRNA synthetase is compared to the mobilities of five well characterized proteins (apomyoglobin, pepsin, bovine plasma albumin, Ile-tRNA synthetase, and β-galactosidase) the molecular weight of the enzyme is estimated to be 69,000 ± 2000. This value, although obtained under conditions such that disulfide bond should be reduced and polypeptide chains unfolded, is quite close to that obtained by sucrose gradient sedimentation, gel filtration, or equilibrium sedimentation with the native protein.

TABLE I: Amino Acid Composition of Gln-tRNA Synthetase.

Amino Acid	App Residues/Mole
Aspartic and asparagine	59.4
Threonine	25.4
Serine	24.8
Glutamic and glutamine	56.8
Proline	28.9
Glycine	34.6
Alanine	39.6
Valine	35.2
Methionine	13.4
Isoleucine	31.6
Leucine	41.1
Tyrosine	20.3
Phenylalanine	22.0
Lysine	30.7
Histidine	14.5
Arginine	34.4
Half-cystine	17.7
Tryptophan	ND[a]
Total	530

[a] Not determined.

Isolation of the Gln-tRNA Synthetase-tRNA^{Gln} Complex. I purified the Gln-tRNA synthetase to be able to study its interaction with modified glutamine tRNAs. In preparation for these studies a method was developed to isolate the complex formed between Gln-tRNA synthetase and tRNA^{Gln}, using sucrose gradient sedimentation. This approach has been used to isolate similar complexes between other AA-tRNA synthetases and their cognate tRNAs (Lagerqvist *et al.*, 1966; Yaniv and Gros, 1969; Knowles *et al.*, 1970). The method also allows one to determine how many tRNA molecules are bound to a molecule of enzyme.

In a 5–20% sucrose gradient, Gln-tRNA synthetase has a sedimentation coefficient of 4.1 S. Its sedimentation coefficient is the same at pH 5.5 as at pH 7.0. When Gln-tRNA synthetase is incubated with tRNA^{Gln} prior to centrifugation, and sedimented at pH 5.5 in the presence of Mg^{2+},[2] its sedimentation coefficient increases to 6.1 S (Figure 2). tRNA^{Gln} molecules, which in the absence of enzyme sediment at 4 S, cosediment with the enzyme indicating that a Gln-tRNA synthetase–tRNA^{Gln} complex has been formed.

The sotichiometry of the complex, as determined in three separate experiments, indicates that 1 molecule (±15%) of tRNA^{Gln} is bound to one enzyme molecule of 69,300. In the complex depicted in Figure 2, 97 pmoles of enzyme was associated with 88 pmoles of tRNA^{Gln}.

To demonstrate that the complex is not simply due to a nonspecific interaction between nucleic acid and protein, Gln-tRNA synthetase was preincubated with a mixture of tRNA^{Gln} and tRNA$_I^{Val}$ molecules. Only tRNA^{Gln} molecules sedimented with the enzyme at 6.1 S, whereas all the tRNA$_I^{Val}$

[2] In the absence of Mg^{2+}, a very heterogenous distribution of Gln-tRNA synthetase–tRNA^{Gln} is observed. There is no well-defined peak, as the complex sediments over a region from 6 to 9 S.

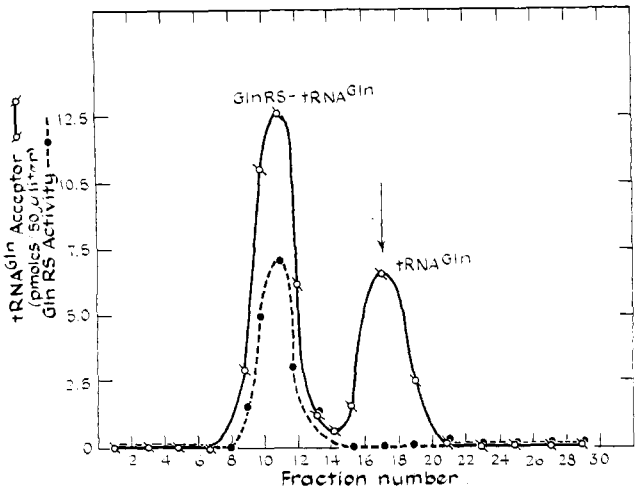

FIGURE 2: Sucrose gradient sedimentation of Gln-tRNA synthetase and $tRNA^{Gln}$. Gln-tRNA synthetase (17 μg) and $tRNA^{Gln}$ (0.2 A_{260}) were mixed with pH 5.5 buffer (see Methods) containing 10 mM $MgCl_2$ and layered on top of a 5% to 20% sucrose gradient containing pH 5.5 buffer. After centrifugation for 11 hr, fractions were collected and assayed for Gln-tRNA synthetase and $tRNA^{Gln}$. The arrow indicates the position of Gln-tRNA synthetase in the absence of $tRNA^{Gln}$. In the region of the Gln-tRNA synthetase–$tRNA^{Gln}$ complex, 97 pmoles of Gln-tRNA synthetase and 88 pmoles of $tRNA^{Gln}$ were found. Gln-tRNA synthetase activity on the ordinate is in arbitrary units.

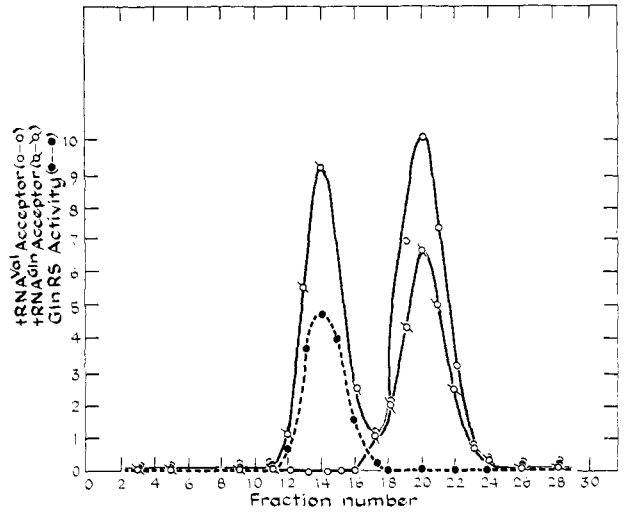

FIGURE 3: Sucrose gradient sedimentation of Gln-tRNA synthetase, $tRNA^{Gln}$, and $tRNA^{Val}$. Gln-tRNA synthetase (17 μg), $tRNA^{Gln}$ (0.2 A_{260}), and $tRNA^{Val}$ (0.3 A_{260}) were mixed with pH 5.5 buffer (see Methods) containing 10 mM $MgCl_2$ and layered on top of a 5–20% sucrose gradient containing pH 5.5 buffer. After 10-hr centrifugation, fractions were collected and assayed for Gln-tRNA synthetase, $tRNA^{Gln}$, and $tRNA^{Val}$. In the region of the Gln-tRNA synthetase–$tRNA^{Gln}$ complex, 80 pmoles of Gln-tRNA synthetase and 87 pmoles of $tRNA^{Gln}$ were found. Ordinates are in arbitrary units.

molecules and excess $tRNA^{Gln}$ molecules sedimented at 4 S (Figure 3).

Previous studies (Ravel *et al.*, 1965) with partially purified preparations of Gln-tRNA synthetase indicated that $tRNA^{Gln}$ must be bound by the enzyme before it will catalyze the exchange of pyrophosphate with ATP. One possible interpretation of this result is that the binding of substrates is ordered, and $tRNA^{Gln}$ must be bound before glutamine or ATP is bound. In agreement with this, I was not able to detect a complex between Gln-tRNA synthetase and [^{3}H]ATP and [^{14}C]glutamine in a sucrose gradient (at pH 5.5 and pH 7.0); nor did ATP (1 mM) and glutamine (5 mM) alter the sedimentation coefficient of the enzyme when both ligands were present throughout the gradient. It is possible, however, that such a complex is too unstable to be isolated by sucrose gradient sedimentation.

Also, preincubation of Gln-tRNA synthetase with $tRNA^{Gln}$, [^{3}H]ATP, and [^{14}C]glutamine resulted in the formation of a complex sedimenting at 6.1 S which contained Gln-tRNA synthetase and [^{14}C]Gln-$tRNA^{Gln}$, but no acid-soluble [^{14}C]-glutamine and [^{3}H]adenosine. This suggests that the ternary complex composed of [^{14}C]Gln-tRNA and [^{14}C]glutaminyl [^{3}H]adenylate bound to Gln-tRNA synthetase either is not formed, or is not stable enough to be isolated under these conditions. The formation of such a complex is thought to occur during the aminoacylation of $tRNA^{Ile}$ by Ile-tRNA synthetase (Yarus and Berg, 1969).

Discussion

The steps employed to purify Gln-tRNA synthetase are similar to ones used for many other AA-tRNA synthetases. A partially purified preparation of Gln-tRNA synthetase has been previously described, in a study demonstrating that separate enzymes esterify glutamic acid and glutamine to tRNAs in *E. coli* (Lazzarini and Mehler, 1964). This study indicates that the Gln-tRNA synthetase from *E. coli*, with a molecular weight of approximately 69,000, is one of the smaller AA-tRNA synthetases (Loftfield, 1971). The molecular weight of the enzyme under conditions where it would be expected to be fully active is the same as the estimate obtained in the presence of a high concentration of β-mercaptoethanol and SDS, suggesting that the native form is monomeric, and probably consists of a single polypeptide chain. Further studies will be necessary to confirm this point.

Other AA-tRNA synthetases from *E. coli* vary widely in size and structure. The Val- and Ile-tRNA synthetases consist of single polypeptide chains containing the catalytic site and the binding sites for all three substrates (Bertholot *et al.*, 1971; Arndt and Berg, 1970). A number of other AA-tRNA synthetases, such as the Phe- (Kosakowski and Böck, 1970) and Gly- (Ostrem and Berg, unpublished observations) tRNA synthetases, are large oligomeric proteins whose activities are dependent on subunit interactions. The Met-tRNA synthetase is oligomeric, but can be dissociated into subunits which are still active (Cassio and Waller, 1968; Bruton and Hartley, 1968). These wide size and structure differences in proteins catalyzing the same basic chemical reactions might result from the requirement for absolute specificity between the AA-tRNA synthetase, the amino acid, and its cognate tRNA, or from additional functions that some of these enzymes might perform in regulating cellular metabolism (Neidhardt, 1966).

The complex formed between Gln-tRNA synthetase and $tRNA^{Gln}$ is stable enough to be isolated by sucrose gradient sedimentation. Similar complexes have been detected, with other AA-tRNA synthetases, by electrophoresis (Okamoto and Kawade, 1967; Seifert *et al.*, 1968), by sucrose gradient sedimentation (Lagerqvist *et al.*, 1966; Yaniv and Gros, 1969; Knowles *et al.*, 1970), and binding to nitrocellulose filters (Yarus and Berg, 1967; Yaniv and Gros, 1969). Quantitation of the amount of active enzyme and the amount of $tRNA^{Gln}$

in the complex indicates that one enzyme molecule of 69,300 binds one $tRNA^{Gln}$.

One interesting feature of Gln-tRNA synthetase is its requirement for $tRNA^{Gln}$ to be able to catalyze the glutamine-dependent ATP–pyrophosphate-exchange reaction (Ravel *et al.*, 1965). This might be due to ordered binding of substrates, or $tRNA^{Gln}$ might activate the enzyme. I could not detect a glutaminyl adenylate–Gln-tRNA synthetase complex by sucrose gradient sedimentation. Either such a complex is not formed in the absence of $tRNA^{Gln}$, or it is not stable enough to be isolated by this method. Conceivably, $tRNA^{Gln}$ might activate Gln-tRNA synthetase by causing it to dimerize, or form higher order promoters.[3] The stoichiometry of binding measured by sucrose gradient sedimentation cannot differentiate between a monomer of 69,300 binding one $tRNA^{Gln}$ molecule, or a dimer of 138,600 binding two $tRNA^{Gln}$ molecules. Alternatively, the low yield of enzyme recovered from the gradient (usually 35–40%) introduces the possibility that a dimer of 138,600 might bind one $tRNA^{Gln}$ molecule, with only half the molecules enzymatically active (and therefore undetectable).

Both of the latter possibilities are unlikely, however, for the change in sedimentation coefficient between Gln-tRNA synthetase alone, and Gln-tRNA synthetase complexed with $tRNA^{Gln}$, is not great enough to be attributable to a complex containing two enzyme molecules and one or more $tRNA^{Gln}$ molecules. A doubling in molecular weight of the enzyme alone would result in a shift in sedimentation coefficient from 4.1 to 6.5 S (Martin and Ames, 1961), and the contribution of a $tRNA^{Gln}$ molecule might increase the value to 8S (Yaniv and Gros, 1969). This sedimentation coefficient is clearly much greater than what is found.

In order to substantiate the argument that one molecule of Gln-tRNA synthetase binds one $tRNA^{Gln}$, an attempt was made to chemically cross-link Gln-tRNA synthetase using dimethyl suberimidate (Davies and Stark, 1970). This reagent has been shown to react specifically with the ϵ-amino groups of lysine residues in proteins, and thereby cross-link the subunits of oligomeric proteins; under denaturing conditions, aggregates of the monomeric units are easily detected.

Gln-tRNA synthetase was treated with dimethyl suberimidate in the presence of $tRNA^{Gln}$ under conditions where the protein is enzymatically active (150 mM triethanolamine buffer, pH 8.5, containing 7 mM $MgCl_2$ and 60 μm $tRNA^{Gln}$). If the Gln-tRNA synthetase dimerized under such conditions, and if it were susceptible to cross-linking by the reagent, a protein of 138,600 would have been seen on SDS polyacrylamide gels. However, absolutely no such species was formed. Although this is a negative result, it is consistent with the postulate that one active Gln-tRNA synthetase of 69,300 binds to one $tRNA^{Gln}$ molecule.

Now that a purified preparation of Gln-tRNA synthetase is available, it should be possible to study its interactions with wild-type and altered $tRNA^{Gln}$ molecules. Recently, because $tRNA^{Gln}$ molecules can be separated from other tRNA species by sucrose gradient sedimentation with Gln-tRNA synthetase, Yaniv and I have been able to purify and sequence the two major species of $tRNA^{Gln}$ from *E. coli* (M. Yaniv and W. R. Folk, unpublished data).

[3] Recently, $tRNA^{Phe}$ has been reported to cause rat liver Phe-tRNA synthetase to aggregate (Sciscenti *et al.*, 1970).

Acknowledgments

I wish to thank Dr. Moshe Yaniv for his suggestions and criticism, and Dr. Paul Berg for his encouragement and support.

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