Circular Dichroism Study of the Interaction of Glutamyl-tRNA Synthetase with $tRNA^{Glu}_2$†

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ABSTRACT: The interaction of glutamyl-tRNA synthetase with $tRNA^{Glu}_2$ has been studied. The enzyme was purified to apparent homogeneity, and consists of a single chain with a molecular weight of 59 000. The sedimentation coefficient ($s°_{20,w}$) was found to be 3.7 S and suggests this enzyme is quite asymmetric. The enzyme binds 1 mol of $tRNA^{Glu}_2$ and has a binding constant of 5×10^6 M^{-1} at pH 7.0 in 0.1 M sodium chloride. A circular dichroic study of the interaction under the same solvent conditions implied both the synthetase and $tRNA^{Glu}_2$ underwent a change in conformation as the complex was formed. In the case of the enzyme, there appears to be some loss of α-helical structure. The $tRNA^{Glu}_2$ results can be interpreted to indicate a change in the conformation of one or more of the helical regions of this molecule. A residue in the anticodon loop, 5-methylaminomethyl-2-thiouridine, has a distinct circular dichroic band at 340 nm in the free $tRNA^{Glu}_2$. As the complex is formed this band is shifted to the blue. This was interpreted to indicate that the enzyme forms a hydrogen bond with this residue in the anticodon loop, with a change in the conformation of the loop possibly also having occurred.

The glutamyl-tRNA synthetase from *Escherichia coli* has been studied previously (Lapointe and Söll, 1972a,b) and reported to consist of a holoenzyme of two nonidentical subunits. The larger one, the catalytic subunit, had a molecular weight of 56 000, and the smaller one, which appeared to be a regulatory factor, had a molecular weight of 46 000. The evidence for the function of the 46 000 molecular weight subunit relied on kinetic data; no apparent physical interaction could be demonstrated. They were able to isolate the 56 000 molecular weight subunit in low yields by isoelectric focussing.

Since we were interested in studying the interaction of $tRNA^{Glu}$ and glutamyl-tRNA synthetase, we sought to purify the catalytically active subunit reported by Lapointe and Söll (1972a). We here report the purification of glutamyl-tRNA synthetase and some physical properties of the enzyme and its complex with $tRNA^{Glu}_2$. The method yields the enzyme pure as judged by sodium dodecyl sulfate gel electrophoresis and sedimentation equilibrium analysis and with an estimated molecular weight of 59 000.

The low molecular weight of the enzyme plus the particular properties of $tRNA^{Glu}_2$ make this system especially attractive for the study of the complex by its circular dichroism. $tRNA^{Glu}_2$ has a modified nucleotide at the 5′ end of the anticodon, mnm^5s^2U[1], which has an isolated CD band centered at about 340 nm (Willick et al., 1973). Thus, this system can be used as a probe for any changes in the anticodon region as the specific complex is formed. While the assignment cannot be made unambiguously, the rest of the CD spectrum can be analyzed for probable changes in the conformation of the protein and the tRNA. The results to be presented here suggest that both GluRS and $tRNA^{Glu}_2$ undergo some conformational change when the complex is formed. In addition, the results indicate that the anticodon region is likely involved in the specific binding to the enzyme.

Materials and Methods

Chemicals. Uniformly ^{14}C-labeled glutamic acid was purchased from Amersham-Searle and had a specific activity of 265 mCi/mmol. ATP was purchased from Terochem. The bovine serum albumin used was a standard solution purchased from Worthington Biochemicals. DEAE-cellulose (Whatman DE-32) was obtained from Mandel Scientific and hydroxylapatite (HT) from Bio-Rad Laboratories. Dextran T-500 was obtained from Pharmacia and polyethylene glycol 6000 from J. T. Baker. *E. coli* $tRNA^{Glu}_2$ was purchased from Boehringer-Mannheim and had a reported acceptor activity of 1200 pmol/A_{260}. Unfractionated *E. coli* tRNA was obtained from Sigma. All other chemicals were reagent grade.

Bacteria Growth. E. coli K12 (ED2612) (R17 resistant) was kindly provided by Dr. W. Paranchych. It was grown in a 300-l. fermentor at 37 °C in a 15 g/l. trypticase soy broth (BBL) medium. Yields were normally 800–900 g of cells (wet wt).

Enzyme Assay. The aminoacylation assay was carried out according to the procedure of Lapointe and Söll (1972a). One unit of enzyme is defined as that amount which forms 1 nmol of aminoacyl-tRNA in 5 min at 30 °C under the conditions of this assay.

Protein Concentration. The protein concentration in the crude fractions was estimated according to Lowry et al. (1951) using BSA as a standard. The concentration of purified GluRS was determined refractometrically, using the analytical ultracentrifuge. Using a value of 4.1 fringes equalling 1 mg/ml of solution, a value of $E_{280}^{mg/ml} = 1.35$ was determined for purified GluRS.

Ultracentrifuge Studies. Molecular weights were estimated by sedimentation equilibrium in a standard buffer containing 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M Na Hepes, 0.001 M DDT, 10% glycerol, pH 7.0. The molecular weight of GluRS was determined by a meniscus depletion method, using 7-mm solution columns (Millar et al., 1969). Sedimentation coefficients and the apparent molecular weight of the complex were determined using the photoelectric scanner at 280 nm.

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[1] Abbreviations used are: GluRS, glutamyl-tRNA synthetase; mnm^5s^2U, 5-methylaminomethyl-2-thiouridine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DEAE, diethylaminoethyl; DTT, dithiothreitol; ATP, adenosine 5′-triphosphate; BSA, bovine serum albumin; CD, circular dichroism.

TABLE I: Procedure Used to Give Purified GluRS.

Fraction	V (ml)	Total Protein (mg)	Total Units	Sp Act. (units/mg)	Recovery (%)	Relative Purification
I. Crude extract	900	11 400	8000	0.70	100	1
II. Liquid polymer extract	900	4 700	9300	2.0	116	2.9
III. DEAE-cellulose (pH 8.0)	200	240	4860	20	61	28.6
IV. Hydroxylapatite (pH 6.8)	36	61	4990	82	62	117
V. DEAE-Sephadex A-50 (pH 7.0)	11.6	21 (9.9[a])	3000	140 (303[a])	37	200(430[b])

[a] Concentration and specific activity based on extinction coefficient reported. [b] Relative purification based on specific activity given in *a*.

Viscosities of the solvent employed relative to that of water were measured using Ubbelhohde viscometers.

Amino acid Analysis. The GluRS was dialyzed extensively against water and lyophilized. Samples were hydrolyzed under reduced pressure in 6 N HCl, 0.5% phenol for 24 and 48 h, and the results were extrapolated to zero time. The value for tryptophan, relative to tyrosine, was determined in 6 M guanidine-HCl (Edelhoch, 1967).

Fluorescence Measurements. Fluorescence measurements were made in a Baird Atomic or Turner Model 210 spectrophotofluorimeter, with the cells thermostatically controlled at 19 °C. The quantum yield of GluRS was determined using the Turner Model 210 in the absolute mode with tryptophan used as a standard. The value of ϕ_{Trp} was taken as 0.13 (Chen, 1967). The correction used for the inner filter effect when absorbing ligands were added was $A(1 - 10^{-A_0})/A_0\ (1 - 10^{-A})$, where A_0 = absorbance of the enzyme alone at the exciting wavelength and A = total absorbance at this wavelength after the addition of ligand (Helene et al., 1971). Total absorbances were less than 0.2 at the final concentration, and the volume correction was less than 6%.

Circular Dichroic Studies. The CD spectra of GluRS, $tRNA^{Glu}_2$, and the complex were measured with a Cary Model 60 spectropolarimeter, equipped with a Model 6001 attachment. For complexing experiments, the GluRS was mixed with the appropriate amount of $tRNA^{Glu}_2$, while simultaneously the GluRS and $tRNA^{Glu}_2$ solutions were diluted with the appropriate buffer to the same final volume. Duplicate experiments gave the same qualitative results. A value of $\epsilon_{260}^{0.1\%}$ = 23.4 ml/mg for $tRNA^{Glu}_2$ was used (Willick et al., 1973). Unless otherwise stated, CD data is presented in terms of the appropriate mean residue weight. For GluRS a value of 115 was used and for $tRNA^{Glu}_2$ a value of 320. A value of 26 300 was used for the molecular weight of sodium $tRNA^{Glu}_2$ when results were expressed in molar quantities. Experiments were carried out at the ambient temperature of the instrument, 27 °C.

Results

Purification Procedure. General Remarks. The starting point of the purification procedure was a modification of that of Lapointe and Söll (1972a). Since the procedure finally used to give purified GluRS differs substantially from that of Lapointe and Söll (1972), the entire procedure is outlined below, and summarized in Table I. Concentrations were carried out by dialyzing against 30% polyethylene glycol 6000, with the exception of the purified enzyme. In this case, concentration was carried out under reduced pressure using Sartorious membrane filters (BDH). All steps were carried out at 4 °C.

Preparation of Cell Extract. Frozen *E. coli* cells (450 g wet wt) were suspended in two volumes of 0.01 M Tris-HCl, 0.01 M $MgCl_2$, 0.01 M NH_4Cl, 0.0005 M DTT, 10% glycerol, pH 8.0, and passed through a Sorvall-Ribi automatic cell fractionator (5–10 °C, 20 000 psi). Cell debris was removed by centrifuging in a Spinco Rotor 21 at 20 000 rpm for 30 min. The supernatant (900 ml) (fraction I) was used in the next step.

Liquid Polymer Extraction. Fraction I was adjusted to pH 8.0 with NH_4OH and 1 M potassium phosphate was added to give a final concentration of 0.1 M, final pH 8.0. An equal volume of an emulsion of 28% polyethylene glycol 6000 and 6% Dextran T-500 in 0.1 M potassium phosphate, 10% glycerol, 0.001 M DTT (pH 8.0) was then added and the mixture stirred for 2 h. After centrifuging at 6000*g* for 30 min to separate the phases, the upper phase was collected (1600 ml). Then the solution was stirred overnight with a thick slurry (800 ml) of DE-32 equilibrated with 0.01 M NH_4Cl, 0.01 M $MgCl_2$, 0.01 M Tris-HCl (pH 8.0). Bound protein was then eluted with 1 M NH_4Cl, 0.01 M $MgCl_2$, 0.01 M Tris-HCl, pH 8.0. The DE-32 was removed by centrifugation and the supernatant was dialyzed vs. the equilibrium buffer in the following step to give fraction II (final volume 900 ml).

DEAE-Cellulose Fractionation (pH 8.0). Fraction II was applied to a 6 × 30 cm column of DE-32 equilibrated with 0.05 M NH_4Cl, 0.01 M $MgCl_2$, 0.01 M Tris-HCl, 0.0005 M DTT, 10% glycerol (pH 8.0), and eluted with a linear gradient (4 l.) of NH_4Cl (0.05–0.4 M) in the equilibration buffer. A flow rate of 150 ml/h was maintained, and 15-ml fractions were collected. The bulk of the activity was eluted in two peaks in the range 0.2–0.3 M NH_4Cl. Approximately 70% of the fractions containing GluRS activity was collected, concentrated, and dialyzed against the equilibration buffer in the following step (fraction III) (final volume 200 ml).

Hydroxylapatite Fractionation (pH 6.8). Fraction III was applied to a 4.1 × 32 column of hydroxylapatite equilibrated with 0.05 M potassium phosphate, 0.0005 M DTT, 10% glycerol (pH 6.8). The column was eluted with a linear gradient (2 l.) of potassium phosphate (0.05–0.25 M). A flow rate of 75 ml/h was maintained and 10-ml fractions were collected. Fractions containing about 90% of the total activity were collected, concentrated, and dialyzed against the equilibration buffer of the next step to give fraction IV (final volume 36 ml). Since we found the hydroxylapatite resolution decreased with repeated use, the preparation reported used fresh hydroxylapatite.

DEAE-Sephadex A-50 Fractionation (pH 7.0). Fraction IV was applied to a 1.6 × 30 column of DEAE-Sephadex A-50 equilibrated against 0.2 M KCl, 0.01 M Na Hepes, 0.01 M $MgCl_2$, 0.001 M DTT, 10% glycerol (pH 7.0), and eluted with a linear gradient (600 ml) of KCl (0.2–0.35 M). A flow rate of 12 ml/h was maintained and 4-ml fractions were collected.

TABLE II: Amino Acid Compositions of GluRS.

Amino Acid	No. of Mol/59 000 g
Alanine	47
Arginine	36
Aspartic acid and asparagine	62
Glutamic acid and glutamine	70
Glycine	33
Histidine	21
Isoleucine	27
Leucine	49
Lysine	30
Methionine	13
Phenylalanine	16
Proline	24
Serine	28
Threonine	27
Tryptophan	11[a]
Tyrosine	19
Valine	30

[a] Estimated spectrophotometrically by method of Edelhoch (1967).

TABLE III: Summary of K_m Values for GluRS.[a]

Substrate	K_m (×10^6 M) This Work	Lapointe and Soll (1972)[b]	
ATP	340	40	250
Glutamic acid	60	5	86
tRNA$^{Glu}_2$	0.13	0.3	0.12

[a] K_m values were obtained in the presence of known saturating amounts of the remaining two cosubstrates at 30 °C. [b] First column is that of the holoenzyme reported by Lapointe and Soll; second column that of the 56K subunit.

The peak of GluRS activity was eluted at about 0.3 M KCl.

Purity of GluRS. GluRS obtained by this procedure gave a single band when examined by sodium dodecyl sulfate gel electrophoresis according to Weber and Osborn (1969). Our best preparations are in excess of 95% pure by this criteria. These preparations also gave a linear log C vs. r^2 plot in the ultracentrifuge over a range of 0.25–2.5 mg/ml. This latter method is also a sensitive indicator of molecular weight homogeneity.

Molecular Weight of GluRS. The molecular weight of GluRS as indicated by sodium dodecyl sulfate gel electrophoresis (Weber and Osborn, 1969) is 58 000 (±2000). Experiments in the analytical ultracentrifuge indicated a molecular weight of 59 000 (±3000), using a value for the partial specific volume ($\bar{v}$) of 0.73 ml g^{-1} calculated from the amino acid composition (Table II). The main source of error in this experiment is the choice of $\bar{v}$ in the solvent containing 10% glycerol. Since both techniques give essentially the same result, we feel safe in assigning a molecular weight of 59 000 (±3000) to the GluRS. This result agrees with that estimated by Lapointe and Söll (1972) for the catalytically active "subunit".

K_m Values for GluRS with ATP, Glutamic Acid, and tRNA$^{Glu}_2$. In order to compare this enzyme preparation with that of Lapointe and Söll (1972a), the K_m values for each of the substrates in the presence of saturating amounts of the cosubstrates were measured (Table III). While the K_m values do not agree identically with those reported for the 56K subunit of Lapointe and Söll (1972), it is clear that the results are much closer to the 56K subunit than to the holoenzyme. The synthetic substrate, 1,N^6-ethenoadenosine triphosphate was tried as a replacement for ATP. Although this substrate will replace ATP in many systems (Secrist et al., 1972), no aminoacylation was observed in its presence with this reaction.

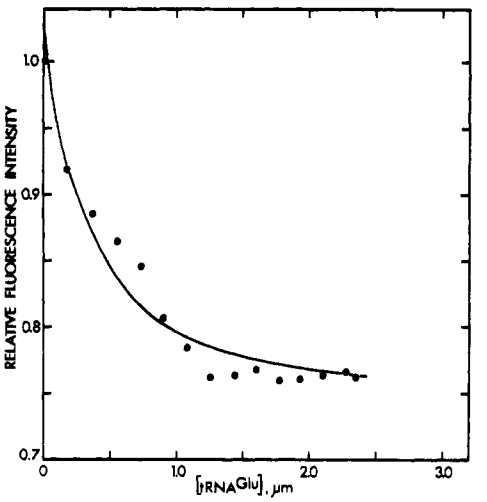

FIGURE 1: Fluorescence quenching of GluRS (0.23 μM) on addition of tRNA$^{Glu}_2$. The solvent was 0.1 M NaCl, 0.01 M Na Hepes, 0.01 M $MgCl_2$, 0.001 M DTT, 10% glycerol, pH 7.0. The solid line was calculated using a value for $K_{ass} = 5 \times 10^6$ M^{-1}.

Binding of tRNA^{Glu} to GluRS. The binding of tRNA^{Glu} to GluRS was measured by using the fact that the tryptophan fluorescence of the enzyme is partially quenched when the tRNA$^{Glu}_2$ is added (Figure 1). The stoichiometry of the binding was measured by monitoring the A_{280} distribution in a sedimentation equilibrium experiment. The maximum value of the apparent molecular weight, which was observed using a value for $\bar{v} = 0.67$ for the complex, was about 80 000. This value for $\bar{v}$ was calculated from the weighted average of the protein and tRNA$^{Glu}_2$ in the complex, using a value for $\bar{v}$ tRNA = 0.53 ml/g. Lapointe and Söll (1972b) also found a 1:1 complex by independent measurements. It was therefore assumed that the binding was 1:1 and the fluorescence quenching curve was analyzed by the method of Lam and Schimmel (1975). A value for K_{ass} for a 1:1 association can be estimated from the formula:

$$K = \frac{2}{2[\text{tRNA}]_{0,m} - [\text{E}]_0}$$

where $[\text{E}]_0$ = total enzyme concentration and $[\text{tRNA}]_{0,m}$ = value of the total tRNA concentration at the midpoint of the titration curve. If α = degree of association

$$= \frac{[\text{E-tRNA}]}{[\text{E}] + [\text{E-tRNA}]}$$
$$= \Delta F/\Delta F_\infty$$

then the F vs. [tRNA]$^{Glu}_2$ curve can be calculated using the formula given in Lam and Schimmel (1975). The curve drawn in Figure 1 utilized a value for $K_{ass} = 5 \times 10^6$ M^{-1}. Such a value is equivalent within experimental error to the value observed by Lam and Schimmel (1975) for the binding of tRNA^{Ile} to a cognate synthetase under similar conditions of ionic strength and pH.

Circular Dichroism of GluRS, tRNA$^{Glu}_2$, and the Complex

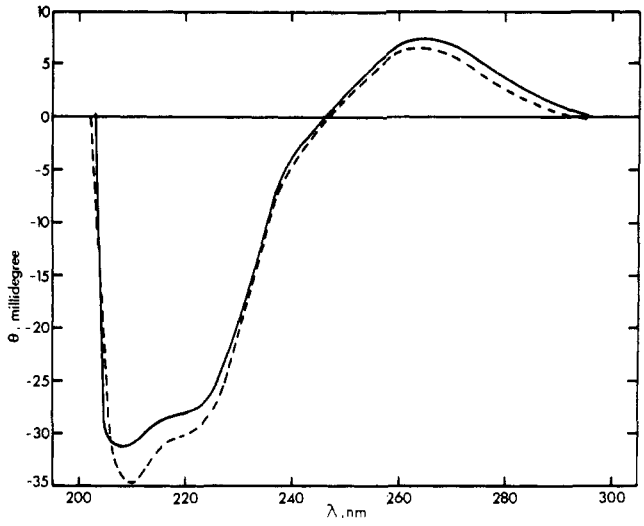

FIGURE 2: CD of the GluRS-$tRNA^{Glu}_2$ complex (—), and the sum of the spectra of RNA^{Glu}_2 and GluRS (- - -). The solvent was the same as in Figure 1. The concentration of GluRS was 8.6 μM and of $tRNA^{Glu}_2$ 6.6 μM. The calculated value for α is 0.72. Data is expressed in terms of millidegrees of ellipticity observed with each of the three samples.

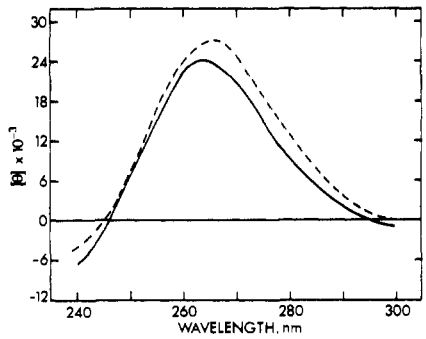

FIGURE 3: CD spectrum of $tRNA^{Glu}_2$ (—) and the spectrum of the complex minus the spectrum of GluRS presented on the basis of the mean residue weight of $tRNA^{Glu}_2$ (- - -). Other conditions were as in Figure 2.

(*200–300 nm*). Solutions of GluRS, $tRNA^{Glu}_2$, and the mixture of these two were prepared such that the final concentrations of the GluRS and $tRNA^{Glu}_2$ in the complex were the same as in the solutions of GluRS and $tRNA^{Glu}_2$. Therefore, if no change in the CD occurred on formation of the complex, the CD of the complex should be the same as the sum of the spectra of the GluRS and $tRNA^{Glu}_2$. Figure 2 shows the results obtained. The two spectra do not superimpose and, therefore, some conformational changes have probably occurred in the GluRS, $tRNA^{Glu}_2$, or in both of these components. In the region above about 245 nm the GluRS is essentially optically inactive when compared to the $tRNA^{Glu}_2$. Figure 3 shows the results obtained by subtracting the spectrum of GluRS from that of the complex and expressing results in terms of the mean residue weight of the $tRNA^{Glu}_2$. Both Figures 2 and 3 demonstrate that an increase in ellipticity of the positive band centered at about 265 nm, accompanied by a red shift of about 5 nm, has occurred as $tRNA^{Glu}_2$ binds to GluRS. A possible explanation for this will be given later.

The results of subtracting the $tRNA^{Glu}_2$ spectrum from that of the complex and expressing both this and the GluRS data on the basis of the mean residue weight of GluRS are shown in Figure 4. The results suggest the loss of some secondary structure in GluRS has occurred when the complex is formed.

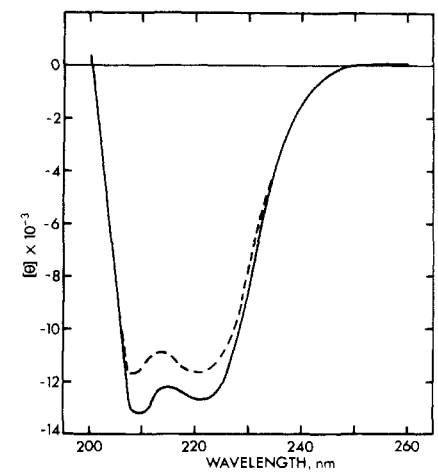

FIGURE 4: CD spectrum of GluRS (—) and the spectrum of the complex minus the spectrum of $tRNA^{Glu}_2$ presented on the basis of the mean residue weight of GluRS (- - -). Other conditions were as in Figure 2.

However, it must be emphasized that the results of the change in this region cannot be interpreted unambiguously, since both $tRNA^{Glu}_2$ and GluRS contribute to the spectrum.

Past experience has indicated that any conformational change giving rise to an increase in the major tRNA band at 260 nm is generally compensated by an increase in the absolute value of the negative band at 209 nm (Willick et al, 1973). Thus, the decrease in the GluRS spectrum shown in Figure 4 is a minimal value. In addition, since the calculated value for α is 0.72 under the conditions of this experiment, almost 30% of the GluRS is not bound. This also means the difference shown in Figure 4 is a minimum value.

The spectra shown in Figure 4 were analyzed for secondary structure using the methods of Chen et al. (1974). One analysis used a constrained linear fit, minimized in the least-squares sense, with the constraint being that $0 < f_\alpha, f_\beta, f_R < 1$, where f_α, f_β, and f_R are the fractions of α helix, β structure, and random structure, respectively. The results, with error value of the fitting procedure in brackets, were: $f_\alpha = 0.42$ ($\pm$0.01), $f_\beta = 0.21$ ($\pm$0.06), and $f_R = 0.36$ ($\pm$0.02) for GluRS. If the spectrum of the GluRS-$tRNA^{Glu}_2$ complex is analyzed assuming the change is essentially all due to GluRS, then the results were 0.38 ($\pm$0.01), 0.27 ($\pm$0.04), and 0.35 ($\pm$0.03) for f_α, f_β, and f_R. Method 2 of Chen et al (1974) was also used. This method allows for variation in the α-helical chain length. It was found that a value of 42 residues for the average chain length gave the best fit for the experimental curve; the estimated values for f_α, f_β, and f_R were 0.47 ($\pm$0.05), 0.33 ($\pm$0.05), and 0.18 ($\pm$0.3), respectively. Thus, it appears that the absolute value of 40–50% for the α-helical content of GluRS is reasonable. These methods cannot be relied on at present to give reasonable values for f_β and f_R. We can also say with a reasonable certainty that some secondary structure has been lost by the GluRS when it binds $tRNA^{Glu}_2$, and that this is probably a small amount of the α-helical structure.

CD Spectra of GluRS, $tRNA^{Glu}_2$, and Complex (300–380 nm). The formation of the complex results in a marked change in the CD spectrum in this region (Figure 5). The minimum at 305 nm decreases in absolute magnitude, probably due to the apparent red shift and increase in the major band shown in Figures 2 and 3. The band due to mnm^5s^2U with a minimum at 335 nm, appears to undergo a blue shift of about 10 nm, with a possible decrease in absolute magnitude. A tentative explanation for this result will be discussed later.

Discussion

The results of this work indicate that GluRS is a single chain polypeptide with a molecular weight of about 59 000. This enzyme is therefore one of the smallest active units reported so far (Söll and Schimmel, 1974), although the tyrosyl-tRNA synthetase from yeast has a molecular weight of 46 000 (Beikirch et al., 1972). The GluRS has a $s^{\circ}_{20,w}$ of about 3.7 S. This value, coupled with a value of $\bar{v} = 0.73$ ml/g, gives a frictional coefficient (f/f_0) ratio of 1.47 when compared to an unhydrated sphere of the same volume. The estimated axial ratio, if the molecule is treated as a prolate ellipsoid, is then 7.5:1. If we assume an approximate value for the effect of hydration on the value of $\bar{v}_{Hyd} = \bar{v}_{GluRS} + \delta\bar{v}_{H_2O}$ (Tanford, 1961), then the frictional ratio drops to 1.38. This calculation assumes a value for $\delta = 0.2$. The axial ratio for the corresponding prolate ellipsoid is then 6:1. Thus, the data suggests the enzyme is quite asymmetric, with a long axis in the range 150–200 Å. The distance from the anticodon to the 3′ end in yeast tRNA^{Phe} crystals is 75 Å (Suddath et al, 1974). Recent work indicates the synthetase must bind to a large region of the cognate tRNA (Budzik et al, 1975; Schoemaker et al, 1975). It is not surprising, therefore, that a synthetase as small as GluRS would be quite asymmetric.

The CD results in Figures 2, 3, and 4 suggest there is possibly some conformational change occurring in both GluRS and tRNA$^{Glu}_2$. An earlier study by Ohta et al (1967) on the tyrosyl-tRNA synthetase interaction with tRNA^{Tyr} indicated a loss of secondary structure in the enzyme as the tRNA^{Tyr} was bound. This study also strongly suggests the GluRS suffers some loss of secondary structure, probably α helical. Such a result is not unreasonable, since some of the binding sites for the tRNA$^{Glu}_2$ may have been involved in a secondary structure of the free enzyme. An extensive loss of secondary structure does not occur.

The apparent conformational change in the tRNA$^{Glu}_2$ indicated by Figures 2 and 3 must be viewed with caution. The change observed could be accounted for by only a minor change in the helical structure of the tRNA$^{Glu}_2$ when it is bound by the GluRS. A recent nuclear magnetic resonance study on this same complex indicated that all hydrogen bonds present in the uncomplexed tRNA$^{Glu}_2$ remained intact on complex formation (Shulman et al, 1974). These results are not necessarily in contradiction with our CD observations. Although we believe the CD results are most easily explained by assuming a change in the conformation of one or more helical regions of tRNA$^{Glu}_2$ as the complex is formed, such subtle changes can occur without disrupting the hydrogen bonds, yet give significant changes in the CD spectrum.

The CD spectra involving the mnm^{5}s^{2}U residue in the anticodon can perhaps be interpreted less ambiguously. This band has been examined more closely previously (Willick et al, 1973). Reaction of the thiolated residue with cyanogen bromide eliminated this band and demonstrated the remainder of the tRNA$^{Glu}_2$ molecule had virtually no ellipticity above 320 nm. When different divalent cations were used as counterions, there were quite clear indications of varying conformations of this molecule. In no case, however, was there any indication of any shift in the CD minimum associated with the mnm^{5}s^{2}U residue; only decreases in the magnitude of the band were observed. In the case of tRNA$^{Glu}_2$ binding to GluRS, there at the very least has occurred a blue shift of the band of up to 10 nm, and possibly a change in the rotational strength as well. This band has a very low absorption and a high rotational strength (Willick et al, 1973), which is characteristic of a n → π^* transition. Thus, such a shift in the band might be anticipated to occur if, for example, a hydrogen bond with some residue in the GluRS was formed with this thiolated nucleotide. Determining the absolute position of the band in the complex and its rotational strength is complicated by the fact that the CD spectra associated with the remaining residues has undergone a small red shift. Nonetheless, it seems clear that the anticodon area of the tRNA$^{Glu}_2$ is somehow bound to the GluRS. Binding of the anticodon loop to the cognate synthetase has been indicated by photo-cross-linking studies of tRNA^{Tyr} with Tyr-tRNA synthetase (Schoemaker and Schimmel, 1974). This study provides some direct physical chemical evidence that a portion of the anticodon is involved in the binding of the tRNA$^{Glu}_2$ to GluRS.

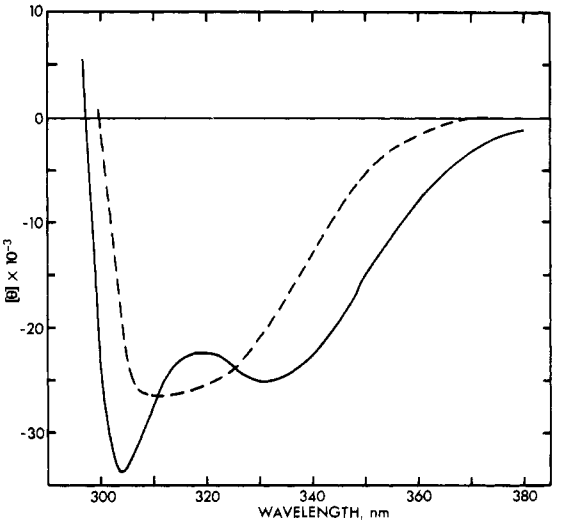

FIGURE 5: Spectrum of tRNA$^{Glu}_2$ (—) and the complex (- - -), presented on the basis of the molar concentration of the mnm^{5}s^{2}U residue. Solvent conditions were as in Figure 1. The concentration of GluRS was 30 μM and of tRNA$^{Glu}_2$ 23 μM. The calculated value for α is 0.75.

Acknowledgments

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