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Articles

tRNA^{fMet}-Induced Conformational Transition at the Intersubunit Domain of Fluorescent-Labeled Methionyl-tRNA Synthetase†

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ABSTRACT: Conformational transition in methionyl-tRNA synthetase upon binding of tRNA^{fMet}, whose binding shows strong negative cooporativity, was analyzed by fluorescence spectroscopy. The fluorescent probe *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (1,5-I-AEDANS) reacts with native methionyl-tRNA synthetase in a nearly stoichiometric amount (2 per dimer) without affecting enzyme activity. The probe is shown by controlled trypsinization to be located in a 130 amino acid fragment at the C-terminus joining the subunits. The emission and excitation spectra, rotational freedom, and solvent accessibility of the fluorophore in AEDANS-methionyl-tRNA synthetase are analyzed. The results suggest that the probe is localized in a nonpolar environment, nearly immobile relative to methionyl-tRNA synthetase yet fully accessible to the solvent. Upon binding of tRNA^{fMet}, the fluorescence intensity in AEDANS-methionyl-tRNA synthetase was appreciably reduced without a shift in the emission or excitation spectra. Lifetime measurement shows that a static mechanism accounts for the observed quenching. Furthermore, the remaining emitting AEDANS becomes effectively shielded from solvent molecules. These results suggest an unsymmetric conformational transition at the intersubunit domains of the two subunits in methionyl-tRNA synthetase upon binding one molecule of tRNA^{fMet}.

Methionyl-tRNA synthetase catalyzes the methionylation of tRNA^{Met} and tRNA^{fMet} and methionine-dependent ATP–PP_i exchange. Methionyl-tRNA synthetase from *Escherichia coli* is a dimeric enzyme with a subunit molecular weight of 76 000. The enzyme exhibits strong negative cooperativity toward binding of tRNA^{fMet} at low concentration of magnesium (Blanquet et al., 1979). The dimeric enzyme dissociates to a fully active monomeric form upon cleavage of a 130 amino acid fragment from the C-terminus by controlled trypsinization (Cassio & Waller, 1971). The three-dimensional structure of the trypsinized form of methionyl-tRNA synthetase has been resolved at 2.5 Å (Zelwer et al., 1982). The amino acid sequence of methionyl-tRNA synthetase has been recently determined from the nucleotide sequence of cloned genes (Barker et al., 1982; Dardel et al., 1984). Thus, the interaction of tRNA and methionyl-tRNA synthetase (Ackerman et al., 1985) provides a good model for nucleic acid–protein interaction as well as negative cooperativity. Conformational changes in methionyl-tRNA synthetase have been observed by using fluorescence (Blanquet et al., 1973) and neutron scattering spectroscopy (Dessen et al., 1978, 1982). However, the structure of the tRNA–synthetase complex and the dynamics of methionyl-tRNA synthetase are not well understood. In this paper, we report the specific covalent attachment of a fluorescent probe at the intersubunit domain of methionyl-tRNA synthetase and its conformational changes upon binding of tRNA^{fMet}. A preliminary report has appeared (Ferguson & Yang, 1981).

Materials and Methods

Dansylhydrazine and *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (1,5-I-AEDANS)[1] were ob-

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[1] Abbreviations: MetRS, methionyl-tRNA synthetase; AEDANS, *N*-[(acetylamino)ethyl]-5-naphthylamine-1-sulfonic acid; 1,5-I-AEDANS, *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid; dansyl, 5-(dimethylamino)naphthalene-1-sulfonyl; ME-AEDANS, *N*-[[[(2-mercaptoethoxy)acetyl]amino]ethyl]-5-naphthylamine-1-sulfonic acid; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride.

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tained from Molecular Probes. ME-AEDANS was prepared by the reaction of 1,5-I-AEDANS with 2-mercaptoethanol (Hudson & Weber, 1973). Acrylamide was recrystallized from ethyl acetate before use. Spectrophotometric-grade guanidine hydrochloride and dimethyl sulfoxide were obtained from Heico and Aldrich, respectively. Cibacron Blue F3GA was obtained from Pierce. All other chemicals were reagent grade or the purest form available from standard sources.

Methionyl-tRNA synthetase was prepared according to Lemoine et al. (1968) with an additional step of column chromatography on Cibacron Blue–Sepharose (Ferguson & Yang, 1986). Fluorescence measurements including quantum yield, fluorescence lifetime, and dynamic quenching are carried out and analyzed as previously described (Ferguson & Yang, 1986). All fluorescence measurements are carried out in the standard buffer, 10 mM imidazole hydrochloride (pH 7.5), 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol.

Preparation of AEDANS-MetRS. AEDANS-MetRS was prepared by the reaction of native, dimeric MetRS with *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (1,5-I-AEDANS). The reaction contained, in a total volume of 150 μL, 3.5 mM 1,5-I-AEDANS, 2.5 mg/mL Met RS, 50 mM sodium phosphate (pH 7.0), and 20% glycerol. The reaction contained about a 250-fold molar excess of 1,5-I-AEDANS with respect to MetRS. Prior to reaction, MetRS was extensively dialyzed at 4 °C against the 50 mM sodium phosphate (pH 7.0) and 20% glycerol buffer. 1,5-I-AEDANS solution was prepared, just prior to its use, in the 50 mM sodium phosphate (pH 7.0) and 20% glycerol buffer. The 1,5-I-AEDANS concentration was determined from the absorption at 336 nm and by using $\epsilon_{336nm} = 6.1 \times 10^3$ M^{-1} cm^{-1} (Hudson & Weber, 1973). 1,5-I-AEDANS is photosensitive and was therefore protected from light at all times (Hudson & Weber, 1973). The reaction was incubated for 4 h at 25 °C, in the dark. The reaction was terminated by passage of the reaction mix through a Sephadex G-25 column (9.0 cm × 0.5 cm) equilibrated with a buffer containing 20 mM imidazole hydrochloride (pH 7.5), 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol. Fractions of about 130-μL volume were collected at 4 °C. The MetRS-containing fractions were identified by a blue fluorescence and separated by thin-layer chromatography on silica gel with ethanol as the solvent. AEDANS-MetRS remained at the origin, while free 1,5-I-AEDANS has an R_f value of 0.70. The AEDANS-MetRS-containing fractions were pooled and concentrated from about 800 μL to about 400 μL by using an Amicon microconcentrator and PM-10 membrane. The concentrated sample was dialyzed extensively against a buffer containing 25 mM potassium phosphate (pH 7.5), 10 μM 2-mercaptoethanol, and 50% glycerol, at 4 °C. The free AEDANS, remaining after the G-25 filtration, was removed during this dialysis step. No free AEDANS could be detected by thin-layer chromatography in the purified preparation of AEDANS-MetRS. The sample containing AEDANS-MetRS was stored at −20 °C, at a concentration of about 1.5 mg/mL. In this preparation of AEDANS-MetRS, the recovery of protein is about 85% and the overall dilution of the initial stock MetRS concentration is about 3-fold.

Fluorescence Titration of AEDANS-MetRS. The interaction of MetRS with $tRNA^{fMet}$ is accompanied by a large decrease in the AEDANS-MetRS emission intensity, monitored at 490 nm with an excitation wavelength at 348 nm.

The functions used to fit the data were calculated with (Pachmann et al., 1973; Engel, 1974)

$$\alpha = \frac{\Delta F}{\Delta F_{max}} = \frac{(nE_0 + x_0 + K) - [(nE_0 + x_0 + K)^2 - 4nE_0x_0]^{1/2}}{2nE_0} \quad (1)$$

where

$$\alpha = \frac{\Delta F}{\Delta F_{max}} = \frac{F_0 - F}{F_0 - F_{max}} = \frac{x_b}{nE_0}$$

F_0, F, and F_{max} are the AEDANS-MetRS emission intensities in the absence of $tRNA^{fMet}$, in the presence of limiting $tRNA^{fMet}$, and in the presence of saturating $tRNA^{fMet}$, respectively. x_0 is the total concentration of $tRNA^{fMet}$, x_b is the concentration of bound $tRNA^{fMet}$, n is the number of binding sites per MetRS molecule, and E_0 is the total MetRS concentration.

Steady-State Fluorescence Anisotropy. The emission anisotropy, r, was measured according to the definition

$$r = \frac{F_{VV} - F_{VH}(F_{HV}/F_{HH})}{F_{VV} + 2F_{VH}(F_{HV}/F_{HH})}$$

where F_{12} is an emission intensity and the subscripts 1 and 2 indicate the position of the excitation and emission polarizers, respectively. The polarizers are in either the vertical, V, or horizontal, H, position.

The steady-state anisotropy, r, for a rigid sphere is related to the rotational correlation time by (Yguerabide, 1972; Cantor & Tao, 1971)

$$1/r = 1/r_0 + (1/r_0)(\tau/\phi) \quad (2)$$

where r_0 is the limiting anisotropy in the absence of molecular rotations, τ is the lifetime of the excited singlet state, and ϕ is the rotational correlation time.

The steady-state anisotropy is measured as a function of solvent viscosity. The solvent viscosity of a sample was varied, at 20 °C, by decreasing the sucrose concentration by dilution with standard buffer or by increasing the sucrose concentration by dilution with standard buffer containing 64% sucrose. The viscosity values for sucrose in water at 20 °C were taken from the *Handbook of Biochemistry and Molecular Biology* (Fasman, 1975). It was verified that the emission spectra of the fluorophore were not shifted as the sucrose concentration was varied. The limiting anisotropy was determined as the intercept of a linear Perrin plot, and an apparent rotational correlation time was determined from the slope of a linear Perrin plot by using eq 2.

Results

Modification of MetRS with AEDANS. Modification of MetRS with 1,5-I-AEDANS was carried out with a 250-fold molar excess of the modifying reagent at pH 7.0 and 25 °C. Free dye was removed by gel filtration and extensive dialysis.

The labeling stoichiometry in AEDANS-MetRS was determined from the difference spectrum and the absorbance at 337 nm by using $\epsilon_{337nm} = 6.0 \times 10^3$ M^{-1} cm^{-1}, which is the molar extinction coefficient for the product of the reaction of 1,5-I-AEDANS with *N*-acetylcysteine (Hudson & Weber, 1973). The labeling stoichiometry for AEDANS-MetRS was found to be 2.2 mol of AEDANS per mole of MetRS or 1.1 mol of AEDANS per mole of subunit MetRS.

The AEDANS-MetRS labeling stoichiometry was also determined from the fluorescence measurements. In the presence of 6 M guanidine hydrochloride, the excitation and emission spectra for ME-AEDANS and AEDANS-MetRS are identical, suggesting that the quantum yield of the fluorophore is the same for ME-AEDANS and AEDANS-

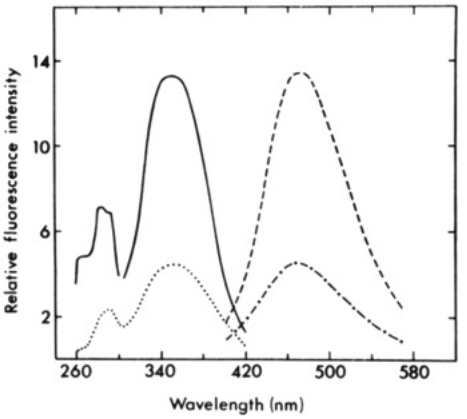

FIGURE 1: Fluorescence excitation (···, —) and emission (-·-, - - -) spectra for 0.43 μM AEDANS-MetRS in the absence (—, - - -) and presence (···, -·-) of 1.1 μM tRNA^{fMet}. The spectra have not been corrected for the wavelength dependence of the lamp intensity or the detection system sensitivity.

MetRS. The molar concentration of AEDANS in AEDANS-MetRS was determined by comparing the emission intensity of ME-AEDANS with that of AEDANS-MetRS in 6 M guanidine hydrochloride at 500 nm (λ_{ex} = 350 nm). On the basis of fluorescence measurements, the labeling stoichiometry was determined to be 1.5 mol of AEDANS per mole of MetRS or about 0.8 mol of AEDANS per mole of subunit MetRS.

In the presence of 6 M guanidine hydrochloride, the peak at 290 nm in the excitation spectrum of AEDANS-MetRS (Figure 1) is completely eliminated, indicating that the peak at 290 nm is due to Forster energy transfer between the intrinsic fluorescence and AEDANS in the native conformation of MetRS.

Location of the Fluorophore in AEDANS-MetRS. In order to locate the AEDANS label in AEDANS-MetRS, AEDANS-MetRS was subjected to limited proteolysis. Controlled trypsinization of native, dimeric MetRS leads to the release of enzymatically inactive fragments corresponding to 20% of the original protein, with concomitant dissociation of the dimer into fully active monomer (Cassio & Waller, 1971). The trypsin-modified MetRS has the same amino-terminal sequence as the native enzyme (Koch & Bruton, 1974). Thus, the two subunits in native MetRS associate through interactions of the carboxyl-terminal structural domain of each subunit (Gulik et al., 1976; Dessen et al., 1982). A similar conclusion was reached for alanyl-tRNA synthetase by employing in vitro mutagenesis (Jasin et al., 1983).

The results of the limited trypsinization of AEDANS-MetRS followed by SDS–polyacrylamide gel electrophoresis are shown in Figure 2. The AEDANS fluorophore was detected in the polyacrylamide gel by the fluorescence under ultraviolet light. Clearly, the AEDANS label is not retained on the M_r 64000 trypsin-modified monomer of MetRS. From scans of the negatives of the photographs of gels, it was determined that <10% of AEDANS is retained on the trypsin-modified MetRS. After trypsinization, a new fluorescent band (Figure 2, lane 5) appeared at the dye front, which corresponded to a molecular weight of less than 25000. Since untrypsinized AEDANS-MetRS that had undergone the same treatment for SDS gel electrophoresis did not show any fluorescence at the dye front, the new fluorescent band likely corresponded to the remaining M_r 12000 fragment or its subfragments. These results suggest that the AEDANS label is located predominantly in the intersubunit domain at the carboxyl terminus of MetRS.

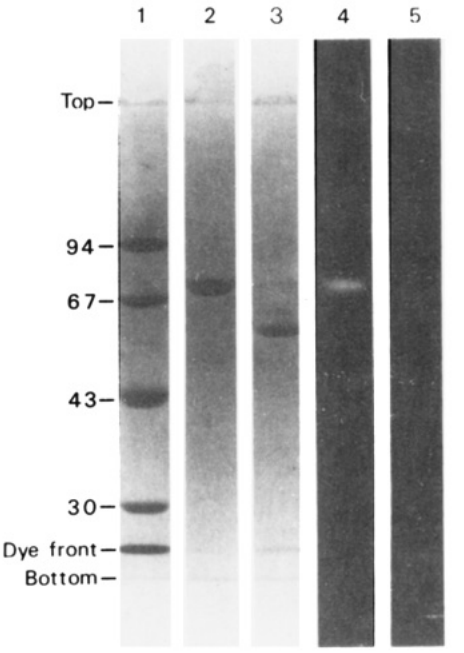

FIGURE 2: Analysis by 8.5% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate of the limited proteolysis of AEDANS-MetRS by trypsin. AEDANS-MetRS (0.13 mg/mL in standard buffer) was incubated at 37 °C in the presence of trypsin (1:250 w/w). At 0 (lanes 2 and 4) and 15 min (lanes 3 and 5), 20-μL aliquots were withdrawn, and the reaction was stopped by the addition of 15 μL of denaturing solution containing 0.14 M Tris-HCl (pH 7.0 at 25 °C), 2.9% sodium dodecyl sulfate, 29% glycerol, and 2 M 2-mercaptoethanol. The sample was vortexed and kept at 4 °C prior to electrophoresis. Electrophoresis was performed according to the method of Laemmli (1970). Following electrophoresis, the gel was washed in ice-cold 70% ethanol–water in order to prevent the protein from diffusing out of the gel and in order to enhance the AEDANS fluorescence. Lanes 4 and 5 show the gel treated with 70% ethanol–water and photographed under ultraviolet light illumination. Lanes 2 and 3 show the same gel as in lanes 4 and 5 following staining with Coomassie Brilliant Blue R-250 (Vesterberg, 1971) Lane 1 shows the following Coomassie Brilliant Blue R-250 stained molecular weight standards: phosphorylase *a* (94K), bovine serum albumin (67K), ovalbumin (43K), and carbonic anhydrase (30K).

The specific activity of aminoacylation by AEDANS-MetRS was found to be 80% of that for unmodified MetRS. The small difference may be due to the extensive process of the preparation. The fact that the catalytic activity is not affected by the modification is also consistent with the label being located at the intersubunit domain of MetRS.

Fluorescence Properties of AEDANS-MetRS. The excitation and emission spectra (Figure 1) and the emission decay time course (Figure 3) of the AEDANS fluorophore in AEDANS-MetRS are consistent with the AEDANS group localized in a relatively nonpolar environment, which is effectively equivalent to an 80% ethanol–water mixture (Hudson & Weber, 1973).

The solvent accessibility of AEDANS is examined by the quenching of emission by acrylamide. As shown in Figure 4, the Stern–Volmer plot is linear, indicating that acrylamide quenches ME-AEDANS by a bimolecular, collisional or dynamic mechanism. Acrylamide also efficiently quenches AEDANS-MetRS. The collisional quenching rate constants, k_q, determined from the Stern–Volmer plot and the emission lifetime, are shown in Table I. The fluorophore in AEDANS-MetRS is quenched by acrylamide just 2-fold less efficiently than ME-AEDANS. This result suggests that the fluorophore in AEDANS-MetRS is fully accessible to solvent, rather than being buried in the interior of the protein.

The rotational correlation time for the AEDANS fluoro-

Table I: Fluorescence Properties of ME-AEDANS, AEDANS-MetRS, and tRNA^{fMet}-Complexed AEDANS-MetRS

	ME-AEDANS	AEDANS-MetRS	AEDANS-MetRS complexed with tRNA^{fMet}
λ_{max}^{ex} (nm)	343	348	348
λ_{max}^{em} (mm)[a]	520	490	490
rel emission intensity F/F_0 at 480 nm (λ_{ex} = 350 nm)	nd[i]	1.0	0.35[b]
emission decay parameters[c] A, τ (ns)	1.0, 10	0.31, 19	0.12, 19
anisotropy r[d]	0.002	0.165	0.175
limiting anisotropy[e] r_0	0.052	0.216	(0.216)
rotational correlation time[f] ϕ (ns)	0.45	62	80
Stern–Volmer quenching constant[g] K_q (M^{-1})	7.4	7.4	1.8
bimolecular quenching rate constant[h] k_q (M^{-1} ns^{-1})	0.74	0.37	0.095

[a] Corrected for the wavelength dependence of the fluorometer detection system sensitivity as described under Materials and Methods in Ferguson and Yang (1986). [b] Determined from the titration of AEDANS-MetRS with tRNA^{fMet}. [c] Determined from the decay data shown in Figure 3. [d] Measured at 490 nm (λ_{ex} = 350 nm) in standard buffer at 20 °C. [e] Determined from the intercept of the Perrin plot, shown in Figure 5. [f] Calculated from the Perrin plot slope and intercept; τ was 19 ns. [g] Determined from the slope of the Stern–Volmer plot for acrylamide quenching (Figure 4). [h] Calculated by using τ_0 = 19 ns for MetRS and 10 ns for ME-AEDANS. [i] nd, not determined.

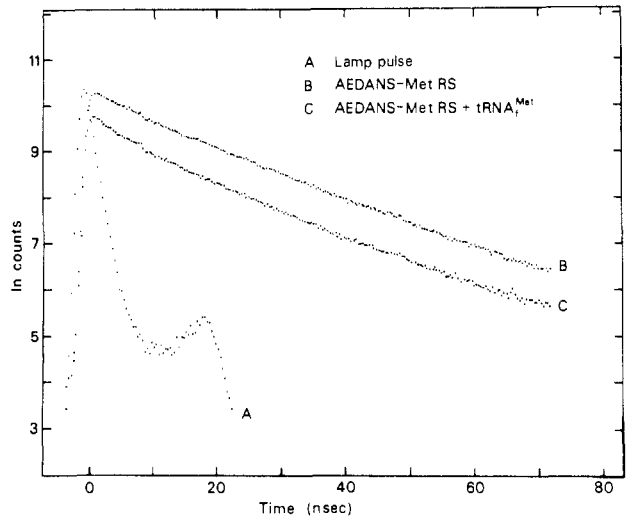

FIGURE 3: Nanosecond emission decay data for 0.43 μM AEDANS-MetRS in the absence (B) and in the presence of 1.8 μM tRNA^{fMet} (C). The excitation pulse (A) is also shown. The excitation light and emission light were selected by using Corning 7-60 and 3-73 filters, respectively.

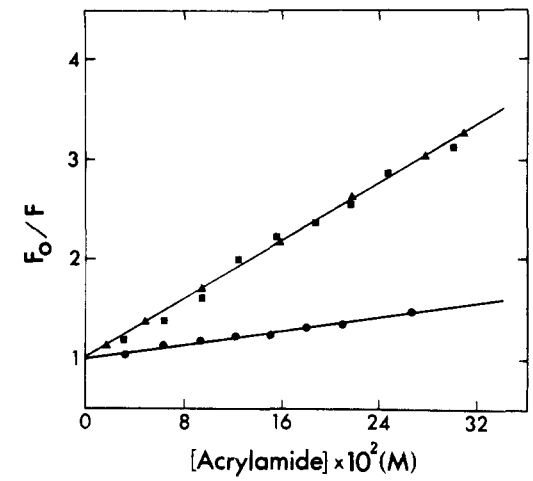

FIGURE 4: Stern–Volmer plot for the acrylamide quenching of ME-AEDANS (▲) and 0.43 μM AEDANS-MetRS in the absence (■) and presence (●) of 1.1 μM tRNA^{fMet}. The emission intensity, F, was monitored at 480 nm (λ_{ex} = 350 nm). F_0 is the initial emission intensity in the absence of acrylamide. The Stern–Volmer quenching constants, K_q, were determined from the slopes of the lines fitting the data and are given in Table I.

phore in AEDANS-MetRS was determined from the slope of the Perrin plot and limiting anisotropy (Figure 5 and Table I). The corresponding rotational correlation time for AEDANS in AEDANS-MetRS is calculated to be 62 ns, with the AEDANS emission lifetime being 19 ns.

Interaction of AEDANS-MetRS with tRNA^{fMet}. As shown in Figure 1, the binding of tRNA^{fMet} to AEDANS-MetRS quenches more than half of the emission of the AEDANS fluorophore. The AEDANS excitation and emission spectra are not shifted upon the binding of tRNA^{fMet} to AEDANS-MetRS. The binding affinity and stoichiometry of tRNA^{fMet} for AEDANS-MetRS (K_d = 0.1 μM), as measured by the fluorescence titration of AEDANS emission intensity (Figure 6), is close to that expected for unmodified MetRS (Ferguson & Yang, 1986; Dessen et al., 1978). Similar results were obtained by monitoring the intrinsic fluorescence.

The emission lifetime is not changed when AEDANS-MetRS complexes with tRNA^{fMet} (Figure 3). The preexponential term for the decay curve in lifetime measurement is decreased by close to the same extent (65%) as the steady-state emission intensity, (Figure 6 and Table I). This result shows that tRNA^{fMet}-induced quenching of AEDANS emission occurs primarily by a static mechanism instead of dynamic processes. The accessibility of the remaining emission of AEDANS in AEDANS-MetRS is substantially reduced upon

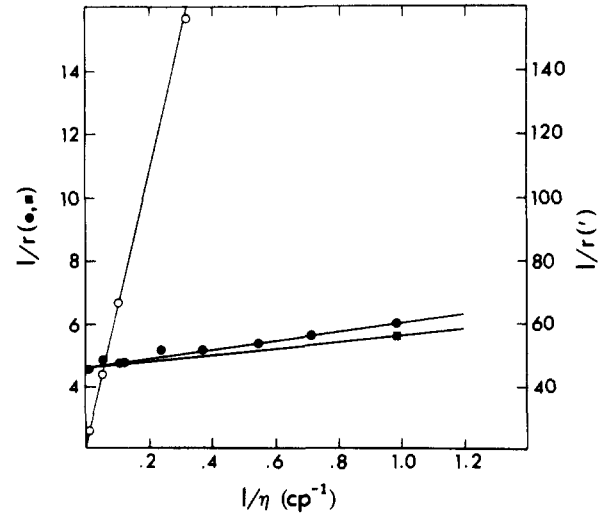

FIGURE 5: Perrin plot for ME-AEDANS (○) and AEDANS-MetRS (●) at 20 °C. The viscosity, η, was varied by changing the concentration of sucrose in the sample, as described under Materials and Methods. The anisotropy, r, was monitored at 490 nm (λ_{ex} = 350 nm). Also indicated is the anisotropy for 0.19 μM AEDANS-MetRS in the presence of 2.3 μM tRNA^{fMet} (■). Addition of sucrose solution to the tRNA–MetRS complex apparently shifted the equilibrium of complex formation due to changes in the conditions and was not considered.

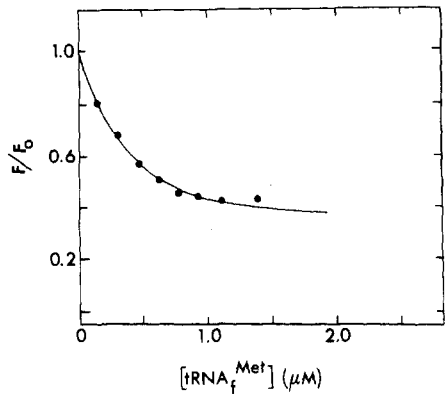

FIGURE 6: Fluorescence titration of 0.43 μM AEDANS-MetRS with tRNA^{fMet} (●). The emission intensity, F, was monitored at 480 nm (λ_{ex} = 350 nm); F_0 is the initial emission intensity in the absence of added tRNA^{fMet}. The data were corrected for inner filter absorption by using correction factors determined by the parallel titration of 0.8 μM ME-AEDANS with tRNA^{fMet}. The correction for inner filter absorption was small, and the correction factors never exceeded 1.03. The solid curve fitting the data was calculated by using eq 1. The binding parameters are K = 0.10 μM, $(F/F_0)_{max}$ = 0.35, and n = 1.0.

binding of tRNA^{fMet} (Figure 4), suggesting that the AEDANS fluorophore in AEDANS-MetRS becomes effectively shielded from solvent molecules.

The emission anisotropy of AEDANS in AEDANS-MetRS was increased from 0.165 to 0.175 upon binding of tRNA^{fMet} (Figure 5). Assuming no change in the limiting anisotropy in bound AEDANS-MetRS (no spectral shift), the increase in anisotropy corresponds to an increase in rotational correlation time from 62 to about 80 ns.

Reaction of 1,5-I-AEDANS with MetRS was also examined in the presence of excess of tRNA^{fMet}. The specific activity and labeling stoichiometry of AEDANS-MetRS prepared in the presence of excess tRNA^{fMet} and 10 mM $MgCl_2$ were the same as those prepared in the absence of tRNA^{fMet}. In addition, it was found that the AEDANS fluorophore in AEDANS-MetRS prepared in the presence of tRNA^{fMet} was located outside of the enzymatically active, trypsin-modified MetRS (M_r 64 000). The properties of AEDANS-MetRS prepared in the presence of excess tRNA^{fMet} were not detectably different from the properties of AEDANS-MetRS prepared in the absence of tRNA^{fMet}. Although tRNA^{fMet} induces conformational changes in MetRS, the conformational changes do not appear to alter its reactivity with 1,5-I-AEDANS under the experimental conditions. Evidently, sulfhydryl groups that may be essential for the MetRS aminoacylation activity are not reactive with 1,5-I-AEDANS, either in the absence or in the presence of tRNA^{fMet}.

Discussion

Fluorescent-labeled MetRS has been prepared by the reaction of 1,5-I-AEDANS with MetRS. The labeled MetRS contains about 2 mol of AEDANS per mole of dimer MetRS and retains nearly full aminoacylation activity. The AEDANS fluorophore in AEDANS-MetRS was found to be located outside the M_r 64 000 active fragment of MetRS. AEDANS-MetRS is fully active in the aminoacylation reaction and binds tRNA^{fMet} with the same affinity and stoichiometry as native MetRS. The conclusions drawn from this study may be reasonably extended to the interaction of tRNA^{fMet} with unmodified MetRS.

Unique Characteristics of the AEDANS Site in MetRS. The fluorophore in AEDANS-MetRS exhibits excitation and emission wavelength maxima and emission lifetime close to those for ME-AEDANS in 80% ethanol (Hudson & Weber, 1973). However, the acrylamide quenching indicates that the AEDANS fluorophore in AEDANS-MetRS is virtually fully accessible to acrylamide molecules. These observations reveal that MetRS provides a unique local site that is effectively nonpolar yet is nearly fully accessible to acrylamide.

It appears unlikely that tRNA^{fMet} interacts by direct contact with AEDANS in labeled MetRS. AEDANS is shown to be located in a region of the MetRS molecule that is not required for its aminoacylation activity, and in addition, tRNA^{fMet} does not protect MetRS from reacting with 1,5-I-AEDANS. These results suggest that the probe is located at a site outside the tRNA^{fMet} binding site on MetRS. Furthermore, since removal of the fragment containing AEDANS by limited proteolysis dissociates MetRS to fully active monomer, the results suggest that AEDANS is located in the region of contact between subunits in the native dimeric MetRS.

The precise location of the probe is not known at present. The amino acid sequence deduced from the nucleotide sequence of the *E. coli* MetRS gene (Dardel et al, 1984) showed that the most C-terminal cysteine is at position 478 (out of 680 residues) in a region required for the catalytic activity, although 1,5-I-AEDANS is known to be fairly specific for sulfhydryl groups in proteins (Hudson & Weber, 1973). The present results evidently showed that there are two 1,5-I-AEDANS-reactive groups in MetRS and none in trypsinized MetRS. This is in good agreement with the findings of Cassio and Waller (1971) that there are two 5,5′-dithiobis(nitrobenzoic acid) (DTNB) reactive groups in native MetRS and none in trypsinized MetRS. In view of the sequencing data, the probe may be attached to an unusually reactive nucleophilic group other than the sulfhydryl group, such as the two imidazole groups in ribonuclease A (Heinrikson et al., 1965), the carboxyl group in ribonuclease T1 (Takahashi et al., 1967), or the methionine residue in galactose receptor protein (Zukin et al., 1977).

Conformational Changes at the Intersubunit Domain. The tRNA^{fMet}-induced changes in the AEDANS emission intensity and solvent accessibility in AEDANS-MetRS, and the known negative cooperativity in tRNA binding, indicate that the binding of tRNA^{fMet} to a single site on the dimeric enzyme may alter both subunits of the enzyme. The excitation and emission wavelength maxima and emission decay parameter measurements indicate that AEDANS-MetRS is quenched by a static mechanism through binding of tRNA^{fMet}. The remaining fluorescent AEDANS in AEDANS-MetRS became effectively shielded from acrylamide. The emission intensity of the AEDANS fluorophore in AEDANS-MetRS is quenched by a maximal extent of 65% in the presence of tRNA^{fMet}. Under that condition, the number of sites occupied by tRNA^{fMet} is expected to be 1.35 per dimer, using association constants $K_1 = 1 \times 10^7$ M^{-1} and $K_2 = 6.7 \times 10^5$ M^{-1} (Ferguson & Yang, 1986). Assuming AEDANS in the tRNA^{fMet}-bound subunit is quenched completely and equally for the first and second bound tRNA, the extent of quenching is expected to be 68%. The good agreement suggests that one of the subunits in tRNA^{fMet}-bound AEDANS-MetRS is statically quenched through conformational changes at the tRNA binding sites in the same subunit. AEDANS in the remaining fluorescent subunit apparently is not quenched but is effectively shielded from acrylamide. Scheme I denotes the unsymmetric conformational changes at the two subunits of MetRS (E) upon binding of tRNA^{fMet} (A).

Scheme I

$$E{\cdot}E \overset{A}{\rightleftharpoons} E''{\cdot}E'A \overset{A}{\rightleftharpoons} (E'A)_2$$

These results suggest the delocalized nature of the conformational changes in MetRS induced by $tRNA^{fMet}$. The conformational changes are likely to be initiated at the tRNA binding site and transmitted to the intersubunit domain of the same subunit and finally to the entire MetRS molecule. These changes likely mediate the negative cooperativity of tRNA binding to MetRS.

Segmental Flexibility in MetRS. The rotational correlation times are 26 and 80 ns for free $tRNA^{fMet}$ and the $tRNA^{fMet}$–MetRS complex, respectively, as determined by using fluorescent-labeled $tRNA^{fMet}$ (Ferguson & Yang, 1986). With AEDANS-MetRS, the rotational correlation times are 62 and 80 ns for free MetRS and the tRNA–MetRS complex, respectively. These measurements are in good agreement with those obtained by using fluorescent-labeled $tRNA^{fMet}$. Using neutron scattering, Dessen et al. (1978, 1982) have shown that the radii of gyration are 44.7 and 43.9 Å for free MetRS and the tRNA–MetRS complex, respectively, and concluded a contraction of the MetRS molecule upon binding of tRNA. As the protein is approximated as a rigid sphere, the decrease in the radius of gyration should have resulted a decrease in the rotational correlation time upon complex formation. However, an appreciable increase in rotational correlation time is observed. An obvious and reasonable hypothesis to reconcile these observations is that there exists in MetRS segmental flexibility, likely mediated by a molecular hinge. The increase in the rotational correlation time upon complexing with tRNA can be best interpreted to reflect tightening of the hinge, rendering a more rigid protein molecule. Further studies of the rotational mobility of MetRS using time-resolved fluorescence spectroscopy will be useful to confirm the nature of the hinge structure. Such hinge structure may conceivably exist between the subunits in MetRS or between the intersubunit domain and the catalytic domain, since trypsin-modified MetRS behaves normally (Dessen et al., 1982). The present analysis cannot resolve these possibilities. In either case, part of the hinge involves the intersubunit domain. The possible role of such a hinge structure in the function of enzymes is yet to be elucidated.

Conformational changes at the intersubunit domain of proteins must play critical roles in numerous phenomena involving protein–protein interactions. Unsymmetric conformational changes at the neighboring subunits provide additional supporting evidence for the premises of the observed negative cooperativity. Further studies by fast kinetics may elucidate the mechanism of conformational transition in MetRS during tRNA–MetRS interactions. Studies of the solution structure of the tRNA–synthetase complex should be useful for our understanding of protein–nucleic acid interactions in general.

Registry No. MetRS, 9033-22-1; ME-AEDANS, 101493-66-7.

References

Ackerman, E. J., Joachimiak, A., Klinghofer, V., & Sigler, P. (1985) *J. Mol. Biol. 181*, 93–102.

Barker, D. B., Ebel, J P., Jakes, R., & Bruton, C. J. (1982) *Eur. J. Biochem. 127*, 449–459.

Blanquet, S., Iwatsubo, M., & Waller, J. P. (1973) *Eur. J. Biochem. 36*, 213–226.

Blanquet, S., Dessen, P., & Fayat, G. (1979) in *Transfer RNA: Structure, Properties and Recognition* (Abelson, J., Schimmel, P., & Soll, D., Eds.) pp 281–299, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Bruton, C. J., & Hartley, B. S. (1970) *J. Mol. Biol. 52*, 165–178.

Cantor, C. R., & Tao, T. (1971) *Procedures in Nucleic Acid Research* (Cantoni, G. C., & Davies, D. R., Eds.) Vol. 2, pp 31–93, Harper and Row, New York.

Cassio, D., & Waller, J.-P. (1971) *Eur. J. Biochem. 20*, 283–300.

Dardel, F., Fayat, G., & Blanquet, S. (1984) *J. Bacteriol. 160*, 1115–1122.

Dessen, P., Blanquet, S., Zaccai, G., & Jacrot, B. (1978) *J. Mol. Biol. 126*, 293–313.

Dessen, P., Fayat, G., Zaccai, G., & Blanquet, S. (1982) *J. Mol. Biol. 154*, 603.

Engel, G. (1974) *Anal. Biochem. 61*, 184–191.

Fairclough, R. H., & Cantor, C. R. (1978) *Methods Enzymol. 48*, 347–379.

Fasman, G., Ed. (1975) *Handbook of Biochemistry and Molecular Biology*, 3rd ed., Vol. I, CRC Press, Cleveland, OH.

Ferguson, B. Q., & Yang, D. C. H. (1981) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 40*, 1807.

Ferguson, B. Q., & Yang, D. C. H. (1986) *Biochemistry 25*, 529–539.

Gulik, A., Monteilhet, C., Dessen, P., & Fayat, G. (1976) *Eur. J. Biochem. 64*, 295–300.

Heinrikson, R. L., Stein, W. H., Crestfield, A. M., & Moore, S. (1965) *J. Biol. Chem. 240*, 2921–2934.

Hudson, E. N., & Weber, G. (1973) *Biochemistry 12*, 4154–4161.

Jasin, M., Regan, L., & Schimmel, P. (1983) *Nature (London) 306*, 441–447.

Koch, G. L. E., & Bruton, D. J. (1974) *FEBS Lett. 40*, 180–182.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lemoine, F., Waller, J.-P., & van Rapenbusch, R. (1968) *Eur. J. Biochem. 4*, 213–221.

Pachmann, U., Cronvall, E., Rigler, R., Hirsch, R., Wintermeyer, W., & Zachau, H. G. (1973) *Eur. J. Biochem. 39*, 265–273.

Scott, T. G., Spencer, R. D., Leonard, N. J., & Weber, G. (1970) *J. Am. Chem. Soc. 92*, 687–695.

Takahashi, K., Stein, W. H., & Moore, S. (1967) *J. Biol. Chem. 242*, 4682.

Yguerabide, J. (1972) *Methods Enzymol. 26*, 498–578.

Zelwer, C., Risler, J. L., & Brunie, S. (1982) *J. Mol. Biol. 155*, 63–81.

Zukin, R. S., Hartig, P. R., & Koshland, D. E., Jr. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1932–1936.