Terbium(III) Luminescence Study of the Spatial Relationship of Tryptophan Residues to the Two Metal Ion Binding Sites of *Escherichia coli* Glutamine Synthetase†

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ABSTRACT: The luminescence of Tb(III) was used to explore the topography of the metal ion sites of *Escherichia coli* glutamine synthetase and the relationship between these sites and tryptophan residues of the enzyme. By irradiation of tryptophan residues at 295 nm and measurement of the resulting Tb(III) luminescence at 544 nm, a biphasic curve was obtained upon titrating apoenzyme with Tb(III) indicating sequential binding of Tb(III) ions to the two binding sites of glutamine synthetase. The luminescence intensity was greater in the second region of the titration curve which is mostly due to energy transfer from Trp-158 to the second Tb(III) binding site of the enzyme. By use of the Förster equation for energy transfer from donor Trp to acceptor Tb(III), distances from Trp-57 to Tb(III) at the n_1 and n_2 sites were calculated, by using a mutant enzyme in which Trp-158 was replaced by Ser, to be 16.4 and 15.7 Å, respectively; distances from Trp-158 to Tb(III) at the n_1 and n_2 sites were calculated, by using a mutant enzyme in which Trp-57 was replaced by Leu, to be 16.8 and 9.5 Å, respectively. All the distances are in reasonably good agreement with the crystal structure distances from *Salmonella typhimurium* glutamine synthetase except the distance from Trp-158 to the second Tb(III) binding site. The discrepancies may result from a slightly different conformation of glutamine synthetase in solution and in the crystal and/or a slightly different conformation for trivalent Ln(III) binding compared to divalent Mn(II) binding.

Structural studies of metalloenzymes by spectroscopic techniques often involve the use of probe metal ions with unique spectroscopic properties. In this work, lanthanide(III) ions are investigated as probes of the two metal ion sites of *Escherichia coli* glutamine synthetase (GS). The advantage of using lanthanide ions as probes lies in the great variety of spectroscopic properties available within this series of ions (Reuben, 1979; Horrocks, 1982). All of the ions, except La(III) and Lu(III), are paramagnetic and therefore useful in magnetic resonance experiments. Gd(III), which has a long electron spin relaxation time, is a useful probe in EPR and NMR relaxation enhancement experiments (Reuben, 1971). The other paramagnetic lanthanides have very short electron spin relaxation times, which render these ions useful as NMR shift probes.

In addition to their utility in magnetic resonance experiments, the paramagnetic lanthanide ions also have chromophoric and luminescent properties (Horrocks, 1982). Useful information can be obtained from detailed studies of these properties including determination of metal ion hydration numbers by luminescence lifetime measurements and determination of site to site distances by fluorescence energy transfer (Horrocks & Collier, 1981; Breen et al., 1985).

E. coli glutamine synthetase contains two divalent cation binding sites per subunit, n_1 and n_2, which are essential for activity. Although Mg(II) is the normal physiological metal ion of GS, unadenylylated GS is also active with Co(II) and Ca(II) in the biosynthetic assay (Stadtman & Ginsburg, 1974). The physical properties of Ln(III) ions differ in several respects from those of Mg(II). The most apparent difference, the trivalent versus divalent positive charge, accounts for the tighter binding of lanthanides to proteins. It is believed, however, that macromolecules are able to adjust to the gain of a single positive charge without significant structural consequences. Several studies involving Ln(III) ions as replacements for Mg(II) have previously been reported with *E. coli* GS (Eads et al., 1985; Eads, 1985). GS was found to bind Tb(III) sequentially as illustrated by a biphasic Tb(III) luminescence titration curve. This sensitized Tb(III) luminescence is believed to result mainly from energy transfer from Trp residues to Tb(III) since the emission of phenylalanine and tyrosine in proteins is usually very small. GS contains two Trp residues, Trp-57 and Trp-158, per subunit. Mutants with Trp-57 replaced by leucine (W57L GS) or Trp-158 replaced by serine (W158S GS) have been constructed to study the individual effect of each Trp residue on eneregy transfer to Tb(III) and their spatial relationships to the two metal ion binding sites.

Experimental Procedures

Materials. *E. coli* glutamine synthetase in a low state of adenylylation was prepared by the method of Miller et al. (1974). Wild-type GS was purified from YMC10 containing pgln6, a pBR322 clone of *glnA*. Mutant W158S GS was purified from YMC11 containing pgln35, a pBR322 clone containing *glnA* and *glnG*, a contiguous *glnA* activator. The preparation of mutant enzymes W158S and W57L is given in the preceding paper (Atkins et al., 1991). The Kunkel method of mutagenesis involving single-stranded uracil-containing DNA template (Kunkel et al., 1986) was performed on a M13mp9 subclone of the *glnA* gene. A DNA fragment of the *glnA* gene containing the W158S mutation was isolated and ligated into pgln35 to obtain a complete *glnA* gene for expression of mutant glutamine synthetase. The plasmids pgln6 and pgln35 were obtained from Boris Magasanik at MIT. Enzyme concentration and state of adenylylation were

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determined spectrophotometrically (Shapiro & Stadtman, 1970). All enzyme concentrations were given as subunit concentrations. Metal-free enzyme was prepared by dialysis against 3 mM EDTA, 10 mM Hepes, and 100 mM KCl, pH 7.0, followed by extensive dialysis against the same buffer without EDTA. A Tb(III) solution was prepared from the chloride salt obtained from Aldrich.

Methods. The fluorescence spectra of GS and Tb(III) were measured on a Perkin-Elmer MPF-44B fluorescence spectrophotometer equipped with a differential corrected spectra unit. Fluorescence spectra were recorded after conformational changes induced by metal binding were complete, at least 20 min after metal addition.

Förster Theory. Intramolecular distances can be calculated from the efficiency of energy transfer assuming a mechanism of dipole–dipole radiationless energy transfer. According to Förster theory (Förster, 1948, 1965), the donor–acceptor distance is related to the efficiency of energy transfer, E, by

$$r = R_0[(1/E) - 1]^{1/6} \quad (1)$$

where R_0 is the critical distance for 50% energy transfer and is given by

$$R_0^6 = (8.78 \times 10^{-25})\kappa^2\phi_D n^{-4} J \quad (2)$$

where κ is an orientation factor, ϕ_D is the quantum yield of the energy donor in the absence of acceptor, n is the refractive index of the medium, and r is the distance between the donor and the acceptor. The spectral overlap integral, J, is defined as

$$J = \left[\int F(\lambda)\epsilon(\lambda)\lambda^4\, d\lambda\right] \Big/ \left[\int F(\lambda)\, d\lambda\right] \quad (3)$$

where $F(\lambda)$ is the fluorescence intensity of the donor at wavelength λ and $\epsilon(\lambda)$ is the extinction coefficient of the acceptor at that wavelength.

For Tyr → Tb(III) energy transfer, E can be determined from eq 4 (Beardsley & Cantor, 1970; Yang & Soll, 1974)

$$E = (A_{Tb(III)}/A_{Trp})(\phi_{Trp}/\phi_{Tb(III)}) \quad (4)$$

where $A_{Tb(III)}$ and A_{Tyr} are the integrated areas of luminescence emission of Tb(III) ion and tyrosine in the enzyme, respectively, and $\phi_{Tb(III)}$ and ϕ_{Tyr} are their respective quantum yields.

Quantum Yield Determinations. Corrected fluorescence emission spectra of an aqueous solution of free L-tryptophan (40 μM) at pH 6.0 were recorded at 23 °C using excitation wavelengths of 280 and 295 nm. In addition, fluorescence emission spectra of 10 μM wild-type $GS_{2.8}$, W158S GS_1, and W158S GS_8 (where the subscript refers to the average state of adenylylation of the dodecamer) were recorded at 25 °C using an excitation wavelength of 295 nm. Absorbances of the L-Trp and GS solutions were measured with a Cary 210 UV/VIS spectrophotometer. The fluorescence emission spectra were digitized by using a Sumagraphics MM1200 tablet digitizer interfaced to an IBM CS9000 computer, and the peak areas were estimated by using a computer program employing Simpson's method.

The quantum yield of Tb(III) bound to GS was determined by measuring the luminescent lifetimes of enzyme-bound Tb(III) as the percentage of D_2O in the buffer was varied. The quantum yield of Tb(III) bound to GS, $\phi_{Tb(III)}$, was estimated from

$$\phi_{Tb(III)} = k(D_2O)/k_{obsd} \quad (5)$$

where $k(D_2O)$ is the exponential decay constant for GS-bound Tb(III) in pure D_2O and k_{obsd} is the observed exponential decay constant.

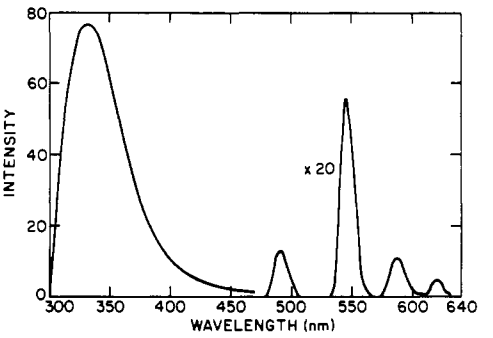

FIGURE 1: Luminescence emission spectrum (λ_{ex} = 295 nm) of 20 μM wild-type $GS_{2.8}$ containing 2.0 equiv of Tb(III) per subunit enzyme in 10 mM Hepes/0.1 M KCl, pH 7.0, at 25 °C. The Tb(III) luminescence has been corrected for the base line due to the tryptophan fluorescence tail.

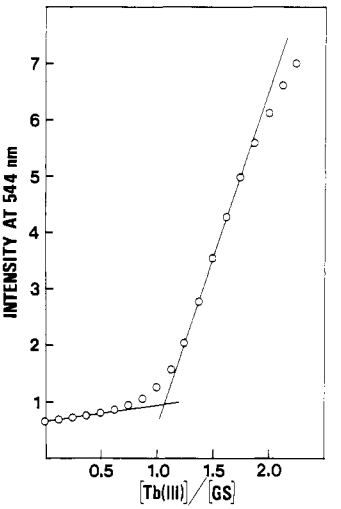

FIGURE 2: Titration of wild-type glutamine synthetase with Tb(III) followed by sensitized Tb(III) luminescence at 544 nm. The solution contained 16.6 μM enzyme in 10 mM Hepes/0.1 M KCl, pH 7.0, at 25 °C.

RESULTS

Sensitized Tb(III) Luminescence of GS-Bound Tb(III). Tb(III) ion exhibits a strong fluorescence when bound to GS. Figure 1 shows the luminescence emission spectrum obtained from a solution containing wild-type GS in the presence of 2 equiv of Tb(III). The dominant peak at 333 nm is due to intrinsic emission from tryptophan residues. The other peaks at 489, 544, 585, and 621 nm are characteristic of the luminescence emission bands of Tb(III). At the excitation wavelength used to obtain this spectrum, 295 nm, absorbance by Tb(III) is negligible, which implies that the luminescence energy emitted by Tb(III) was transferred to this ion by an indirect process such as Förster energy transfer. This phenomenon, known as sensitized terbium luminescence, is known for a number of systems to arise from energy transfer from Trp residues to Tb(III) ions bound nearby.

Stoichiometry of Ln(III) Ion Binding to Glutamine Synthetase. Figure 2 shows a plot of the Tb(III) luminescence emission intensity at 544 nm as a function of Tb(III) concentration in a solution initially containing apoenzyme. The curve consists of two linear phases. The first phase represents binding to a high-affinity site with a stoichiometry of one metal ion per enzyme subunit. The second linear phase represents binding to a second site, also with a stoichiometry of one per enzyme subunit. This second, lower affinity site is closer to a tryptophan residue than the first site since Tb(III) binding results in a significant amount of sensitized Tb(III) lu-

Table I: Tryptophan Quantum Yields for *E. coli* Glutamine Synthetase and Its Mutants Determined in 10 mM Hepes/0.1 M KCl, pH 7.0, at 23 °C

enzyme	donor Trp	equiv of Tb(III)	ϕ_{Trp}
W57L GS_{12}	158	0	0.07
		1	0.07
		2	0.07
W158S GS_1	57	0	0.14
		1	0.11
		2	0.10
W158S GS_8	57	0	0.15
		1	0.12
		2	0.11
(WT $GS_{2.8}$)-(W158S GS_1)	158	0	0.05
		1	0.06
		2	0.07
(WT $GS_{2.8}$)-(W158S GS_8)	158	0	0.04
		1	0.04
		2	0.05
WT $GS_{2.8}$	57, 158	0	0.11

minescence. An independent demonstration for the existence of two types of Ln(III) sites was previously obtained by observing the effects of Gd(III) ion binding on the fluorescence emission intensity of the enzyme (Eads et al., 1985).

A slight deviation from linearity is apparent in the second phase of the titration shown in Figure 2 as the Tb(III) level approaches 2 equiv. This curvature most likely arises from metal ion binding to additional sites since a significant population of these sites results in precipitation of the enzyme. A similar phenomenon is found with other ions, and the aggregation of GS induced by Zn(II) (Miller et al., 1974) may result from binding of Zn(II) to the same sites.

Quantum Yields of Trp and GS-Bound Tb(III). The fluorescence quantum yields of the Trp residues of wild type and mutant GS in the absence of energy transfer, ϕ_{Trp}, were determined from the relative areas of the corrected emission spectra of the enzyme solutions excited at 295 nm and an aqueous L-Trp solution excited at 280 nm. From the ratios of the areas, the absorbances at the excitation wavelengths, and the fluorescence quantum yield of 0.13 for aqueous L-Trp at 23 °C, pH 6.0 (Chen, 1967), the quantum yields for wild type, W158S GS, and W57L GS were calculated and are summarized in Table I.

The quantum yields of GS-bound Tb(III) were estimated to be 0.21 and 0.36 for 1 and 2 equiv of Tb(III) per subunit from the change in the luminescent exponential decay constants as the H_2O/D_2O ratio was varied.

Sensitized Tb(III) Luminescence Titration of GS Mutants. Because there are two Trp residues, Trp-57 and Trp-158, per subunit (Colombo & Villafranca, 1986), it is important to determine the contributions of the individual Trp residues to the sensitized Tb(III) luminescence. Figure 3 shows the Tb(III) luminescence titrations of W57L and W158S GS with Tb(III). Both titration curves are biphasic with a break point at 1 equiv, indicating the sequential binding of Tb(III) ions. It is evident from Figure 3 that most of the sensitized Tb(III) luminescence is from Trp-158 to Tb(III) at the n_2 site and Trp-158 is expected to be closer to the n_2 site than Trp-57. The sensitized luminescence of Tb(III) at the n_1 site is always much weaker, indicating that both Trp residues are far away from the n_1 site. In addition, Trp-158 is expected to be much closer to the n_2 site than the n_1 site while the distances from Trp-57 to both binding sites are not much different as seen from the titration curves.

Determination of Trp to Tb(III) Distances. In order to calculate distances, peak areas were determined of tryptophan fluorescence and sensitized Tb(III) luminescence as GS was

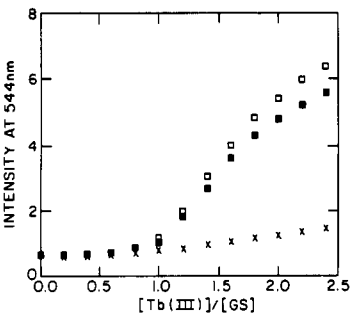

FIGURE 3: Sensitized Tb(III) luminescence titration of 20 μM (□) wild type, (■) W57L, and (×) W158S glutamine synthetase in 10 μM Hepes/0.1 M KCl, pH 7.0, at 25 °C.

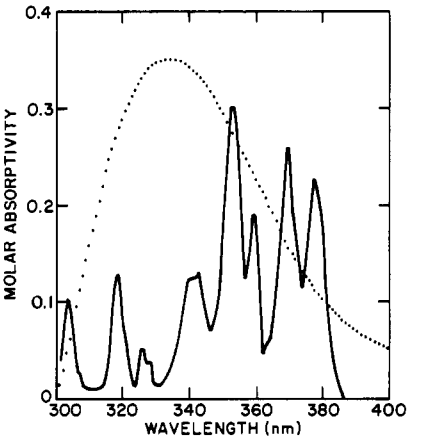

FIGURE 4: Spectral overlap of the emission spectrum [(···) λ_{ex} = 295 nm] of wild-type $GS_{2.8}$ and the absorption spectrum (—) of a 1/1 Tb(III)/EDTA complex. ϵ_{353nm} = 0.3 M^{-1} cm^{-1}.

titrated with Tb(III). For calculations done at various ratios of Tb(III)/enzyme subunit, $A_{Tb(III)}$ was corrected for the fractional occupancy of each metal ion binding site. The overlap in the fluorescence spectrum of wild-type GS and the absorption spectrum of the Tb(III)/EDTA complex are illustrated in Figure 4. The Tb(III)/EDTA absorption spectum was used due to the weak absorption properties of Tb(III) which precludes use of the spectrum of Tb(III) bound to GS. The overlap integral estimated from eq 3 was 1.4×10^{-19} cm^6 mol^{-1}. The overlap integrals for W57L and W158S GS were also determined similarly to be 1.4×10^{-19} and 1.1×10^{-19} cm^6 mol^{-1}, respectively.

The critical distance for 50% energy transfer, R_0, was calculated from eq 2 by using the quantum yields in Table I. The orientation factor, κ^2, was taken as $^2/_3$, the value for an isotropic donor and an isotropic acceptor. According to Horrocks and Collier (1981), it is unlikely that the true value of κ^2 deviates from this value sufficiently to cause a significant error in the calculation of R_0. A value of 1.36 was used for the refractive index n. The distances from Trp to the two Tb(III) binding sites were then estimated by eq 1, and the resulting R_0 values and distances are summarized in Table II. Uncertainties in the distances may arise from several sources. All of the factors which contribute to R_0 have uncertainties associated with them, the largest being κ^2. Using the two possible extremes for this value leads to uncertainties in the estimated distances of ±12%. These may be considered reasonable limits for the uncertainty inherent in the distance measurements.

Since W57L GS exhibits an intense tyrosine emission (Lin et al., 1991a), the determined Trp-158 to Tb(III) distances may be affected by possible energy transfer from Tyr residues to the Tb(III) ions. Therefore, an alternative way of deter-

Table II: Summary of Efficiencies of Energy Transfer, E, Critical Distances for 50% Energy Transfer, R_0, and Tb(III)–Trp Distances, r, for *E. coli* Glutamine Synthetase and Its Mutants

enzyme	donor Trp	equiv of Tb(III)	E^a	R_0 (Å)	r (Å)
W57L GS_{12}	158	1	7.52×10^{-5}	3.46	16.8
		2	2.28×10^{-3}	3.45	9.5
W158S GS_1	57	1	1.30×10^{-4}	3.70	16.4
		2	1.51×10^{-4}	3.64	15.8
W158S GS_8	57	1	1.48×10^{-4}	3.77	16.4
		2	1.80×10^{-4}	3.70	15.6
(WT $GS_{2,8}$)–(W158S GS_1)	158	1	2.59×10^{-4}	3.45	12.0
		2	2.53×10^{-3}	3.57	9.7
(WT $GS_{2,8}$)–(W158S GS_8)	158	1	5.21×10^{-4}	3.31	11.7
		2	2.40×10^{-3}	3.46	9.5

[a] With an error of ±10%.

Table III: Comparison of the Experimentally Determined Trp to Tb(III) Distances and the Crystal Structure Distances from *S. typhimurium* GS

Experimental Results			
sample	donor	acceptor	r (Å)
W158S GS	Trp-57	Tb(III), site 1	16.4
	Trp-57	Tb(III), site 2	15.7
WT GS–W158S GS	Trp-158	Tb(III), site 1	11.8
	Trp-158	Tb(III), site 2	9.6
W57L GS	Trp-158	Tb(III), site 1	16.8
	Trp-158	Tb(III), site 2	9.5

Crystal Structure Distances		
site 1	site 2	r (Å)
Mn(II) at n_1	Trp-57	9.2
Mn(II) at n_2	Trp-57	13.9
Mn(II) at n_1	Trp-158	20.7
Mn(II) at n_2	Trp-158	25.8

mining Trp-158 to Tb(III) distances has been performed by subtracting the Trp-57 contribution obtained from W158S GS from the data of the wild-type GS. The results are also summarized in Table II.

Discussion

The binding behavior of lanthanide(III) ions to glutamine synthetase closely parallels that of divalent metal ions. The n_1 and n_2 metal ion sites differ enough in structure (Yamishita et al., 1989) so that their affinity for metal ions is different and they are populated sequentially during titrations of the enzyme (Hunt et al., 1975; Denton & Ginsburg, 1969; Villafranca et al., 1976). The n_1 site has three glutamyl residues as metal ion ligands while the n_2 site has one histidyl and two glutamyl residues. Both divalent metal ions and lanthanide(III) ions exhibit sequential binding to GS, although the lanthanide(III) ions bind more tightly. These data suggest that binding of the first two lanthanide(III) ions occurs at the n_1 and n_2 divalent metal ion sites.

The luminescence property of Tb(III) was used for topographical determinations of Trp–Tb(III) distances. The tryptophan–metal distances obtained are compared with distances from the X-ray crystal structure of *S. typhimurium* GS (Almassy et al., 1986; Yamashita et al., 1989), which differs by only 10 residues per subunit from *E. coli* GS (Colombo & Villafranca, 1986; Janson et al., 1986) (Table III). The distances from Trp-57 to the two Tb(III) binding sites determined experimentally with the W158S GS mutant were 16.4 and 15.7 Å, respectively, which are in resonable agreement with the crystal structure distances of 19.2 and 13.9 Å. The slight discrepency in distances may be attributable to the mobility of Trp-57. In the crystal structure, Trp-57 is pointed away from the two metal binding sites. According to Eisenberg (personal communication), the loop containing Trp-57 is characterized as having a high degree of motion within the crystal, and the position of Trp-57 is not clearly defined. Thus, rotation of Trp-57 around the bonds connecting the α and β carbons and the β and γ carbons will result in differences in the obtained distances. The closest distances possible were 17.4 and 14.9 Å for Mn(II) at the n_1 and n_2 site, respectively, which are resonably close to the experimentally determined distances.

The distances from Trp-158 to the two Tb(III) binding sites determined with the W57L GS mutant or from the subtraction method are very similar, although the distance to the n_1 site obtained with the W57L GS is somewhat longer than that obtained from the subtraction method. The distance of 16.8 Å is probably more accurate because the Tb(III) luminescence at the n_1 site is very weak and the subtraction method will lead to substantial error. The distances from Trp-158 to the n_2 site obtained from the two methods are in excellent agreement with each other (9.5 and 9.6 Å), indicating that the obtained distances are not affected significantly by the Tyr emission. The determined distance of 16.8 Å from Trp-158 to Tb(III) at the n_1 site is in resonable agreement with the crystal structure distance of 20.7 Å. On the other hand, the experimental distance of 9.5 Å to the second Tb(III) binding site does not agree well with the crystal structure distance of 25.8 Å. This discrepancy may result from a slightly different conformation of GS in solution and in the crystal and/or a slightly different conformation for trivalent Ln(III) binding compared to divalent Mn(II) binding.

It is likely that a Tyr residue (Tyr-179) (Lin et al., 1991a) is being excited in addition to a Trp residue by illumination at 295 nm in both wild-type and W57L glutamine synthetase, leading to an additional energy-transfer contribution. The experimental result is that the W158S mutant enzyme gives metal ion to Trp distances comparable to crystallographic distances, while wild type and W57L do not. With W158S, the mutation at position 158 may alter the location of the Tyr residue and thus diminish possible energy-transfer contributions from Tyr resulting in only Trp-57 to Tb(III) energy transfer. Contributions from Tyr residues in energy-transfer experiments have been observed for other protein systems (Willis & Szabo, 1989), and in GS, the distances [based on the crystal structure (Yamishita et al., 1989)] from the benzene ring of Tyr-179 to the n_1 and n_2 metal ion sites are 7.6 and 11.9 Å, respectively (Lin et al., 1991a).

The discrepancy in the data analysis is in the n_2–donor distance yet Tyr-179 is closer to the n_1 site. This raises the question of whether Tb(III) initially binds to the crystallographically defined n_1 or n_2 sites. Since the n_1 site has three glutamyl residues whereas the n_2 site has two glutamyl and one histidyl residue, our assumption is that Tb(III) binds to the n_1 site first. Direct metal ion binding studies (Lin et al., 1991b) support the conclusion that Ln(III) and Mn(II) bind to identical sites but that the final conformation of the enzyme is different for each metal ion (unpublished observations). The question will remain unresolved until structures of enzyme with Ln(III) and other metal ions are available.

Tb(III) luminescence is greater for more highly adenylylated enzyme for wild-type and mutant enzymes (data not shown). This is most likely due to the Trp quantum yields being higher for the adenylylated enzymes. However, the Trp to Tb(III) distances obtained from GS with different adenylylation states are very similar. Thus, the adenylylation of a specific Try residue does not affect the distances between the Trp residues

to the two Tb(III) binding sites of GS.

Registry No. GS, 9023-70-5; L-Trp, 73-22-3; Tb, 7440-27-9.

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Fluorescent Probes for Measuring the Binding Constants and Distances between the Metal Ions Bound to *Escherichia coli* Glutamine Synthetase†

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ABSTRACT: TNS, 2-*p*-toluidinylnaphthalene-6-sulfonate, has been used as a fluorescent probe to determine the binding constants of metal ions to the two binding sites of *Escherichia coli* glutamine synthetase (GS). TNS fluorescence is enhanced dramatically when bound to proteins due to its high quantum yield resulting from its interactions with hydrophobic regions in proteins. The fluorescence energy transfer from a hydrophobic tryptophan residue of GS to TNS has been detected as an excitation band centered at 280 nm. Therefore, TNS is believed to be bound to a hydrophobic site on the GS surface other than the active site and is located near a hydrophobic Trp residue of GS. GS binds lanthanide ions [Ln(III)] more tightly than either Mn(II) or Mg(II), and the binding constants of several lanthanide ions were determined to be in the range (2.1–4.6) × 10^{10} and (1.4–3.0) × 10^{8} M^{-1} to the two metal binding sites of GS, respectively. The intermetal distances between the two metal binding sites of GS were also determined by measuring the efficiencies of energy transfer from Tb(III) to other Ln(III) ions. The intermetal distances of Tb(III)–Ho(III) and Tb(III)–Nd(III) were 7.9 and 6.8 Å, respectively.

Escherichia coli glutamine synthetase (GS) is a dodecameric enzyme which catalyzes the formation of glutamine from glutamate, NH_4^+, and ATP. The enzyme contains two essential metal binding sites per subunit—a higher affinity n_1 site and a lower affinity n_2 site (Ginsburg, 1972; Hunt et al., 1975). The metal–metal interaction for the two binding sites of GS has been investigated by using different chromophoric metal ions [e.g., Mn(II), Co(II), Cr(III), and lanthanide ions (Ln(III))] and by different spectroscopic methods [e.g., NMR, EPR, fluorescence energy transfer, and sensitized Tb(III) luminescence] (Villafranca & Ash, 1976; Villafranca et al., 1977, 1978; Balakrishnan & Villafranca, 1979; Gibbs et al., 1984; Eads et al., 1985).

Tb(III) luminesces in solution at room temperature, and its luminescence is significantly enhanced when bound to proteins due to Förster-type energy transfer. The energy relay system

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