organometallic substrate and the first actinide compounds of any type to be coordinated in a η^1 rather than a η^5 fashion to the cyclopentadienyl moiety (from ferrocene). II is also the first example of a compound to contain two η^5-Cp_3U moieties bound to the same ligand.

Compounds I and II are formed by the reaction in THF at −30° of $(\eta^5\text{-}C_5H_5)_3UCl$[5] with ferrocenyllithium[6] or 1,1′-dilithioferrocenetetramethylethylenediamine,[7] respectively. Both compounds are very sensitive to oxygen and moisture; II is a green crystalline solid formed in 60% yield and I is a brown solid, forming in 75% yield.[8]

II is nonvolatile, decomposing above 190° to give ferrocene and a brown intractable solid while I sublimes easily under vacuum above 180°. Mass spectra of I show several important peaks at *m/e* 618 (M^+), 553 (M − Cp), 433 (Cp_3U), 368 (Cp_2U), 186 (Cp_2Fe), and 121 (CpFe). The spectrum is completely consistent with a structure based on π-bonding of three of the Cp rings to the uranium and σ-bonding of a Cp ligand from ferrocene to uranium.

Infrared spectra[9] of both I and II are also consistent with the proposed structures. Absorptions are present which are derived from both η^1 and η^5 coordinated cyclopentadienyl vibrations.[10]

The nmr data for I are also in accord with the above formulation. The 15 protons of the η^5-Cp_3U group appear as a sharp singlet at δ −2.33[11] which is in the region found for these protons by others.[4] The five protons of the unsubstituted cyclopentadienyl ligand on ferrocene appear as a sharp singlet at δ −1.64, a shift of over 5.7 ppm from free ferrocene. The signals for the four protons on the substituted ring which come much further upfield were found but could not be located exactly, coming roughly at δ −13 and −30. The furthest upfield signal is assigned to the two α protons. Marks[4b] and Streitwieser[12] have explained these shifts in terms of large contact contributions. A mechanism which involves the bonding of the R groups (ferrocene here) to uranium is then seen as a covalent contribution from filled ligand MO's to vacant f orbitals.

No nmr data could be obtained for II due to its low solubility. It is insoluble in almost all organic solvents except THF, in which it is only very sparingly soluble.

Magnetic susceptibility data are listed in Table I. The susceptibility for the monosubstituted derivative is in the same range as other Cp_3UR derivatives.[4] The susceptibility of II presents an interesting contrast. Preliminary results at variable temperatures indicate that, in the range from 300 to 60°K, there is continual increase in susceptibility as temperature decreases. It has been shown that other Cp_3UR derivatives display temperature-independent paramagnetism below 100°K. This phenomenon could be due to large spin–orbit coupling constants, strong ligand field splitting due to the ferrocene moiety, or perhaps to effects arising from having two Cp_3U groups bound in relatively close proximity to the same ligand.

Table I. Magnetic Susceptibility Data

Compound	$10^6\chi$	μ_{eff}
I[a]	2708	2.60
II[b]	5852	3.67

[a] Data at 310°K. [b] Data at 288°K.

Further studies with this type of compound are in progress to clarify the nature of the uranium–carbon σ bond, particularly with respect to f orbital involvement.

Acknowledgment. We wish to thank the United States Air Force Office of Scientific Research, AFOSR 71-2017, for financial support. We thank Dr. Erik Pederson and Mrs. Solveig Kallesøe for the determination of magnetic susceptibility.

(13) Work done in partial requirement for the Ph.D. degree at Texas A&M University.

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Paramagnetic Rare Earth Ion Probes of Transfer Ribonucleic Acid Structure

Sir:

Numerous recent investigations[1–6] have demonstrated that chelated rare earth ions may be used as nmr shift reagents to study the structure of molecules in organic solvents. Recently, however, Williams, *et al.*,[7] studied the binding of bare rare earth ions to the enzyme lysozyme in aqueous solution.[8] In this communication we report the first use of bare lanthanide ions as nmr shift reagents in an investigation of the structure of tRNA molecules in H_2O. These molecules are very important biologically since they are responsible for translating the genetic code, and consequently, there is considerable interest in their structure in solution. We have already shown in a series of high resolution nmr studies that the cloverleaf model is the correct description of the base pairing structure of at

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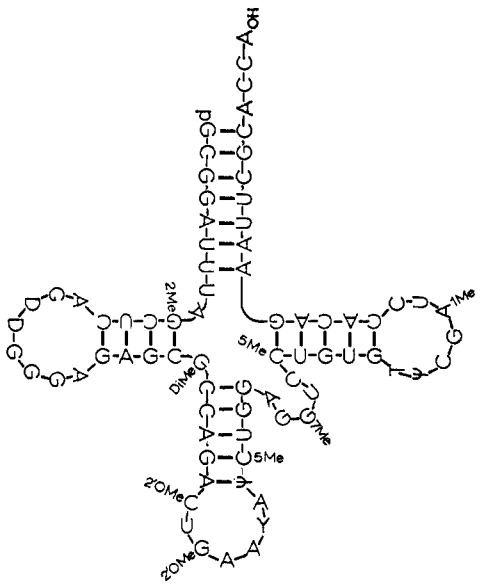

Figure 1. The primary sequence of yeast $tRNA^{Phe}$ arranged in the cloverleaf model: U. L. RajBhandary, S. A. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, and H. G. Khorana, *Proc. Nat. Acad. Sci. U. S.*, **57**, 751 (1967).

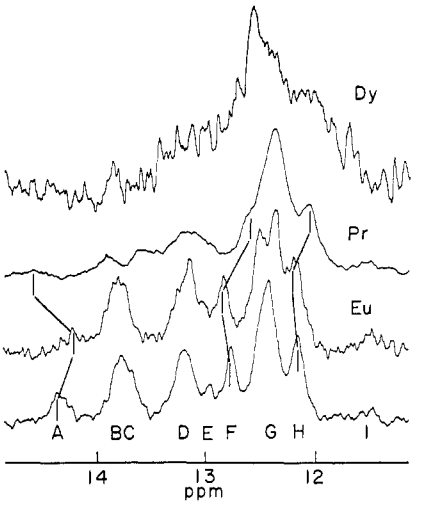

Figure 2. The proton nmr spectra of $tRNA^{Phe}$ at 300 MHz with no added lanthanide ions, with added Eu^{3+} ions, with added Pr^{3+} ions, and with added Dy^{3+} ions. The three spectra with added lanthanide ions have an ion/tRNA ratio of about 1:1.

least six different tRNA molecules,[9-13] including yeast $tRNA^{Phe}$ ($tRNA^{Phe}$ denotes the transfer RNA responsible for reading the codon for the amino acid phenylalanine). (See Figure 1.) Since the four helical arms of the cloverleaf structure are presumed to be regular "A" form RNA helices,[14] most of the important tertiary structure of the molecule would be established if the relative orientation of the four short double helical stems in the molecule could be determined. The fact all four stems intersect in a common region greatly constrains the number of different ways the molecule may be folded, and therefore relatively little additional information is needed. Paramagnetic lanthanide metal ions could be used to provide some of this information if the necessary experimental conditions are satisfied.[15] In this communication we show that lanthanide metal ions do bind to RNA molecules and that the bound ions give rise to observable paramagnetic shifts of proton nmr resonances and we identify one of the binding sites.

Chromatography on benzolated DEAE-cellulose[16,17] was used to isolate $tRNA^{Phe}$ from soluble RNA of brewer's yeast. $tRNA^{Phe}$ in a 0.1 *M* NaCl and 0.01 cacodylate buffer at pH 6.0 was put in a specially constructed Wilmad nmr micro cell and a measured amount of the lanthanide chloride, dissolved in doubly distilled water, was added. Spectra were obtained with a Varian HR 300 field sweep spectrometer and a Nicolet 1020A computer for signal averaging. The nmr spectra of $tRNA^{Phe}$ with no added lanthanide metal ions, with Eu^{3+} ions added, with Pr^{3+} ions added, and with added Dy^{3+} ions (approximately 0.001 *M*) are shown in Figure 2.

Resonances in the low field (11–15 ppm) spectrum of $tRNA^{Phe}$ are due to ring nitrogen protons[18] which are slowly exchanging with the solvent because of their involvement in Watson–Crick base pairs. These resonances have previously been assigned to specific base pairs.[11] The addition of small amounts of lanthanide ions noticeably shifts many, but not all, of these resonances, as can be seen in Figure 2. It was possible that the observed shifts were due to a structural change rather than a paramagnetic effect, but this possibility is ruled out by comparing the effects of Eu^{3+} and Pr^{3+} ions in Figure 2. Focusing on peak A it can be seen that Eu^{3+} caused an upfield shift whereas Pr^{3+} ions caused a downfield shift of peak A, and this is expected since these two ions are known to give paramagnetic shifts in opposite directions.[5,6,15] Dy^{3+} usually produces shifts *ca.* ten times larger than Eu^{3+} and Pr^{3+}, but it is 40 times worse in causing line broadening.[19] Therefore, the fact that Dy^{3+} ions, at a low Dy^{3+}/tRNA ratio (1/1), completely changed the entire spectrum is not unexpected.

At the present time we cannot make specific assignments of all the resonances which are shifted by the lanthanide ions; however, the lowest field resonance at 14.4 ppm has been unambiguously assigned to base pair AU_6. Since this is one of the resonances most affected by addition of the paramagnetic ions, we conclude that the strongest metal binding site is located close to AU_6.

The work described here demonstrates it is feasible to use rare earth ions as paramagnetic probes of tRNA structure. Since the binding of just one lanthanide

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metal per tRNA shifts more than one resonance, it is evident the shifts in the nmr spectra contain information about the three-dimensional structure of the tRNA molecule. When the nmr shift data are combined with the assignment of resonances to specific Watson–Crick base pairs, the known distance and directional dependence of the paramagnetism of the lanthanide metal ions, and information derived from optical studies,[20] we may be able to determine the complete folding pattern of the tRNA molecules in solution.

Acknowledgments. The support of a U. S. Public Health Service Grant (GM 10449) and a Biomedical Science Support Grant (RR 07010-06) from the General Research Branch Division of Research Resources, Bureau of Health Professions, Education and Manpower Training, National Institutes of Health, is gratefully acknowledged. We also thank Mr. Frank Bosco of Wilmad Glass Co. for his cooperation.

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Europium as a Fluorescent Probe of Metal Binding Sites on Transfer Ribonucleic Acid. I. Binding to *Escherichia coli* Formylmethionine Transfer Ribonucleic Acid

Sir:

Rare earth ions have been used in different ways to study the structure and function of tRNA.[1-5] Kayne and Cohn, for example, have demonstrated that rare earth ions can substitute for divalent Mg^{2+} ion in promoting the aminoacylation of tRNA molecules.[1] In studies of the X-ray diffraction of yeast $tRNA^{Phe}$, Kim, *et al.*, used rare earth ions to obtain isomorphous replacement of the Mg^{2+} ion in their $tRNA^{Phe}$ crystals.[2,3] Formoso recently studied the binding of Tb^{3+} to mononucleotides and mixed RNA.[4] The accompanying communication describes the first application of rare earth ions as shift reagents in a high resolution nuclear magnetic resonance investigation of tRNA structure.[5] In the present communication we show how optical emission spectroscopy can be used to obtain information about the locations of binding sites and both kinetic and equilibrium data on the interaction of Eu^{3+} with tRNA molecules. In the experiments described here we have examined the binding of Eu^{3+} to *E. coli* $tRNA^{fMet}$.

E. coli $tRNA^{fMet}$ (lot No. 15290, aminoacylation activity 100%) was kindly provided by Dr. A. D. Kelmers, Oak Ridge National Laboratory, Oak Ridge, Tenn. For present studies the tRNA was reprecipitated from ~1 mg/ml solutions two–three times with ethanol. To remove metal cations, pellets containing 3–5 mg were dissolved in 1 ml of solution containing 25 m*M* (Na) EDTA, 0.5 *M* NaCl and 50 m*M* (Na) cacodylate, chilled in water, dipped into a 70–75° water bath for 20 sec, and then plunged into ice water. The tRNA was dialized first against NaCl and buffer solution and then against pure H_2O. After flash evaporation down to 10 mg/ml (based on the standard 1 mg = 20 A_{260} units = 40 nmol, in this experiment A_{260} was measured in 0.1 *M* NaCl and 50 m*M* cacodylate, pH 7). The concentrated, metal free, desalted tRNA was then renatured by slowly dializing in 0.1 *M* NaCl and 50 m*M* cacodylate at pH 7 at 5°. Renatured tRNA in water was then dialized against a 25-fold larger volume of NaCl-buffer in D_2O (99.7%). A 10^{-2} *M* stock $EuCl_3$ solvent was prepared from the hexahydrate (Alfa Inorganics, lot No. 113072) which was diluted in either D_2O or H_2O to a standard solution 10^{-4} *M* Eu^{3+} with 100 m*M* NaCl and 50 m*M* cacodylate, pH 7.0.

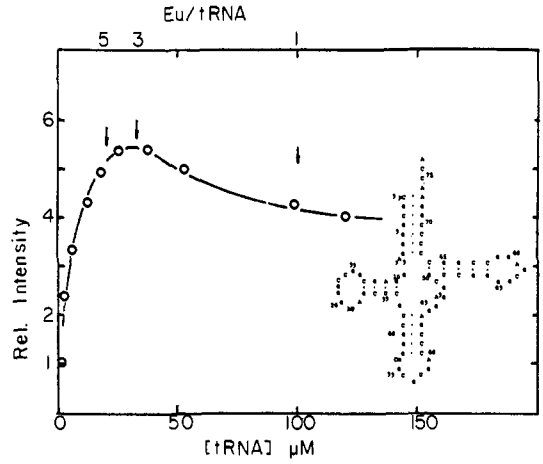

Figure 1. The effect of *E. coli* $tRNA^{fMet}$ on the steady state Eu^{3+} fluorescence in H_2O (Eu^{3+} = 10^{-4} *M*). The insert shows the primary sequence of *E. coli* $tRNA^{fMet}$ arranged in the cloverleaf structure.

The variation in the steady state fluorescence intensity with the Eu^{3+}/tRNA ratio (Figure 1) indicates that the peak intensity is obtained when there are three Eu^{3+} per tRNA molecule and that there is a decrease in the intensity when the ratio is either raised or lowered. The complex behavior of the steady state intensity indicates that more than one type of binding site is involved, and this is clearly demonstrated by the lifetime measurements.

In the absence of tRNA, the Eu^{3+} emission decayed exponentially with a lifetime of τ = 1.44 msec in D_2O. In the presence of tRNA, the decay could be fitted by a sum of exponentials using a single long lifetime of 1.95 msec and a range of shorter lifetimes (0.8–1.3 msec). The results of the lifetime measurements (obtained in D_2O) are presented in Figure 2 where the integrated intensity due to the shorter lived and to the long lived (1.95 msec) component are plotted separately. From these data we see that, as the Eu^{3+} solution is titrated with tRNA, there is a steep rise in the intensity of the short-lived emission until a peak value is reached for a Eu^{3+}/tRNA ratio of 3:1. Further addition of tRNA leads to a decrease in the intensity of the short-lived component and an increase in the long-lived component which plateaus at a ratio of Eu^{3+}/tRNA = 1.0.

There are additional experimental observations which

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