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.

(20) See communication by J. M. Wolfson and D. R. Kearns, *J. Amer. Chem. Soc.*, **96**, 3653 (1974).

Claude R. Jones, David R. Kearns*
Department of Chemistry, University of California
Riverside, California 92502
Received February 1, 1974

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.

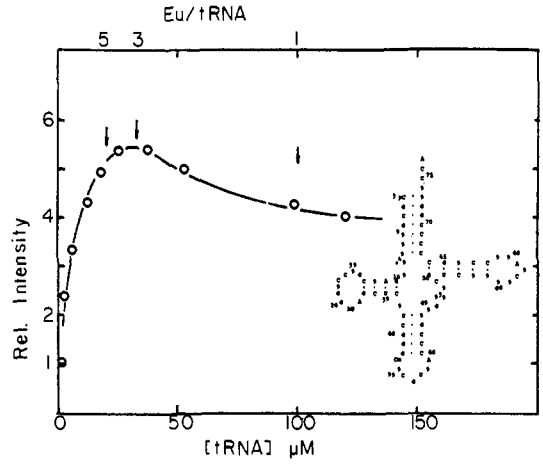

Figure 1. The effect of *E. coli* $tRNA^{fMet}$ on the steady state Eu^{3+} fluorescence in H_2O ($Eu^{3+} = 10^{-4}$ *M*). The insert shows the primary sequence of *E. coli* $tRNA^{fMet}$ arranged in the cloverleaf structure.

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

(1) M. S. Kayne and M. Cohn, *Biochem. Biophys. Res. Commun.*, **46**, 1285 (1972).
(2) S. H. Kim, G. Quigley, F. L. Suddath, A. McPherson, D. Sneden, J. J. Kim, J. Weinzierl, P. Blattman, and A. Rich, *Proc. Nat. Acad. Sci. U.S.*, **69**, 3746 (1972).
(3) S. H. Kim, G. Quigley, F. L. Suddath, A. McPherson, D. Sneden, J. J. Kim, J. Weinzierl, and A. Rich, *Science*, **179**, 285 (1973).
(4) C. Formoso, *Biochem. Biophys. Res. Commun.*, **53**, 1084 (1973).
(5) C. R. Jones and D. R. Kearns, *J. Amer. Chem. Soc.*, **96**, 3651 (1974).

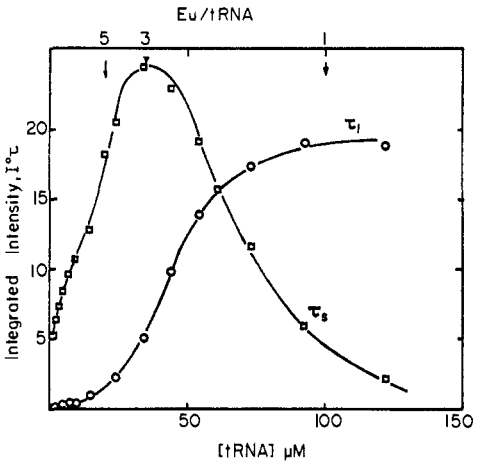

Figure 2. Effect of *E. coli* $tRNA^{fMet}$ on the fluorescence decay of 10^{-4} *M* Eu^{3+} in D_2O. The curves depict the variation in the integrated intensities, $I_0\tau$, for the short (τ_s = 0.8–1.3 msec) and the long lived (τ_ℓ = 1.95 msec) contributions to the decay. (See text.) These curves show that the maximal contribution from the short-lived component occurs for a Eu^{3+}/tRNA ratio of 3:1.

are useful in interpreting these data. The excitation spectrum of the Eu^{3+} luminescence, obtained with Eu^{3+}/tRNA ratios of 1:1 *and* 3:1, shows a very large peak at 340 nm corresponding to the maximum in the 4-thiouridine absorption but a minimum in the Eu^{3+} ion absorption. Very little emission (factor of over 100 smaller) was produced by direct excitation of the Eu^{3+} at 395 nm. When Mg^{2+} was added to the solution containing Eu^{2+} (10^{-4} *M*) and tRNA (5×10^{-6} *M*) the Eu^{3+} luminescence was suppressed but a large excess (0.08 *M*) was required to significantly reduce the intensity.

On the basis of these observations we draw the following conclusions regarding the binding of Eu^{3+} *E. coli* $tRNA^{fMet}$. (1) There are at least two different types of strong Eu^{3+} binding sites, and this is indicated by the observation of more than one emission lifetime. (2) The exchange of Eu^{3+} between the two different types of binding sites is slow compared with 1.9 msec; otherwise only a single exponential decay would have been observed. (3) The number of strong binding sites is approximately three. (4) The binding of Eu^{3+} is much stronger than the binding of Mg^{2+}. (5) The very large enhancement of both the short and long-lived components is due to 4-thiouridine sensitized energy transfer and this is confirmed by the excitation spectrum. Since this type of energy transfer is extremely short ranged, at least two of the Eu^{3+} binding sites are located very close (within 5–10 Å) to the 4-thiouridine residue at position 8 in *E. coli* tRNA (see insert, Figure 1).[6-10]

These preliminary studies illustrate, but by no means exhaust, the different ways in which rare earth ions may be used to probe metal binding sites on tRNA. Perhaps the most significant result is that there are two strong binding sites located near the 4-thiouridine residue at position 8 and it is interesting to note that the nmr experiments indicate a similar location for a Eu^{3+} binding site in yeast $tRNA^{Phe}$.[5]

(6) A. Heller and E. Wasserman, *J. Chem. Phys.*, **42**, 949 (1965).
(7) S. I. Weissman, *J. Chem. Phys.*, **10**, 214 (1942).
(8) P. Yuster and S. I. Weissman, *J. Chem. Phys.*, **17**, 1182 (1949).
(9) S. I. Weissman, *J. Chem. Phys.*, **18**, 1258 (1950).
(10) A. Lamola and J. Eisinger in "Molecular Luminescence," E. Lim, Ed., W. A. Benjamin, New York, N. Y., 1969, p 801.

Acknowledgments. The support of the U. S. Public Health Service (Grant GM 10449) is most gratefully acknowledged. We particularly thank Professor Brian Reid for providing laboratory facilities for some of this work.

J. M. Wolfson, D. R. Kearns*
Department of Chemistry, University of California
Riverside, California 92502
Received February 1, 1974

A General Ketone Synthesis.[1] Reaction of Organocopper Reagents with *S*-Alkyl and *S*-Aryl Thioesters

Sir:

Among the more useful organometallic reagents in synthetic chemistry are the organocopper(I) complexes.[2] We now wish to report our results for the reaction of organocopper(I) complexes with *S*-alkyl and *S*-aryl thioesters (eq 1–3) which gives ketones in high yield

$$^1/_2R_2CuLi + R'COSR'' \longrightarrow R'COR \quad (1)$$

$$RMgX \cdot CuI + R'COSR'' \longrightarrow R'COR \quad (2)$$

$$(RCu)_n \cdot LiI + R'COSR'' \longrightarrow R'COR \quad (3)$$

with efficient utilization of organometallic reagent.[3] This reaction appears to be general in scope (Table I).

The reaction of *S*-ethyl decanethioate (**1**) with various organometallic reagents was examined in some detail (eq 4). Treatment of **1** with either 1 equiv of *n*-butyl-

$$\underset{\mathbf{1}}{CH_3(CH_2)_8COSCH_2CH_3} \xrightarrow{RM} \underset{\mathbf{2}}{CH_3(CH_2)_8COR} + \underset{\mathbf{3}}{CH_3(CH_2)_8C(OH)R_2} \quad (4)$$

magnesium bromide (0°) or 1 equiv of *n*-butyllithium (−78°) gave the tertiary carbinol **3** (R = *n*-Bu) and recovered **1** in about equal amounts; lower reaction temperatures did not increase ketone formation from **1** and the Grignard reagent.[4] However, when **1** re-

(1) Contribution No. 18 from the Research Laboratory of Zoecon Corporation.
(2) (a) G. H. Posner, *Org. React.*, **19**, 1 (1972); (b) J. F. Normant, *Synthesis*, **4**, 63 (1972).
(3) Ketones have been prepared, in variable yields from acid chlorides and *excess* organocuprates: (a) G. M. Whitesides, C. P. Casey, J. San Filippo, Jr., and E. J. Panek, *Trans. N. Y. Acad. Sci.*, **29**, 572 (1967); (b) C. Jallabert, N. T. Luong-Thi, and H. Riviere, *Bull. Soc. Chim. Fr.*, 797 (1970); (c) G. H. Posner and C. E. Whitten, *Tetrahedron Lett.*, 4647 (1970); (d) N. T. Luong-Thi, H. Riviere, J.-P. Begué, and C. Forestier, *ibid.*, 2113 (1971); (e) G. H. Posner, C. E. Whitten, and P. E. McFarland, *J. Amer. Chem. Soc.*, **94**, 5106 (1972); see also ref 5; (f) N. T. Luong-Thi, H. Riviere, and A. Spassky, *Bull. Soc. Chim. Fr.*, 2102 (1973); from acid chlorides and Grignard derived organocopper(I) complexes, (g) J.-E. Dubois and M. Boussu, *Tetrahedron Lett.*, 2523 (1970); (h) N. T. Luong-Thi and H. Riviere, *ibid.*, 587 (1971); (i) J.-E. Dubois, M. Boussu, and C. Lion, *ibid.*, 829 (1971); (j) J. A. MacPhee and J.-E. Dubois, *ibid.*, 467 (1972); see also ref 3d and 3f; from acid chlorides and 1:1 CuX·RLi complexes, (k) H. Gilman and J. M. Straley, *Recl. Trav. Chim. Pays-Bas*, **55**, 821 (1936); (l) A. E. Jukes, S. S. Dua, and H. Gilman, *J. Organometal. Chem.*, **21**, 241 (1970); (m) J. F. Normant and M. Bourgain, *Tetrahedron Lett.*, 2659 (1970); (n) M. Bourgain and J. F. Normant, *Bull. Soc. Chim. Fr.*, 2137 (1973); from carbon monoxide and organocuprates; (o) J. Schwartz, *Tetrahedron Lett.*, 2803 (1972); and from certain esters and organocuprates, (p) S. A. Humphrey, J. L. Herrmann, and R. H. Schlessinger, *Chem. Commun.*, 1244 (1971); (q) G. H. Posner and D. J. Brunelle, *J. Chem. Soc., Chem. Commun.*, 907 (1973).
(4) *Cf.* M. S. Newman and A. S. Smith, *J. Org. Chem.*, **13**, 592 (1948); for preparation of ketones from acid anhydrides and Grignard reagents at −78°.