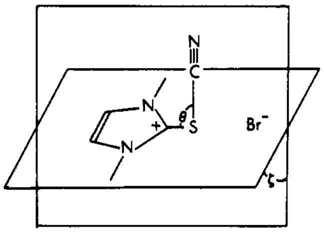

Figure 5. Solid state structure of salt **4a**.

dec, result from the reaction of **1** in CH_2Cl_2 solution at 0 °C with chlorine and iodine monobromide, respectively. The latter sulfurane begins to decompose in the solid state after ca. 1 h at room temperature which, nevertheless, represents extraordinary thermal stability considering the low dissociation energy of a normal two electron S-Br and S-I bond which here has an even lower bond order.

In contrast, the reaction of **1** with cyanogen bromide give the salt **4a,** mp 220-221 °C dec, whose crystal structure was determined similarily by x-ray diffraction: monoclinic; a = 7.288, b = 9.502, c = 15.206 Å; β = 122.459°; V = 1053 Å^3; Z = 4; ρ_{calcd} = 3.381 g/cm^3; space group $P2_1/C$ from systematic absences. A total of 1248 nonzero reflections were refined to a crystallographic R factor of 0.043 and the resultant structure is shown in Figure 5.

The covalent attachment of the cyano group is characterized by an axial S-C bond length of 1.757 Å orthogonal (θ = 96.20, ζ = 90.0) to the ring system while the equatorial bromide ion occupies a lattice position 3.270 Å distant from sulfur. A second neighbor bromide ion was found at a lattice position 3.587 Å in the axial direction from C2 anti to the cyano group.

Treatment of **1** with cyanogen chloride gives **4b,** mp 215-216 °C dec and the common ionic structure of these derivatives was evident from their superimposible IR spectra.

The observed incongruity between the appearance of a hypercovalent or an ionic molecule as a function of ligand structure is clearly independent of ligand electronegativity. If one considers an *electron-rich linear* multicenter bonding array with the hypervalent center participating by predominately p-orbital bonding two general patterns of occupied, symmetry-adapted, delocalized molecular orbitals appear. For $2n + 3$ (n = 0, 1, 2, . . .) atomic orbitals combined to give molecular orbitals with $2n + 4$ electrons there are $1n + 1$ occupied *nonbonding* levels with respect to the ligands which do not contribute to vicinal bonding in the array and for isolable hypervalent molecules requires electronegative ligands to stabilize the resultant electron density as is observed for **3.** Conversely, $2n + 4$ atomic orbitals combined to give molecular orbitals for $4n + 6$ electrons result in $4n + 2$ occupied *antibonding* levels noded with respect to the ligands which are destabilizing regardless of ligand electronegativity, as for example, the hypervalent possibility related to **4.** In other words, if the sum of the one central hypervalent atom plus the coaxial ligand atoms is odd the system is stable and, if even, destabilized. This argument may be extrapolated to account for the origin of the unusually high energy barrier observed for the gas-phase nucleophilic displacement at carbon associated the reaction of CN^- with CH_3Cl and C_2H^- with CH_3F which requires a linear transition state described by a combination of $2n + 4$ atomic orbitals.[7] Likewise the observed highly concerted decomposition (ΔH^* = 17.6 kcal/mol) of di-*tert*-butyl *O,O'*-phenylbisthioperbenzoate proceeds via a linear 6-electron 5-center hypervalent sulfur bonding array with one occupied bonding and two occupied nonbonding levels in the transition state and is an example of a stabilized $2n + 3$ atomic orbital combination.[8]

Acknowledgments. We sincerely wish to thank the National Institutes of Health and National Science Foundation for research grants and to Professor Charles Liotta and J. C. Martin for bringing ref 7 and 8 to our attention.

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A. J. Arduengo, Edward M. Burgess*
School of Chemistry, Georgia Institute of Technology
Atlanta, Georgia 30332

Received September 24, 1976

Laser-Induced Lanthanide Ion Luminescence Lifetime Measurements by Direct Excitation of Metal Ion Levels. A New Class of Structural Probe for Calcium-Binding Proteins and Nucleic Acids

Sir:

The employment of the fluorescence of organic groups in biochemical research is widespread. In marked contrast, the use of metal ion luminescence in probing structure-function relationships remains a relatively unexploited area. Of all the metallic aquo cations in the periodic table, only certain members of the trivalent lanthanide series, Ln(III), luminesce in solution at room temperature. While Ln(III) ions do not occur naturally in biological systems, they appear to be, in general, capable of replacing calcium ions in biological systems[1,2] with isomorphous substitution having been demonstrated in several instances.[3,4] Unfortunately, in comparison to organic fluorophores, lanthanide ions are only weakly luminescent, principally because they are extremely weak absorbers of electromagnetic radiation. Heretofore, utilization of Ln(III) ion luminescence in biochemical research has been limited to cases in which the metal ion luminescence is greatly enhanced upon binding to a macromolecule. Only for Tb(III) bound to globular proteins[5-8] and for Tb(III) and Eu(III) bound to nucleic acids[9,10] have such intensity enhancements been reported. This enhancement of luminescence results primarily from energy transfer from strongly absorbing organic moieties (aromatic amino acids, nucleotide bases) to excited metal ion levels. The present report represents the initial stage of a program designed to extend the utility of Ln(III) ion luminescence to any macromolecule which binds a Ln(III) ion. The technique described, which does not depend on energy transfer, should allow additional members of the lanthanide series to serve as useful probe ions. The experiments are carried out using radiation of a wavelength at which most proteins and nucleic acids are transparent. We have concentrated initially on the measurement of Ln(III) ion luminescence decay constants which provide a sensitive measure of the number of water molecules coordinated to the metal ion.

In this study we exploit the large photon flux available from a coaxial flashlamp-pumped dye laser (Phase-R, Model 1200

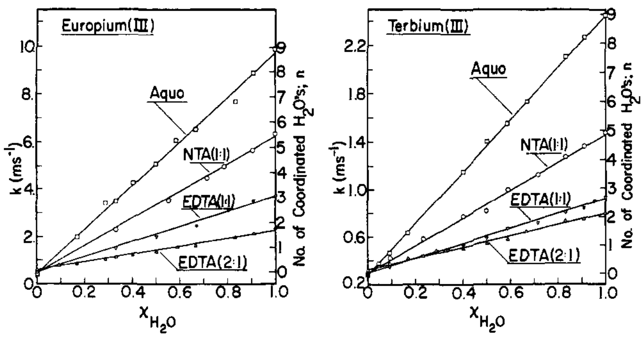

Figure 1. Plots of lanthanide ion luminescence decay constants, k (ms^{-1}), vs. mole fraction of H_2O (χ_{H_2O}) in H_2O-D_2O mixtures for Eu(III) and Tb(III). The number of coordinated H_2O's, n, for the indicated complexes is given on the right-hand ordinates: aquo ions, □; NTA complexes (pH 6.0), ○; EDTA (1:1) complexes (pH 6.0), ▽; EDTA (2:1) complexes (pH 7.5), △.

with a 5-30 mJ per 0.4 μs pulse output) to excite directly the weakly absorbing Eu(III) or Tb(III) ion levels. Eu(III) was excited to the $^5D_2$ state[11] while Tb(III) was excited to the $^5D_4$ emitting level.[12] Fluorescence decays were monitored using a red-sensitive photomultiplier tube. Oscilloscope traces were recorded photographically.

Luminescence of sufficient intensity for lifetime measurements was observable for aqueous solutions of europium(III) and terbium(III) chloride at concentrations as low as 1 μM. The laser-induced Ln(III) luminescence is observed to decay exponentially with a decay constant, k (equal to the reciprocal of the lifetime, τ), which is independent of metal ion concentration. Of particular importance here is the fact that the k values are extremely sensitive to the constitution of the metal ion coordination sphere. Owing to a radiationless path for deexcitation via coupling to OH vibrational overtones, the observed k values in H_2O are much larger than in D_2O.[13-21] Previous work[14,15] has established that k values for the Eu(III) and Tb(III) aquo ions vary linearly with the mole fraction of H_2O (χ_{H_2O}) in H_2O-D_2O mixtures. Furthermore it has been demonstrated[18,20,21] that OH oscillators act independently in effecting radiationless deexcitation and with an efficiency unaffected by the constitution of the remainder of the coordination sphere. These findings suggest that luminescence lifetime measurements should provide a reliable measure of the number of water molecules in the primary coordination sphere of the metal. In Figure 1 are presented plots of k values for the aquo ions of Eu(III) and Tb(III) and their EDTA[22] and NTA[22] chelate complexes vs. χ_{H_2O} in H_2O-D_2O mixtures. The linearity of the plots extends to the chelate systems and it is apparent that the slopes are markedly decreased in the chelate systems owing to the exclusion of varying numbers of water molecules from the primary coordination spheres of the metal ions in the different complexes.

The observed k values may be represented by the equation: $k = k_r + k_x + k_{H_2O}\chi_{H_2O}$, where k_r is the inherent radiative decay constant, k_x is the decay constant for all nonradiative processes other than water OH deexcitation, and k_{H_2O} is the decay constant for deexcitation via water OH vibrations.[23] As the k_{H_2O} values (slopes of plots in Figure 1) are proportional to the number of water molecules, n, in the primary coordination sphere,[20,21] the k_{H_2O}/k_{H_2O} (aquo ion) values represent the ratio of numbers of water molecules bound in a particular complex to that in the aquo ion. Since the intercept $(k_r + k_x)$ is roughly constant for the complexes studied, and assuming $n = 9$ for the aquo ion,[20,24-26] the approximate n values can be obtained from the intercept with the scale along the right-hand ordinates of the graphs in Figure 1. For the 1:1 EDTA complexes n values of about 3 and 2.5 are obtained for Eu(III) and Tb(III), respectively. The value of $n = 3$ is consistent with the known x-ray structure[27] of substances with the composition $KLnEDTA{\cdot}7H_2O$ where three water molecules occupy the first coordination sphere in addition to the sexadentate EDTA ligand. At present our measurements are probably not sufficiently precise to exclude the presence of a certain fraction of a second hydrate species differing in composition by one water molecule which has been indicated for the Eu(III)-EDTA system in several studies.[28-31] Our finding that the Tb(III)-EDTA system coordinates, on average, approximately 0.5 fewer water molecules than does the Eu(III) system is entirely consistent with a like finding[31b] from thermodynamic studies. In solutions with a 2:1 EDTA:Eu(III) ratio our results show that approximately two water molecules remain coordinated consistent with the previously reported[28,32] formation of 2:1 complexes. In the Tb(III) system the change we observe in going from 1:1 to 2:1 complexes is less dramatic, but in the absence of knowledge of the pertinent equilibrium constant little can be concluded from this result. The NTA ion is potentially a quadridentate ligand and one might expect an additional five or six water molecules to occupy the primary coordination sphere to achieve a total coordination number of 9 or 10 (a 10-coordinate protonated EDTA complex of Ln(III) is known[33]). Our result (Figure 1) of ~5.5 and ~5 coordinated water molecules in the Eu(III)- and Tb(III)-NTA systems, respectively, is entirely consistent with this expectation. Furthermore, the reduction in numbers of coordinated water molecules in going from Eu(III) to Tb(III), noted in the EDTA systems, is again found, presumably due to the reduced ionic radius of Tb(III).

In order to assess the utility of lanthanide luminescence lifetimes as probes of enzyme structure we have carried out measurements on Eu(III) and Tb(III) bound at a calcium site in thermolysin.[3] This enzyme (obtained from Calbiochem, San Diego, Calif.) is an endoproteinase of known x-ray structure[34] which binds one zinc and four calcium ions in the native state. Under the conditions used in the present experiments (0.05 M Tris chloride buffer pH 7.5, 0.1 M NaBr, 1 mM $CaCl_2$) an added Ln(III) ion binds at calcium site 1 with no effect on enzymatic activity.[5] Our luminescence decay constant results for Ln(III) ions bound to thermolysin in H_2O and D_2O solution in the concentration range 50-250 μM are as follows: Eu(III) in H_2O, 2.13 ± 0.20 ms^{-1}; Eu(III) in D_2O, 0.57 ± 0.05 ms^{-1}; Tb(III) in H_2O, 0.79 ± 0.05 ms^{-1}; Tb(III) in D_2O, 0.45 ± 0.02 ms^{-1}. These results imply that ca. two water molecules coordinate to Eu(III) and Tb(III) when these ions are bound at calcium site 1 in thermolysin. The lifetime of Tb(III) bound to thermolysin in H_2O (τ, 1.26 ms) is also very close to the value (τ, 1.27 ms) for this ion in transferrin,[7] the only previously reported luminescence lifetime for a protein-bound Ln(III) ion.

This preliminary study demonstrates the feasibility of using direct laser excitation of Ln(III) ion levels to monitor luminescence lifetimes and thus to probe the environment of this class of reporter ion in biological systems. The present study has been limited to Eu(III) and Tb(III), which have relatively long lifetimes; however, it is clear that this technique can be applied to other members of the lanthanide series and can thus expand the number of useful metal ion luminescence probes. It should be noted that experiments of this type are applicable to Tb(III)-protein systems where energy transfer (from the UV-excited aromatic amino acid residues to the bound Tb(III)) is weak or absent[8] and to proteins exhibiting UV photosensitivity.[8] In comparison with the Gd(III) water proton resonance relaxation technique[35,36] for determining the number of coordinated water molecules the present technique is much simpler experimentally and would appear to be more reliable and versatile. Important applications of experiments of this type in monitoring intermetal ion energy transfer for distance measurements[5] and for the examination of potentially environmentally sensitive luminescence emissions under high resolution suggest themselves.

Acknowledgments. We thank the National Science Foundation for support of this work through Grants CHE76-08528 (to W.DeW.H., Jr.) and CHE75-20122 (to R.A.B.) and to the National Institutes of Health through Grant GM23599 (to W.DeW.H., Jr.).

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William DeW. Horrocks, Jr.,* Gregory F. Schmidt
Daniel R. Sudnick, Carter Kittrell, Robert A. Bernheim
Department of Chemistry, The Pennsylvania State University
University Park, Pennsylvania 16802
Received November 15, 1976

Substituent Effects on the Formation Constants of Iron(III) and Iron(II) Tetraphenylporphyrin–Pyridine Complexes

Sir:

In recent years there has been considerable interest in linear free energy relationships involving metalloporphyrins. These have focused on the effect of porphyrin ring basicity in determining polarographic half-wave potentials,[1-4] electron transfer kinetics,[5] spectroscopic properties,[6] and stability constants for axial ligand addition to form 1:1 and 2:1 complexes.[3,7-11] Recent publications have shown that the presence of electron withdrawing groups on a porphyrin ring contribute to substantially increased stability constants for Lewis base complexation of cobalt(II),[3,9] nickel(II),[8,10] vanadyl(II),[10] and zinc(II)[11] para and meta substituted tetraphenylporphyrins. The magnitude of interaction of the central metal with the porphyrin ring was measured using the Hammett linear free energy relationship.[12]

$$\log \frac{K^x}{K^H} = \Sigma\sigma\rho \qquad (1)$$

In all previous studies of +2 metals ρ was positive and found to increase in the order VO(p-X)TPP (0.113) < Co(p-X)TPP (0.166) < Zn(p- or m-X)TPP (0.188) ≪ Ni(p-X)TPP (0.331) < Ni(m-X)TPP (0.413). Of the metals investigated only Ni(p-X)TPP and Ni(m-X)TPP formed 2:1 adducts, and it was therefore suggested that ρ might be dependent on the number of axial ligands.[3] Another suggested possibility was that ρ increased with the number of metal d electrons.[10] However, the differences in ρ are small for the monoliganded complexes containing vanadyl, cobalt, and zinc and one might be tempted to argue that within experimental error, no significant differences exist in the extent of metal, porphyrin ring interaction.

Recently, Walker et al.[13] have reported linear free energy relationships for the addition of Lewis bases to Fe(p-X)TPPCl to form 1:1 and 2:1 complexes. For Fe(p-X)TPP·B_2Cl, where B = N-methylimidazole, plots of log K^X/K^H vs. 4σ gave a negative slope of $\rho = -0.39$ in $CHCl_3$ compared to a positive slope for all of the previously investigated +2 metals. The change in sign of the reaction constant was rationalized on the basis of a stabilization of the positively charged Fe(III) and there appeared to be a clear indication that stabilization of an ion paired product, FeTPP·$B_2{}^+Cl^-$, predominated the reactions of Fe(p-X)TPPCl with axial ligands. However, a comparison of substitutent effects with Fe^{II}(p-X)TPP·B_2 was not presented so that suggestions of charge stabilization could only be made based on comparisons with equilibrium constants for Ni^{II}(p-X)TPP·B_2. Furthermore, no conclusions could be drawn from stability constants of Fe(p-X)TPP·BCl presumably due to the small values of the equilibrium constant and scatter in the data.

In this communication we present the first comparison between substituent effects on Fe^{II}P·B_2, Fe^{III}P·B_2Cl, and Fe^{III}P·BCl in DMF where B is pyridine and P represents (p-X)TPP or (m-X)TPP. Both iron(III) and iron(II) porphyrins are known to form bis coordination complexes with pyridine[14,15] while iron(III) will also form a monoligand adduct in some solvents.[13] At a platinum electrode reversible reductions are observed for both Fe(III) and Fe(II) in DMF.[16] Stability constants for Fe^{III}P·BCl, Fe^{III}P·B_2Cl, and Fe^{II}P·B_2 can then be evaluated from shifts in polarographic peak potentials between the uncomplexed porphyrin in neat DMF and the complexed species in DMF–pyridine mixtures.[17]

A typical cyclic voltammogram of Fe(p-CH_3)TPPCl in neat DMF, 11.5 mM and 1.58 M pyridine–DMF mixtures is shown in Figure 1. On adding pyridine, peak potentials for Fe^{III}P·Cl reduction to Fe^{II}P (peak 1) shifted only slightly, while peak potentials for reoxidation of Fe^{II}P (peak 2) shifted anodically by over 300 mV with increasing pyridine concentration. At the same time, a new peak (identified as peak 3, Figure 1) appeared which was separated by 60 mV from peak 2 and also shifted significantly with changes in the pyridine concentration.

Using the magnitude of the potential shifts Scheme I may be formulated. Assignments of DMF as an axial ligand are based on analogous assignments in Me_2SO and DMA where up to two ligands are coordinated to iron(II) and iron(III).[15]