

Mössbauer, magnetic susceptibility, radiolytic and photochemical studies of europium and lutetium porphyrins

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Abstract

The chemistry of the lanthanide complexes of europium and lutetium with several porphyrin molecules were probed in both the solid state and in solution. Mössbauer and variable temperature magnetic susceptibility studies indicated that the trivalent state of europium was formed under the synthetic conditions. Electron addition to both europium(III) and lutetium(III) porphyrins by photochemical and radiolytic techniques resulted in reduction of the porphyrin ring rather than the coordinated trivalent metal ion, and the resulting π -radical anion disproportionated into the initial metalloporphyrin and the ring reduced metalloporphyrin di-anion. The reduction potentials in solution for mono- and di-anion formation of the lutetium porphyrin were measured by electrochemical procedures. While the lutetium(III) porphyrin is stable for years at pH 7, the metal is rapidly removed from the metalloporphyrin by protons in the di-anion state. The photophysics of the lutetium(III) porphyrin in solution are dominated by the triplet state, and the reactivity of this triplet with dioxygen, NADH and persulfate is reported.

Introduction

Porphyrins are an important class of cyclic conjugated tetrapyrrole pigments that occur widely in nature as metal complexes. Vanadium and nickel porphyrins are in oil shale, iron porphyrins in hemoglobin, myoglobin and the cytochromes. Vitamin B-12 is a cobalt porphyrin derivative and reduced porphyrins bound to magnesium ions constitute chlorophyll [1]. By attaching various substituents to the macrocyclic ring, synthetic porphyrins can be made either water soluble or insoluble. Although techniques are available to incorporate many metals into water insoluble porphyrins, our interest centers on the unique properties of water soluble metalloporphyrins, which have applications as relays in the photochemical

splitting of water [2], as tumor imaging agents for whole body magnetic resonance applications [3], as singlet oxygen producers in photodynamic therapy [4], and as potential *in vivo* anti-cyanide drugs [5].

While much is known about the properties of porphyrins bound to small transition metal ions, little has been done on lanthanide complexes, where the metal is so large that it must sit 1.2–1.8 Å above the mean porphyrin plane [6]. In this communication, the oxidation states of europium in porphyrins were probed by magnetic susceptibility and Mössbauer measurements, where the latter are the first reported on such compounds. Electrochemical and radiolytic studies were carried out to ascertain if added electrons would reduce the coordinated lanthanide ion, or the porphyrin ligand itself. Photochemical procedures were used to examine the nature and reactivity of

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the excited states of a water soluble lutetium porphyrin complex.

Experimental

Europium was inserted into the water insoluble TPP (tetraphenylporphyrin) and CH₃-TPP (tetra(4-methylphenyl)porphyrin) derivatives by an imidazole melt method [6], whereas the lanthanide TMPyP (tetra(*N*-methyl-3-pyridyl)porphyrin) and TPyP (tetra(3-pyridyl)porphyrin) complexes were prepared in dimethylformamide [7]. Satisfactory analytical data was obtained for all derivatives, indicating that the trivalent lanthanide was obtained in each case. For example for HO-Lu(III)-TMPyP-I₄·3H₂O, C₄₄H₃₇N₈OLuI₄·3H₂O; Calc: C, 37.1; N, 7.8; Lu, 12.2. Found: C, 37.1; N, 7.9; Lu, 12.2. The apparatus and techniques for the magnetic susceptibility [8] and radiolytic [2] studies have been described before. Mössbauer experiments [9] employed a 100 mci ¹⁵¹Eu source in a samarium fluoride matrix. Measurements were made at 80 K, and the results were analyzed using a computerized least-squares routine.

Absorption spectra were recorded with an Hitachi U3201 spectrophotometer and fluorescence spectra with a fully corrected Perkin-Elmer LS5 spectrofluorimeter. Quantitative fluorescence studies were performed with optically dilute solutions using Rhodamine 101 in ethanol, for which the fluorescence quantum yield is taken as 1.0 [10]. Fluorescence lifetimes were measured by the single-photon-counting technique using a mode-locked, synchronously-pumped, cavity-dumped Rhodamine 6G laser. Output from the dye laser at 580 nm was attenuated with neutral density filters. Fluorescence was isolated with narrow (10 nm) band-pass filters and detected at right angles with a Hamamatsu microchannel plate photodetector giving an overall instrumental response function of 80 ps. Data analyses were made using the procedures recommended by O'Connor and Phillips [11] and all experiments were made in air equilibrated aqueous solutions at 22 °C using a thermostatted cell holder.

Laser flash photolysis studies were done with a frequency doubled, Q-switched Quantel YG481 Nd-YAG laser. Output from the laser at 540 nm was attenuated with neutral density filters and defocused onto the sample cell. The monitoring beam was provided by a low-intensity xenon arc lamp and was passed through narrow (50 nm) band-pass filters before entering the sample cell in order to avoid undue photolysis of the solution. The triplet state was monitored at 470 nm, with a 450 nm cut-off filter in the monitoring beam before the sample cell. For all measurements, the solution was adjusted to

possess an absorbance at 532 nm of ~0.1 and purged continuously with N₂. The intensity of the laser was calibrated using tetra(3-hydroxyphenyl)porphyrin in methanol as a standard for which the triplet quantum yield is 0.69 and the triplet molar differential coefficient at 440 nm is 44 000 M⁻¹ cm⁻¹ [12]. For all kinetic measurements, 50 individual decay profiles were signal averaged and corrected for any baseline changes before being analyzed by computer iterative procedures. Transient differential absorption spectra were recorded point-by-point with 10 individual laser shots being averaged at each wavelength.

Triplet state molar differential extinction coefficients were determined by the photosensitization method using anthracene 1,5-disulfonate as a sensitizer in N₂-purged aqueous solution. For these experiments, the frequency-tripled 355 nm output from the Nd-YAG laser was used to excite anthracene (3 × 10⁻⁵ M) and the resultant triplet state concentration was measured at 425 nm assuming a triplet quantum yield of 0.71 [13] and a molar differential extinction coefficient of 64 700 M⁻¹ cm⁻¹ [14]. The laser intensity was attenuated with neutral density filters until the decay of the triplet anthracene followed first-order kinetics; typically, the lifetime was around 850 μs. Aliquots of a solution of the porphyrin in water were added and rates of formation of the triplet porphyrin (at 470 nm) were recorded as a function of the total porphyrin concentration. The bimolecular rate constant for energy transfer from triplet anthracene was found to be (4 ± 1) × 10⁹ M⁻¹ s⁻¹. Sufficient porphyrin was added to reduce the lifetime of the triplet anthracene to <10 μs; this ensures quantitative formation of the triplet dye via energy transfer such that the concentration of the triplet dye could be equated to the initial concentration of triplet anthracene.

Triplet quenching studies were made by observing the effect of added substrate on the triplet lifetime in N₂-saturated aqueous solution. At least five different substrate concentrations were used and the bimolecular rate constant was calculated from the gradient of a linear plot of the pseudo first-order decay rate constant versus substrate concentration. Transient absorption spectra of the reaction products were recorded point-by-point after decay of the triplet state. Extinction coefficients for the products were obtained from the pulse radiolysis experiments.

Singlet molecular oxygen was detected by the time-resolved luminescence technique [15] using a silicon photodiode filtered to isolate a spectra window at 1270 ± 30 nm. The detector was calibrated using aluminum phthalocyanine trisulfonate in O₂-saturated D₂O solutions, for which the quantum yield for formation of O₂(¹Δ_g) is 0.34 [16]. Solutions of

the dye and standard in O₂-saturated D₂O were adjusted to possess identical absorbances in the range 0.1–0.3 at 532 nm and were excited with a 10-ns laser pulse from the frequency-doubled Nd-YAG laser. The yield of O₂(¹Δ_g) was determined by computer extrapolation of the decay profile to the center of the excitation pulse after averaging 50 individual decay profiles. A 30-fold range of laser intensities was used and measurements were restricted to cases where the observed signal was a linear function of the laser intensity.

Cyclic voltammetry studies were made with a Rank E611 potentiostat driven by a purpose-built triangle wave generator. A highly-polished glassy carbon disc was used as the working electrode, together with a Pt counter electrode and a saturated calomel reference. Solutions containing the porphyrin (*c.* 10⁻⁴ M) and KCl (0.2 M) were purged thoroughly with N₂ prior to electrolysis.

Results

Magnetic susceptibility

An automated variable temperature Faraday balance was used to measure the magnetic susceptibility of a 24 mg sample of the acetylacetonate complex of europium-tetraphenylporphyrin (Eu-TTP(acac)). At 298 K, the observed gram susceptibility was independent of field strength from 1400–3200 Gauss, indicating a lack of ferromagnetic impurities in the solid. The gram susceptibilities were measured at two degree intervals between 77 and 298 K, and a plot of the observed magnetic moments versus temperature is shown in Fig. 1.

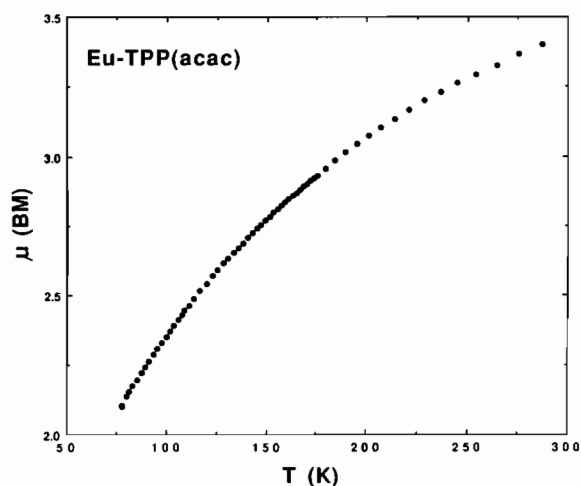


Fig. 1. Plot of the observed magnetic moment vs. temperature for Eu(III)-TTP(acac).

Mössbauer measurements

Typical Mössbauer absorption spectra for the europium porphyrins are shown in Fig. 2. The spectrum for each porphyrin is a singlet in each case, where the variable line widths indicate the presence of unresolved quadrupole splitting. The results at 80 K are shown in Table 1.

Radiolysis and electrochemistry

Reductive pulse radiolysis was done on Lu(III)-TMPyP(Lu(III)-P) in N₂-saturated methanol solutions. The immediate one-electron reduction product was Lu(III)-P⁻, the ring reduced porphyrin π -radical anion, which had absorption bands at 470 and 710 nm, similar [17] to those found for Zn(II)-TMPyP (which has a non redox-active metal ion). This product disproportionates into the two-electron ring reduced di-anion Lu(III)-P²⁻ (780 nm) and Lu(III)-P. Under continuous gamma-radiolysis conditions in N₂-saturated water at pH 7 containing 5% isopropanol, a two-electron ring reduced form was immediately noted, and further radiolysis led

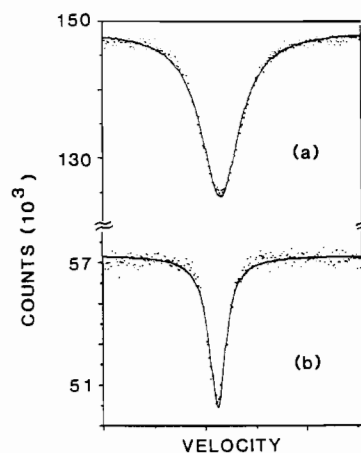


Fig. 2. Mössbauer spectra of (a) Eu-TTP(acac) at 80 K, (b) Eu-T(3-Py)P(acac) at 80 K.

TABLE 1. Mössbauer results

Complex	Isomer shifts ^{a, b} at 80 K (mm/s)	FWHM at 80 K (mm/s) ^b
Eu-T(3-Py)P(acac) ^c	+0.32	1.4
Eu-T(N-Me-3-Py)P(acac)I ₄ ^d	+0.30	1.3
Eu-TTP(acac) ^e	+0.97	3.2
Eu-T(4-CH ₃)PP(acac) ^f	+1.1	2.9

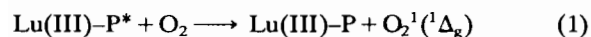
^aIsomer shifts are relative to EuF₃. ^b±0.02 mm/s. ^cEuropium(III)-tetra(3-pyridyl)-porphyrin acetylacetonate. ^dEuropium(III)-tetra(*N*-methyl-3-pyridyl)porphyrin acetylacetonate tetraiodide. ^eEuropium(III)-tetraphenylporphyrin acetylacetonate. ^fEuropium(III)-tetra(4-methylphenyl)porphyrin acetylacetonate.

to the bleaching of the entire spectra as further electrons were added to the porphyrin ring. Upon reoxidation with $O_2(g)$, the absorption spectra indicated that the metal had been removed from the porphyrin, forming the free-base H_2-P compound. The behavior of $Eu(III)-TMPyP$ was similar, and indicated only ring reduction.

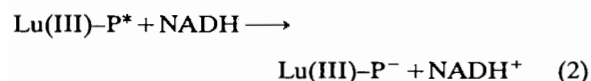
Cyclic voltammetry conducted on $Lu(III)-P$ at pH 9.3 in phosphate buffer shows two clear peaks on reductive scans, which are diffusion controlled but essentially irreversible due to some chemical reaction removing the electrode products before reoxidation can occur. On the reverse scan, there is a sharp peak corresponding to a two-electron oxidation process. In conjunction with the radiolysis results, the first reduction product is the ring reduced π -radical anion ($E_{1/2} = -0.61$ V versus NHE for $Lu(III)-P/Lu(III)-P^-$), and the second peak is the porphyrin di-anion (-0.72 V for $Lu(III)-P^-/Lu(III)-P^{2-}$). The mono- and di-reduced species rapidly protonate, leading to the observed irreversibility. After loss of the metal from the reduced form, the single two-electron peak is due to the oxidation of the di-reduced metal-free porphyrin into the free-base, H_2-P ($E_{1/2} = -0.42$ V for H_2-P^{2-}/H_2-P).

Photophysics and excited state reactivities

The photophysics of the chloride salt of $Lu(III)-TMPyP$ were measured in N_2 -deaerated solutions (0.1 M Na_2SO_4) at pH 7. The fluorescence quantum yield was 0.0015 ± 0.0003 , with a excited singlet state lifetime of <0.1 ns, while the triplet quantum yield was ~ 1.0 , with a triplet lifetime of 27.5 ± 2.0 μs in the absence of O_2 . The triplet ($Lu(III)-P^*$) reacts with the triplet state of dioxygen forming the spectroscopically observed singlet molecular oxygen



with a specific rate constant of $(7.4 \pm 0.8) \times 10^8$ $M^{-1} s^{-1}$. In O_2 -saturated D_2O ($pD=7$), the quantum yield for formation of $O_2(^1\Delta_g)$ was found to be 0.78 ± 0.06 . $Lu(III)-P^*$ is quenched with NADH (reduced nicotinamide adenine dinucleotide) according to the process

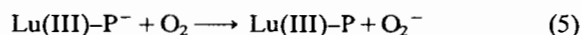


with $k = (1.4 \pm 0.2) \times 10^8$ $M^{-1} s^{-1}$. There is an efficient build-up of $Lu(III)-P^-$ at the end of the reaction, and this species was identified by its characteristic absorption spectrum.

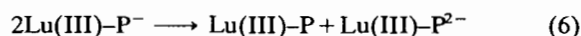
Using a high concentration of NADH (to reduce the triplet lifetime), a second crop of $Lu(III)-P^-$ can be observed, due to the reactions



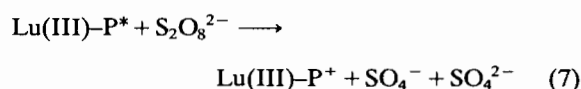
The π -radical anion ($Lu(III)-P^-$) reacts slowly ($k = (2.3 \pm 0.4) \times 10^7$ $M^{-1} s^{-1}$) with dioxygen, where O_2^- is the presumed product



Under N_2 , the π -radical anion decays via a very clean second order process ($k = (1.6 \pm 0.2) \times 10^4$ ϵ_{480} $M^{-1} s^{-1}$) with no sign of any first order competing reaction, and the $Lu(III)-P^{2-}$ is the product consistent with the pulse radiolysis studies.



Under the same pH 7 conditions, the triplet state was found to react with the oxidizing agent, sodium persulfate



The specific rate constant for this process was $(2.3 \pm 0.5) \times 10^7$ $M^{-1} s^{-1}$. The radical cation, $Lu(III)-P^+$ was observed by transient laser flash photolysis, and it rapidly decays to give unidentified products.

Discussion

Metalloporphyrins are perhaps the most widely studied class of bio-inorganic molecules due to their occurrence in natural systems. The present study gives information on the oxidation states and reactivities of lanthanide porphyrin complexes of europium and lutetium. Along with the chemical analyses, the magnetic susceptibility data on the europium porphyrin indicates the high stability of $Eu(III)$. The 298 K moment of 3.40 BM found for $Eu(III)-TPP(acac)$ is in the range 3.12–3.63 BM found for trivalent $Eu(III)$ oxide and sulfate [18] and the dependence of the magnetic moment on temperature (Fig. 1) is similar to that found by Selwood [19] for $Eu_2(SO_4)_3 \cdot 8H_2O$. In contrast, europium(II) in $EuCl_2$ has a temperature independent moment [20] of 7.9 BM. The Mössbauer results are also fully consistent with stable europium(III) oxidation levels. The europium porphyrins in Table 1 have isomer shifts (relative to EuF_3) in the 0–1 mm/s range characteristic [21] of $Eu(III)$, while $Eu(II)$ complexes are found at -15 to -17 mm/s. The observed spectra of $Eu(III)$ compounds [14] are similar to our results shown in Fig. 2, where a broad line results from overlap of eight closely spaced lines,

due to quadrupole interactions. Relative to phenyl and 4-methyl-phenyl groups, the 3-pyridyl or *N*-methyl-3-pyridyl substituents remove electron density from the central nitrogen atoms, and the data in Table 1 indicate that electron donation by the phenyl groups lead to more positive Eu(III) isomer shifts. This is the first report on the Mössbauer parameters of europium porphyrins, and certainly a wider range of porphyrin types need to be studied before any generalizations can be made. While many studies have correlated chemical and Mössbauer properties of iron porphyrins [22, 23], relatively less is known about the corresponding tin(IV) macrocycles [24].

Cobalt(III) and iron(III) complexes of TMPyP can be reduced to the metal +2 and +1 oxidation states under radiolytic conditions [24, 25] and the aquo ions of Lu(III) and Eu(III) can also be reduced to the divalent forms [26]. In the present Lu(III) and Eu(III)-TMPyP systems, the radiolysis and photochemical reduction results are typical of metalloporphyrin complexes (Zn(II), Mg(II), Pd(II), Al(III), Cd(II)) in which the porphyrin ligand itself and not the central metal ion is the initial reduction product [27]. For these cases, the formation of the radical anion is followed by disproportionation into the initial M-P and the two-electron reduced di-anion, M-P²⁻.

The half-life for proton catalyzed metal loss of Lu(III) from Lu(III)-TMPyP (Lu(III)-P) at pH 7 is $\sim 10^7$ years [7], and it is thus surprising that Lu(III) is so readily removed from the reduced lutetium-porphyrin complex. The implication of a clean second order decay of the π -radical anion (Lu(III)-P⁻ (eqn. (6), found both in the photochemical and radiolytic experiments) is that the Lu(III) remains in the porphyrin at the π -radical anion stage, and is lost only at the two-electron Lu(III)-P²⁻ level. This is supported by the observation (eqn. (5)) that O₂ oxidizes the π -radical anion to the original metalloporphyrin without metal ion loss. The electrochemistry indicates that this electron-rich di-anion can protonate more readily than the initial Lu(III)-P, and such facile protonation leads to demetallation. In contrast to the initial M(III)-porphyrin, the geometry of the di-anion may be severely distorted from planarity, thus moving the M³⁺ away from the central nitrogen atoms, which frees them to coordinate with protons.

There is no observable luminescence from the lanthanide ion itself in the lanthanum porphyrins, even in the solid state. The triplet state dominates the photophysics of the Lu(III)-TMPyP complex, and its lifetime is consistent with the atomic number of the Lu(III) ion, about a factor of 5-10 shorter than divalent transition metal ion porphyrins [2]. Both divalent transition metal ion porphyrins [2] and

Lu(III)-TMPyP show similar reactivities of the triplet state with O₂ and S₂O₈²⁻. Photolysis [28] at pH 7 in the presence of NADH and colloidal Pt caused reductive demetallation of the porphyrin with no evolution of H₂(g). Thus, the Pt colloid cannot compete with the fast chemical protonation that removes the Lu(III)-TMPyP dianion. The quantum yield of 0.78 ± 0.06 for the formation of singlet oxygen (eqn. (1)) is among the highest values [4, 12, 16] reported for any known photosystem, suggesting that lanthanide porphyrins could have important applications in the destruction of tumors by photodynamic therapy.

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