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Restoration of Catalytic Activity by Replacement of a Coordinated Amide Group: Synthesis and Laser-Induced Luminescence Studies of the Phosphate Diester Transesterification Catalyst $[Eu(NBAC)]^{3+}$

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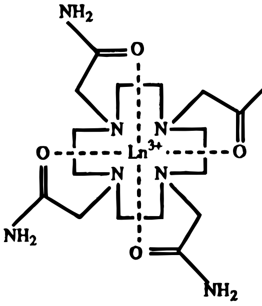
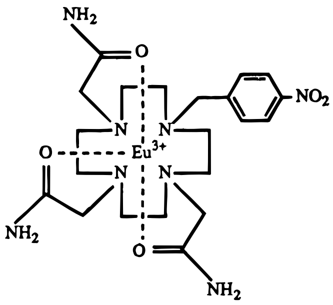
Our recent work with lanthanide(III) complexes of the tetraamide macrocycle TCMC (TCMC = 1,4,7,10-tetrakis-(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane) led us to suggest a correlation between the number of available coordination sites for binding small molecules and RNA-cleavage activity.[1,2] The $[La(TCMC)]^{3+}$ complex contained two available coordination sites and promoted rapid RNA cleavage whereas the $[Eu(TCMC)]^{3+}$ complex contained one available coordination site and did not promote RNA cleavage. To test the correlation between available coordination sites and catalysis, a new heptadentate ligand has been prepared by replacing one of the amide groups of TCMC with a noncoordinating group as shown below. Here we report the preparation, solution characterization, and catalytic properties of the Eu(III) complex of the NBAC ligand (NBAC = 1-(4-nitrobenzyl)-4,7,10-tris-(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane). The nitrobenzyl group was chosen as a precursor to an isothiocyanate group for conjugation of the complex to a modified oligonucleotide.[3] Such catalysts may be useful for the sequence-specific cleavage of RNA.



$[Ln(TCMC)]^{3+}$

Ln = La, Eu, Dy



$[Eu(NBAC)]^{3+}$

The macrocycle 1-(4-nitrobenzyl)-4,7,10-tetraazacyclododecane (NBC) was prepared as reported previously[4] with some minor modifications.[5] NBAC was obtained by the addition of triethylamine and bromoacetamide to a solution of NBC in absolute ethanol, followed by refluxing the solution for 3 h under N_2.[5] The Eu(III) complex was prepared by heating an ethanolic solution of NBAC and $Eu(CF_3SO_3)_3$ to reflux for 1 h under N_2. $[Eu(NBAC)](CF_3SO_3)_3$ was isolated as a powder upon addition of methylene chloride to the ethanol solution.[5] Satisfactory microanalytical data were obtained on all compounds.

The solution chemistry of the $[Eu(NBAC)]^{3+}$ complex was studied by use of laser-induced luminescence spectroscopy.[6,7] At pH 6.33, a major excitation peak appears at 579.95 nm (>95% of the area) and a minor peak is observed at 580.43 nm. As the pH is increased from 6.39 to 8.60, the lower energy peak increases in intensity with a concomitant decrease in the intensity of the higher energy peak. Excitation spectra such as that shown in Figure 1 are similar for solutions containing 20 or 200 μM $[Eu(NBAC)]^{3+}$. Nearly identical luminescence lifetimes are determined for excitation either at 579.95 nm or at 580.43 nm, consistent with rapid interconversion of the two Eu(III) species on the luminescence time scale. The number of water molecules bound to Eu(III) in $[Eu(NBAC)]^{3+}$, as determined by luminescence lifetime measurements in H_2O and in D_2O,[8] decreases with increasing pH. At pH 6.33, lifetimes in H_2O and D_2O are 0.37 and 1.67 s, respectively, suggesting that 2.2 ± 0.5 water molecules (4−5 OH oscillators) are bound, and at pH 8.60, lifetimes in H_2O and D_2O are 0.64 and 1.51 s, respectively, indicating that 1.0 ± 0.5 water molecules (or 2 OH oscillators) are bound to Eu(III).[9] The midpoint of this pH-induced change in bound water molecules occurs at a pH of approximately 6.9. In order to further characterize this acid−base equilibrium, potentiometric titrations were carried out. An end point was observed after 1 equiv of base was added to a solution of $[Eu(NBAC)]^{3+}$, and a pK_a of 6.85 was calculated for this equilibrium. These data suggest that the 580.43 nm peak is due to a $[Eu(NBAC)]^{3+}$ species with a hydroxide ligand. The relative positions of the two bands also support this assignment, as Eu(III) hydroxo complexes generally give rise to excitation bands that are red-shifted with respect to Eu(III) aquo complexes.[6]

That the $[Eu(NBAC)]^{3+}$ complex is predominantly monomeric in solution at pH values pertinent to this study was demonstrated by use of intermolecular energy transfer experiments with $[Nd(NBAC)]^{3+}$ added as an energy acceptor. Quenching studies that compare lifetime measurements (dynamic quenching) with emission intensity measurements (static quenching) have been used to determine whether a lanthanide-(III) complex self-associates in solution.[10] Stern−Volmer dynamic (K^{τ}_{SV}) and static (K^{ϕ}_{SV}) quenching constants are 938 and 3910 M^{-1} (pH 6.30) and 712 and 1980 M^{-1} (pH 7.30), respectively, for 10 μM solutions of $[Eu(NBAC)]^{3+}$ with 5−20

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(1) Amin, S.; Lake, C. H.; Churchill, M. R.; Morrow, J. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 773.

(2) Amin, S.; Voss, D. A., Jr.; Horrocks, W. D. Jr.; Lake, C. H.; Churchill, M. R.; Morrow, J. R. *Inorg. Chem.* **1995**, *34*, 3294.

(3) Amin, S. Ph.D. Dissertation, State University of New York at Buffalo, 1995.

(4) Kruper, W. J., Jr.; Rudolf, P. R.; Langhoff, C. A. *J. Org. Chem.* **1993**, *58*, 3869.

(5) Amin, S.; Chappell, L. L.; Morrow, J. R. *Inorg. Synth.*, submitted.

(6) Albin, M.; Horrocks, W. D., Jr. *Inorg. Chem.* **1985**, *24*, 895.

(7) The instrumentation and methods are described in ref 2. Conditions are given in the caption to Figure 1.

(8) Horrocks, W. D., Jr.; Sudnick, D. R. *J. Am. Chem. Soc.* **1979**, *101*, 334.

(9) The numbers of bound water molecules are 2.0 and 0.71 if contributions due to amide N−H oscillators and unbound but closely diffusing molecules are taken into account as described in: Dickins, R. S.; Parker, D.; de Sousa, A. S.; Williams, J. A. G. *Chem. Commun.* **1996**, 697.

(10) (a) Brittain, H. G. *Inorg. Chem.* **1979**, *18*, 1740. (b) Spaulding, L.; Brittain, H. G. *Inorg. Chem.* **1983**, *22*, 3486. (c) Zhang, X.; Chang, C. A.; Brittain, H. G.; Garrison, J. M.; Telser, J.; Tweedle, M. F. *Inorg. Chem.* **1992**, *31*, 5597.

S0020-1669(95)01646-6 CCC: $12.00

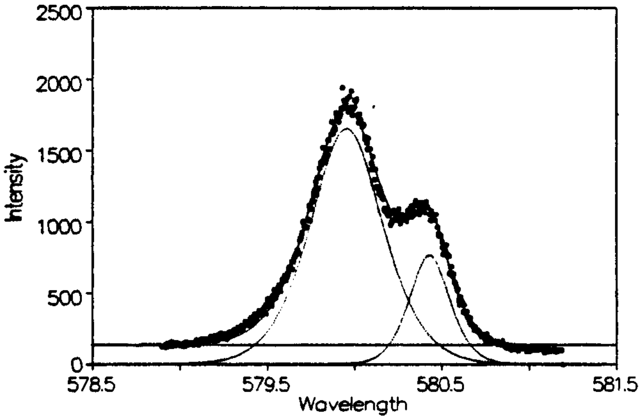

Figure 1. $^7F_0 \rightarrow {}^5D_0$ excitation spectrum ($\lambda_{em} = 614$ nm) of a solution of $[Eu(NBAC)]^{3+}$ (0.2 mM at pH 7.30; [Hepes] = 0.02 M, [NaCl] = 0.1 M). Peak maxima are at 579.95 and 580.43 nm.

μM $[Nd(NBAC)]^{3+}$ added as a quencher. Formation constants of the donor−acceptor complexes calculated as reported previously[11] are 4190 and 1930 M^{-1} at pH values of 6.30 and 7.30, respectively. This indicates that self-association of $[Eu(NBAC)]^{3+}$ ($\leq$3%) is not substantial under these conditions.

It is important that a complex remains intact in solution in the presence of competing ligands if the complex is to be useful as a catalyst. Luminescence experiments were carried out to study the dissociation of $[Eu(NBAC)]^{3+}$ (0.2 mM) in water at 37 °C and pH 7.40 with solutions containing 0.1 M NaCl, 0.02 M Hepes buffer, with and without 1.0 mM competing ligand. The percent dissociation of the complex was determined by monitoring the appearance of a peak for the Eu(III) complex of the competing ligand or by following the disappearance of the peak at 580.43 nm for the $[Eu(NBAC)]^{3+}$ complex as a function of time. The $[Eu(NBAC)]^{3+}$ complex did not dissociate under these conditions over a 3 day period in the absence of other strongly binding competing ligands. However, in contrast to our results with similar Eu(III) macrocyclic complexes,[12] the extent of dissociation of the $[Eu(NBAC)]^{3+}$ complex is accelerated by polyamino carboxylate ligands and is dependent on the competing ligand with 9.6%, 41.4%, and 90.3% dissociation observed after 1.0 h for DTPA, EDTA, and NTA, respectively. The observed order for extraction of Eu^{3+} from the NBAC complex (NTA > EDTA > DTPA) is surprising, given that formation constants for the competing ligands and Eu(III) are in the reverse order.[13] This suggests that the rate-determining step is not dissociation of the Eu(III) complex followed by rapid binding of the competing ligand to the Eu(III) ion. Rather, the competing ligand probably binds to the $[Eu(NBAC)]^{3+}$ complex and displaces the NBAC macrocycle.[14] Differences in percent dissociation then reflect differences in strength of competing ligand binding to $[Eu(NBAC)]^{3+}$ and displacement of NBAC by the competing ligand in the ternary complex. A similar dependence on competing ligand with EDTA more effective than with DTPA is observed in the decomposition of hexadentate Schiff-base complexes of lanthanum(III).[15] The relative lability of the $[Eu(NBAC)]^{3+}$ complex in the presence of competing ligands is in contrast to the highly inert character of the $[Eu(TCMC)]^{3+}$ complex.[1]

$[Eu(NBAC)]^{3+}$ promotes rapid transesterification of the RNA model substrate 2-hydroxypropyl 4-nitrophenyl hydrogen phosphate at pH 7.40 and 37 °C.[16] The reaction is first order in complex over the concentration range 0.3−0.9 mM, and a plot of k_{obs} versus concentration of $[Eu(NBAC)]^{3+}$ gives a second-order rate constant of 3.7 $M^{-1}\ s^{-1}$. This rate constant is 50−260-fold greater than that of other lanthanide(III) macrocyclic complexes under similar conditions.[17] The second-order rate constant for transesterification of the phosphate diester by $[Eu(NBAC)]^{3+}$ is similar in reactions containing DTPA (10%−50% of the macrocyclic complex concentration), suggesting that catalysis is not due to free Eu^{3+} ion. (The europium(III) complex of DTPA is inactive as a catalyst.) Under these conditions, little dissociation of $[Eu(NBAC)]^{3+}$ would be anticipated, since transesterification reactions were complete within 40 min. Multiple catalytic turnovers are observed with 0.11 turnover/s for the $[Eu(NBAC)]^{3+}$ catalyst.

The catalytic activity of the $[Eu(NBAC)]^{3+}$ complex is in sharp contrast to the inability of the $[Eu(TCMC)]^{3+}$ complex to facilitate phosphate diester transesterification.[1,2] This difference in catalytic behavior may be due to the greater number of available coordination sites for small-molecule binding in $[Eu(NBAC)]^{3+}$ as compared to the $[Eu(TCMC)]^{3+}$ complex. In addition, the $[Eu(NBAC)]^{3+}$ complex is a much more powerful Lewis acid, as indicated by the formation of a europium(III) hydroxide complex at neutral pH. In comparison, the $[Eu(TCMC)]^{3+}$ complex does not form a europium(III) hydroxide species at pH values as high as 9.00.[1,2] Studies are underway to further delineate the reactivity of the $[Eu(NBAC)]^{3+}$ complex toward RNA and to prepare sequence-specific ribonucleases.

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Supporting Information Available: A table giving extent dissociation of complexes over time, a figure showing the number of bound water molecules as a function of pH, and plots of luminescence intensity or lifetime as a function of quencher for detemination of Stern−Volmer dynamic and static quenching constants (7 pages). Ordering information is given on any current masthead page.

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(11) Demas, J. N. *J. Chem. Educ.* **1976**, *53*, 657.

(12) Chin, K. O. A.; Morrow, J. R.; Lake C. H.; Churchill, M. R. *Inorg. Chem.* **1994**, *33* 656.

(13) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1989; Vols. 1−6.

(14) Wilkins, R. G. *Kinetics and Mechanism of Reactions of Transition Metal Complexes*, 2nd ed.; VCH: New York, 1991.

(15) Voss, D. A.; Buttrey, L. A.; Marks, C.; Churchill, M. R.; Lake C. H.; Morrow, J. R. Manuscript in preparation.

(16) In a typical kinetics run, solutions contained 0.03 mM phosphate diester, 0.01 M Hepes buffer, 0.1 M $NaNO_3$, and 0.3 mM europium(III) complex at 37 °C. Reactions were monitored for greater than 4 half-lives to obtain first-order rate constants (k_{obs}).

(17) Chin, K. O. A.; Morrow, J. R. *Inorg. Chem.* **1994**, *33*, 5036.