Europium(III) Luminescence Excitation Spectroscopy. Quantitative Correlation between the Total Charge on the Ligands and the $^7F_0 \rightarrow {}^5D_0$ Transition Frequency in Europium(III) Complexes

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A correlation between the total charge, p, on the ligands bound to europium(III) and the energy of the $^7F_0 \rightarrow {}^5D_0$ transition, ν, has been observed in solution and the solid state. The frequency of the transition is given by the equation $\nu = -0.76p^2 + 2.29p + 17273$. As the environment about the europium ion becomes more negative, a shift to lower energy attributable to a nephelauxetic effect is observed. Quantitation of the effect of temperature on the frequency of the $^7F_0 \rightarrow {}^5D_0$ transition allows one to calculate the total coordinated ligand charge from a spectral measurement at any temperature. The use of these correlations in the elucidation of the nature of metal ion binding sites in proteins is discussed.

Introduction

The ability of certain trivalent lanthanide ions, Ln(III), to luminesce in solution at room temperature renders them attractive as substitutional probe ions for the study of Ca(II)- and Mg-(II)-binding species of biological interest.[1,2] The Eu(III) ion is particularly useful in this regard in that it possesses nondegenerate ground ($^7F_0$) and emitting ($^5D_0$) states. Work in this laboratory[3] has focused on the development and exploitation of tunable dye laser excitation of the $^7F_0 \rightarrow {}^5D_0$ transition in the 578–581-nm region while monitoring the $^5D_0 \rightarrow {}^7F_2$ transition near 612 nm. A key feature of this technique is that since neither the ground nor emissive level can be split by a ligand field, there is, in principle, a one-to-one correspondence between the number of peaks in the excitation spectrum and the number of distinct Eu(III) ion environments. For single environments, peak widths at half-maximum of less than 10 cm^{-1} for solutions and as narrow as 1.5 cm^{-1} for crystalline solids have been observed at room temperature. These laser experiments have proven valuable in providing a variety of types of information, including quantitation of the number and stoichiometries of species present,[4,5] the measurement of the number of Eu(III)-coordinated water molecules,[6] binding constants,[4] distances between metal ion binding sites,[7] and ligand-exchange rates.[8] The present research demonstrates the use of $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy of Eu(III) to obtain estimates of the total charge on the ligand atoms in the first coordination sphere.

The basis of the method is the observation[9] that the greater the negative charge on the ligands, the lower the $^7F_0 \rightarrow {}^5D_0$ transition energy. This transition is between levels of different terms, neither of which can be split by the ligand field, and corresponds to the pairing up of two of the six unpaired electrons found in the ground state of Eu(III). The energy of this transition will therefore be a function of the interelectronic repulsion parameters of the ion, and any change in energy can be regarded as a "nephelauxetic" effect.[10] Much more dramatic nephelauxetic

energy level shifts are observed in transition-metal complexes where changes in covalency (delocalization) and ligand polarizability (effective nuclear charge on the central ion) have been implicated in causing interelectronic repulsion parameters to be reduced from the free ion values by as much as 50% in extreme cases. For the present Eu(III) systems, where covalency is expected to be minimal, the nephelauxetic effect is much more subtle, accounting for a range of $^7F_0 \rightarrow {}^5D_0$ transition energies differing by only 45 cm^{-1} out of 17 250 cm^{-1} (0.26%).

In order to allow comparison of $^7F_0 \rightarrow {}^5D_0$ transition energies obtained at different temperatures, we also investigated the effect of temperature on several representative Eu(III) complexes over the temperature range of 77–350 K.

Experimental Section

The compounds used in this study were prepared according to the literature procedures indicated in Table I. EuCl$_3$·6H$_2$O (99.9%) and the organic ligands were obtained from Aldrich Chemical Co. and used without further purification.

Excitation spectra were obtained on a previously described pulsed nitrogen laser–pumped dye laser system.[3] The half-width of the laser line was 0.45 cm^{-1}. Calibration of the system was initially accomplished with use of a He–Ne laser with all subsequently determined peak positions referenced to the two peaks in the $^7F_0 \rightarrow {}^5D_0$ excitation spectrum of Eu(EDTA)$^-$ (579.60 and 580.12 nm). Accurate excitation peak positions (±0.5 cm^{-1}) were obtained by slowly scanning the dye laser (0.2 nm/min) through the transition region.

Temperatures above 298 K were maintained with a Haake D3 water bath (±0.1 °C), which circulated water through a steel block that enclosed a 1-cm cuvette on all four sides (except for the optical path). Temperatures from 77 to 298 K were obtained with an Air Products Model LT-3-110 Heli-Tran apparatus operating with liquid nitrogen as the coolant. The temperature was regulated with an Air Products temperature controller, Model APD-E, employing a chromel/gold/0.07% iron thermocouple (±0.5 K). Samples for the low-temperature measurements were placed in a capillary tube and mounted on a copper block that was in contact with the cryogen. Good thermal conductivity was achieved with use of indium gaskets and conducting grease to attach the sample holder and sample, respectively. A minimum of 10 min was allowed for the sample to equilibrate at each temperature.

Results

$^7F_0 \rightarrow {}^5D_0$ excitation spectra were recorded for 36 Eu(III) complexes in solution or in the solid state with the results tabulated in Table I. The complexes studied involved varying numbers of

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Table I. Position of $^7F_0 \rightarrow {}^5D_0$ Transition of Eu(III) Complexes at 296 K

complex[a]	state[h]	CN	$^7F_0 \rightarrow {}^5D_0$ position, cm^{-1}	ligand charge[b]		ref	
				actual	calcd	prep	data
Na$_3$[Eu(DPA)$_3$]·15H$_2$O	X	9	17 226	6–	6.5–	11	12
Eu(IMDA)(DCTA)$^{3-}$	o		17 226	6–	6.5–	c	9
Eu(DPA)$_3^{3-}$	o	9	17 232	6–	6.0–	c	9, 12
Na$_3$[Eu(ODA)$_3$]·6H$_2$O	X	9	17 235	6–	5.8–	d	e
Eu(IMDA)$_3^{3-}$	o	9	17 235	6–	5.8–	c	e
Eu(chelidamate)$_3^{6-}$	o	9	17 235	6–	5.7–	c	e
Eu(ODA)$_3^{3-}$	o	9	17 238	6–	5.4–	c	9
Eu(DTPA)$^{2-}$	o		17 240	5–	5.3–	c	e
Eu(DOTA)$^-$	o	9	17 247	4–	4.5–	13	14
Eu(DPA)$_2^-$	o	9	17 249	4–	4.3–	c	9, 12
Eu(chelidamate)$_2^-$	o	9	17 251	4–	4.1–	c	e
Na[Eu(EDTA)(H$_2$O)$_3$]·5H$_2$O	X	9	17 251	4–	4.0–	15	e
Eu(IMDA)$_2^-$	o	9	17 252	4–	3.9–	c	e
Eu(ODA)$_2^-$	o	9	17 253	4–	3.8–	c	9
Eu(EDA)$_2^-$	o	11	17 257	4–	3.3–	c	4
Eu(HEDTA)	o		17 255	3–	3.6–	c	e
Eu(dbm)$_3$(H$_2$O)	X	7	17 259	3–	3.3–	16	e, g
Eu(NO$_3$)$_3$·6H$_2$O	X	10	17 262	3–	2.6–	f	e
[Eu(nic)$_3$(H$_2$O)$_2$]	X	8	17 262	3–	2.6–	17	e
[Eu(acac)$_3$(H$_2$O)$_2$]·H$_2$O	X	8	17 262	3–	2.6–	18	g
[Eu(NTA)(H$_2$O)$_2$]·H$_2$O	X	9	17 263	3–	2.4–	19	12
Eu(LAS)$_2^+$	o		17 260	2–	3.4–	5	5
Eu(NO$_3$)$_2$(H$_2$O)$_x^+$	o		17 261	2–	2.8–	20	20
[Eu(H$_2$O)$_6$Cl$_2$]Cl	X	8	17 263	2–	2.5–	d	e
Eu(DPA)$^+$	o	9	17 264	2–	2.2–	c	9, 12
[Eu(S-ODA)(H$_2$O)$_4$]Cl	X	9	17 265	2–	2.1–	21	e
Eu(chelidamate)	o	9	17 265	2–	2.0–	c	e
Eu(ODA)$^+$	o	9	17 266	2–	1.7–	c	9
Eu(IMDA)$^+$	o	9	17 267	2–	1.7–	c	e
Eu(EDA)$^+$	o	11	17 271	2–	0.7–	c	4
Eu(SCN)(H$_2$O)$_x^{2+}$	o		17 268	1–	1.4–	20	20
Eu(NO$_3$)(H$_2$O)$_x^{2+}$	o		17 269	1–	1.1–	20	20
Eu(Cl)(H$_2$O)$_x^{2+}$	o		17 270	1–	1.0–	20	20
Eu(MeOH)$_x^{3+}$	o		17 270	0	1.0–	5	5
[Eu(dtd)$_2$](BF$_4$)$_3$	X	8	17 273	0	0.0	22	22
Eu(H$_2$O)$_x^{3+}$	o		17 275	0	0.9		e

[a] Abbreviations: acac = acetylacetonate; dbm = dibenzoylmethanate; DCTA = 1,2-diaminocyclohexane-N,N,N',N'-tetraacetate; DOTA = 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetate; DPA = dipicolinate; dtd = 4,5-dimethyl-N,N,N',N'-tetrapropyl-3,6-dioxaoctanediamide; DTPA = diethylenetriaminepentaacetate; EDA = (1,2-ethanedioxydiyl)diacetate; EDTA = ethylenediamine-N,N,N',N'-tetraacetate; HEDTA = N'-(2-hydroxyethyl)ethylenediaminetriacetate; IMDA = iminodiacetate; LAS = lasalocid A; nic = nicotinate; NTA = nitrilotriacetate; ODA = oxydiacetate; S-ODA = thiodiacetate. [b] Sum of charges of groups bound to Eu(III). [c] Mixture of appropriate ligand:metal ratio at pH >5. [d] By slow evaporation of appropriate solution at room temperature. [e] This work. [f] Aldrich Chemical Co. [g] Cader, B., unpublished results from this laboratory. [h] Legend: X= solid; o = solution.
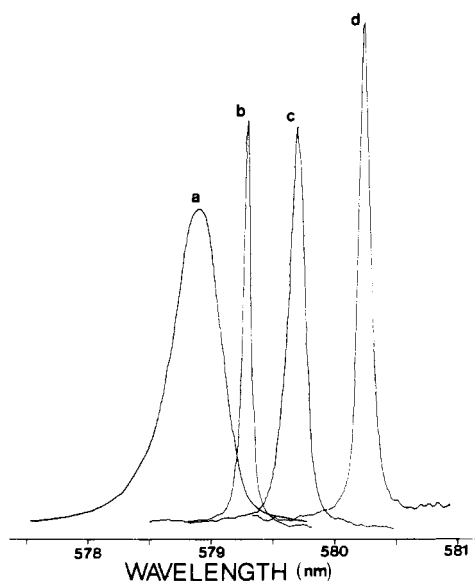

Figure 1. $^7F_0 \rightarrow {}^5D_0$ spectra of (a) [Eu(dtd)$_2$](BF$_4$)$_3$, (b) [Eu(H$_2$O)$_6$-Cl$_2$]Cl, (c) Na[Eu(EDTA)(H$_2$O)$_3$]·5H$_2$O, and (d) Na$_3$[Eu(ODA)$_3$]·6H$_2$O (T = 296 K).
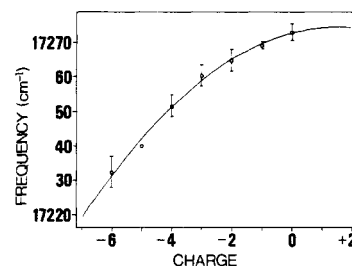

Figure 2. Energy (average from Table I) of the $^7F_0 \rightarrow {}^5D_0$ transition as a function of the effective charge on the Eu(III) ion (see text).

Figure 1 shows the excitation spectra taken at 296 K of four representative complexes spanning this range of total ligand charge. Owing to their sharpness, the peak positions can be determined with good accuracy. The shift to lower energy with increasing negative charge on the ligands is quite evident. In Figure 2 are plotted the average values (points) and standard deviations (error bars) of the $^7F_0 \rightarrow {}^5D_0$ transition frequencies of the complexes listed in Table I as a function of ligand charge. The frequency decreases in a nonlinear manner as the total charge on the ligands, p, becomes more negative. With use of a nonlinear least-squares regression analysis routine the average frequencies, ν in cm^{-1}, at each charge value were fit to the quadratic expression in p given in eq 1. The data are not sufficiently numerous or evenly dis-

neutral and anionic ligands so that the total charge on the ligands in the first coordination sphere of Eu(III) varied from 0 to 6–.

$$\nu = -0.76p^2 + 2.29p + 17\,273 \tag{1}$$
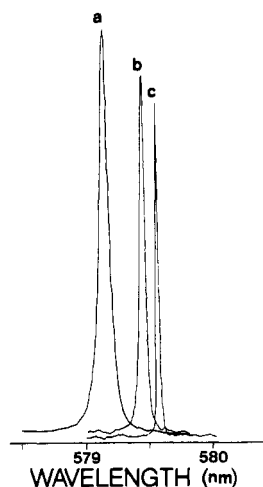
Figure 3. $^7F_0 \rightarrow \,^5D_0$ spectra of a single crystal of $[Eu(H_2O)_6Cl_2]Cl$: (a) 351 K; (b) 201 K; (c) 81 K.

tributed with respect to charge species to warrant separate correlations for solid-state and solution data. In any case, with the exception of charge 3–, the solid-state and solution frequency averages for species of each charge are within 2 cm^{-1} of each other. The solid line predicted by eq 1 is displayed in Figure 2. This equation can be used to predict the total ligand charge in unknown situations (vide infra). The range of actual values obtained for each value of p suggests that an uncertainty in a predicted charge of about ±1 unit should be assigned throughout most of the range. Near neutrality, where the curve is quite flat, a somewhat greater uncertainty may apply. The charge calculated from eq 1 on the basis of the observed $^7F_0 \rightarrow \,^5D_0$ transition frequency is also listed in Table I. With three exceptions, two for charge 2– and one for charge 0, the calculated charges are within one unit of the actual charge. Some idea of the subtlety of the nephelauxetic effect being observed and the precision needed can be obtained when it is realized that eq 1 predicts a change of only 2.6 cm^{-1} (0.09 nm) between complexes of charge 0 and 1– while this difference increases to 10.7 cm^{-1} (0.36 nm) between charges 5– and 6–. Nevertheless, with a tunable dye laser, differences of this order are measurable with good accuracy.

In order to assess the sensitivity of the $^7F_0 \rightarrow \,^5D_0$ transition energy to changes in temperature and to permit comparisons to be made between measurements taken at different temperatures, we investigated the temperature dependence in six representative systems. The spectra for $[EuCl_2(H_2O)_6]Cl$ taken at 351, 201, and 81 K are shown in Figure 3. In addition to a shift to lower energy (longer wavelength) with decreasing temperature a marked narrowing of the peak is observed at lower temperatures. Figure 4 displays the shift in the $^7F_0 \rightarrow \,^5D_0$ transition frequency from its measured position at 296 K (●) as a function of temperature for six crystalline complexes spanning total ligand charges from 0 to 4–. The temperature dependencies are similar and nonlinear in all cases, spanning a maximum change of 13 cm^{-1} over the 81–337 K range. The average shift is 1 cm^{-1}/24 K with the shift per degree being somewhat larger at the higher end of the temperature range studied.

Discussion

The Charge Effect. Since both the $^7F_0$ and $^5D_0$ levels are immune to ligand field splitting and the minor changes in environments are not expected to influence the spin–orbit coupling constant to a measurable degree, we attribute the observed shifts in the $^7F_0 \rightarrow \,^5D_0$ transition energies as neutral ligands are replaced with negative ones to a nephelauxetic effect. That is, the parameters describing interelectronic repulsion decrease very slightly as the negative charge on the complex is increased, thus decreasing the F–D term separation and the transition frequency. It should be noted that although 36 different systems are included in the correlation, only a relatively limited class of Eu(III) complexes has been examined. With three exceptions, the single S atom
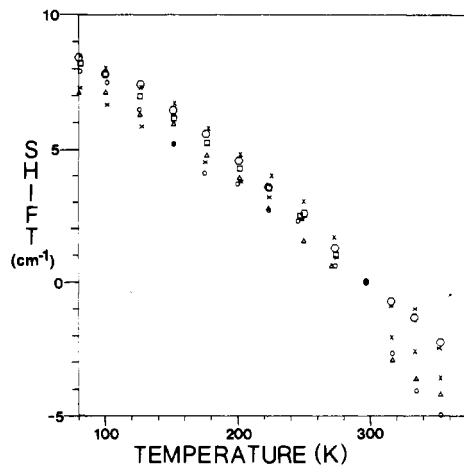

Figure 4. Shift in the $^7F_0 \rightarrow \,^5D_0$ excitation maximum $(\nu(296\ K) - \nu(T))$ as a function of temperature: (○) $[Eu(dtd)_2](BF_4)_3$; (△) $Na[Eu(EDTA)(H_2O)_3]\cdot5H_2O$; (✕) $[Eu(H_2O)_6Cl_2]Cl$; (elongated ✕) $Eu(nic)_3(H_2O)_2$; (◐) $[Eu(S\text{-}ODA)(H_2O)_4]Cl$; (□) $Eu(NO_3)_3\cdot6H_2O$.

donor in $[Eu(S\text{-}ODA)(H_2O)_4]Cl$ and the one or two Cl$^-$ ion donor(s) in $EuCl(H_2O)_x^{2+}$ and $[EuCl_2(H_2O)_6]Cl$, all of the ligand donor atoms are nitrogen or oxygen atoms. For this reason we do not expect significant differences in the covalent character of the bonding amongst the complexes. Nor will differences in polarizability (hardness or softness), which dominate the nephelauxetic effect in transition-metal complexes,[10] explain the observed trend in the present series of complexes.

Some comment on the procedures used to arrive at the total ligand charge, p, is in order. Neutral ligands, of course, contribute zero charge. A net charge of 1– is included for each of the following simple anionic ligands: Cl$^-$, SCN$^-$, and NO$_3^-$, with no correction for any charge delocalization in the last two examples. Our series of complexes does not include coordinated anions such as PO_4^{3-}, SO_4^{2-}, and ClO_4^- or their protonated counterparts, where charge delocalization might be more important. The remaining ligands in the series studied involve chelating ligands that have coordinated carboxylate groups, each of which is considered to contribute a charge of 1– to the total ligand charge. The β-diketonate ligands are each considered to contribute 1– to the total charge. With the exception of the complexes of the chelidamate anion, structure **1**, where the charge on the remote phenolate group

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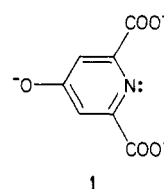
is not counted, the correlation involves the total net negative charge on all the ligands.

There have been a number of fragmentary investigations on the nephelauxetic effect for lanthanide ions reported in the literature,[23–31] only one of which was concerned with Eu(III).[28] As

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Table II. Structural and Spectroscopic Features of Ln(III) Complexes

complex	charge	CN	av Ln–L dist, Å	$^7F_0 \rightarrow {}^5D_0$ freq, cm^{-1}	ref
[Nd(dbm)$_3$(H$_2$O)]	3–	7	2.39	17 258	32
Na$_3$[Nd(ODA)$_3$]·2NaClO$_4$·6H$_2$O	6–	9	2.42	17 238[a]	33
[Nd(acac)$_3$(H$_2$O)$_2$]·H$_2$O	3–	8	2.47	17 262	34
Na$_3$[Nd(DPA)$_3$]·15H$_2$O	6–	9	2.52	17 226	11
[Nd(S-ODA)(H$_2$O)$_4$]Cl	2–	9	2.53[b]	17 265	21

[a] Spectra for Na$_3$[Eu(ODA)$_3$]·6H$_2$O. [b] Includes Nd–S bond length of 3.15 Å; Ln–L = 2.45 without this value, CN = 8.

early as 1956 Jørgensen[23] noted from the spectra of Pr(III), Nd(III), Sm(III), and Gd(III) that electronic transition energy shifts to lower energy of up to about 6% occur for complexes with anionic ligands or in oxide matrices compared to the energies of the aqua ions. He suggested that this shift was due to a decrease in the parameters of interelectronic repulsion arising from either covalency or a decrease in the effective nuclear charge of the central ion upon coordination to the ligands in question. More recently, Jørgensen et al.[24] reported a particularly large nephelauxetic effect on spectra of Ln(III) ions in the ThO$_2$ and Ln$_2$O$_3$ oxide lattices and attributed this to large covalencies in these systems. Other authors, Sinha,[26] Misra,[30] and Singh et al.,[31] noted a nephelauxetic effect in the spectra of various complexes and attributed this phenomenon to variations in covalency between the complexes. Sinha and Schmidtke,[25] in a study of some Pr(III) systems, concluded that the nephelauxetic effect of the high-energy bands was primarily due to a change in the interelectronic repulsion parameter, F_2, with changes in the spin–orbit coupling constant being negligible.

Davidenko and Yatsimirskii[27] reported a correlation of the nephelauxetic effect in Pr(III) and Nd(III) complexes with average metal–ligand distances in the complexes determined by X-ray crystallography. Their results suggest that the shorter the metal–ligand distance, which implies greater covalency, the lower the energy of the electronic transition. Their results also suggested that there might be a correlation of electronic transition frequency with coordination number, since higher coordination numbers generally are associated with greater metal to ligand distances. We explored the possibility of an analogous correlation in the Eu(III) systems but found none. For instance, our single solid-state example of coordination number (CN) 7, [Eu(dbm)$_3$(H$_2$O)], has a $^7F_0 \rightarrow {}^5D_0$ transition frequency of 17 258 cm^{-1} while for CN 10, [Eu(NO$_3$)$_3$(H$_2$O)$_4$], this frequency is 17 262 cm^{-1}. Furthermore, for CN 9, for which we have the most examples (Table I), the $^7F_0 \rightarrow {}^5D_0$ transition frequencies range from 17 226 to 17 267 cm^{-1}. Although crystal structures are not available for most of the Eu(III) complexes we studied, structures of the Nd(III) analogues are available for the five listed in Table II, along with the observed frequencies, total charges, coordination numbers, and average Nd(III)–ligand atom bond distances. Clearly no correlation between transition energy and average bond distance is evident.

The only previous study of the nephelauxetic effect in Eu(III) complexes was that of Goryushko and Davidenko,[28] who noted a correlation between the $^5D_0 \rightarrow {}^7F_0$ emission frequency and β-diketonate substituent electron-withdrawing effects in 1:1 Eu(III)–β-diketonate complexes in aqueous methanol solutions. We were unable to reproduce their results under the conditions they employed, obtaining only spectra characteristic of Eu(III)–chloro complexes. Additionally, the trend they reported runs counter to that reported by Surana et al.,[29] in a study of Nd(III) and Pr(III) complexes of three β-diketonate ligands.

Since no particular covalency trends are to be expected for the limited class of Eu(III) complex studied by us, we attribute the observed correlation with net total ligand charge to a nephelauxetic effect on the parameters of interelectronic repulsion, probably a decrease in the effective nuclear charge on the ion as the total negative charge is increased. It should be emphasized again that

this effect is an extremely subtle one in energy terms and, while perhaps operative also in transition-metal systems, would be completely obliterated by much larger changes in covalency from system to system that can be expected in d-element compounds. It is only because of our ability to measure accurately the frequency of the narrow, unsplit $^7F_0 \rightarrow {}^5D_0$ transition in Eu(III) that the present correlation can be determined at all.

It is possible that an analogous correlation between interterm transition energies and total charges on the ligands exists for other Ln(III) ions. However, since, for Ln(III) ions other than Eu(III), both ground- and excited-state terms will generally be split by ligand fields, there may be some difficulty in defining the transition energy appropriately. Only fragmentary correlations exist. The three Nd(III) systems for which Davidenko and Yatsimirskii[27] report the $^4I_{9/2} \rightarrow {}^2P_{1/2}$ transition energies (which span a range of ∼200 cm^{-1}) appear to follow our correlation; however, the $^3H_4 \rightarrow {}^3P_0$ transitions of three different Pr(III) systems[27] do not conform to the trend observed by us. Clearly more experimental data is needed to determine whether a frequency–ligand charge correlation holds for Ln(III) ions other than Eu(III).

Temperature Dependence of the $^7F_0 \rightarrow {}^5D_0$ Transition Frequency. Our aim in measuring the temperature dependence of the $^7F_0 \rightarrow {}^5D_0$ transition in several representative Eu(III) complexes was to provide a basis for comparison of measurements reported at different temperatures. The temperature dependencies observed for the six compounds we investigated (Figure 4) are all quite similar, with an average shift of 8 cm^{-1} to lower energy on going from room temperature to liquid-nitrogen temperature. A similar temperature dependence was observed by Sovers et al.,[35] for this transition for Eu(III) in various chloride hydrates and in oxide, borate, vanadate, and oxysulfide lattices. Hill and Hüfner[36] made analogous measurements on EuCl$_3$·6H$_2$O, Eu(NO$_3$)$_3$·6H$_2$O, and Eu$_2$O$_3$ down to 4.2 K, obtaining very similar results. The latter authors attributed this behavior to Raman scattering of optical phonons. The theory of this phenomenon[37-39] predicts that the shift will correlate linearly with the internal energy of the crystalline compound and, indeed, an excellent linear correlation of the observed shifts to the internal energy obtained from heat capacity measurements on the analogous Gd(III) compounds was noted. Our interest in this phenomenon is strictly empirical, and we find that the temperature dependencies, particularly in the

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Table III. Structural and Spectroscopic Data for Biological Systems

system	Ca(II) site	no. of carboxylate groups present at metal ion binding site	$^7F_0 \rightarrow {}^5D_0$ position, cm^{-1}	calcd charge	ref
thermolysin	S1	4	17 253	3.8-	40
	S2				
	S3	3	17 265	2.1-	
	S4	2			
parvalbumin carp III	CD	4	17 264	2.2-	41
	EF	4	17 250	4.2-	
cod	CD	5	17 260	2.9-	42
	EF	4			
rabbit	CD	4	17 268	1.4-	42
	EF	4	17 257	3.3-	
calmodulin bovine	I	3			43, 44
brain	II	4	17 261	2.7-	
	III	3			
	IV	3			
troponin C	I	3	17 264	2.2-	45
	II	4			
	III	4	17 273	0.0	
	IV	4			
phospholipase A$_2$ (bovine)		1	17 270	1.1-	46
prothrombin	A	?	17 260	2.9-	47
	B	?	17 270	1.1-	
human factor Xa		?	17 265	2.1-	48
transferrin 298 K, pH 7	A, B (unresolved)	?	17 274	0.4	49
77 K, pH 7	A		17 245	3.8-^{a}	
	B		17 263	0.7-	
77 K, pH 8	A		17 232	5.3-	
	B		17 237	4.8-	

^{a} Calculated by adding 8 cm^{-1} to $^7F_0 \rightarrow {}^5D_0$ transition frequency measured at 77 K.

77–330 K region, are extremely similar for the six systems studied with the spread always less than 2 cm^{-1} for all compounds below 300 K. This finding permits us to use the observed curve to convert a transition frequency measured at any temperature to a 296 K value with an uncertainty of ±1 cm^{-1}.

Metal Ion Binding Sites in Proteins. An important application of the frequency–total ligand charge correlation of the $^7F_0 \rightarrow {}^5D_0$ transition in Eu(III) is in the characterization of metal ion binding sites in proteins. Eu(III) and other lanthanide ions have been shown to replace Ca(II) isomorphously in several calcium-binding proteins and to serve as viable replacement probes for this ion in a variety of such systems.[1] In this section we will explore the application of the frequency–charge correlation in the elucidation of the number and type of protein-supplied ligating groups in biological macromolecules. The only known ligating groups for Ca(II) in proteins are carboxylate groups of aspartic or glutamic acid, hydroxyl groups of serine or threonine, backbone peptide carbonyl groups, and water molecules. Of these, only the carboxylate groups are negatively charged, thus allowing the peak position to provide a means of counting them. First to be considered will be proteins where the Ca(II)-binding site has been elucidated by X-ray crystallography followed by systems for which less information is available. Pertinent data are set out in Table III.

Thermolysin. This thermally stable proteolytic enzyme[50] binds Zn(II) at the active catalytic site as well as four Ca(II) ions, which play a structural role, at sites S(1), S(2), S(3), and S(4). When 1 equiv of Eu(III) is added to a thermolysin solution, it binds at site S(1) with expulsion of the Ca(II) ion nearby (3.8 Å) at site S(2). When crystals of thermolysin are soaked under certain specified conditions,[40] thermolysin with Eu(III) ions occupying sites S(1), S(3), and S(4) can be synthesized. The $^7F_0 \rightarrow {}^5D_0$ peak positions (separate peaks due to Eu(III) in sites S(3) and S(4) are incompletely resolved), calculated (eq 1) total charges, and numbers of coordinated carboxylate groups are shown in Table III. If it is assumed that the three carboxylate groups that bridge

between the Ca(II) ions of sites S(1) and S(2) in the native protein are all coordinated to the Eu(III) at site S(1), the agreement between the charges calculated from the observed $^7F_0 \rightarrow {}^5D_0$ transition frequencies (Table III) and known number of carboxylate groups at the various sites determined by X-ray diffraction[51] is excellent.

Parvalbumin. Small (M_r 12 000), acidic proteins in this class[52] have been isolated from the muscle of a large number of vertebrates. The X-ray structure of parvalbumin isotype III from carp has been determined,[53] and it and other parvalbumins have two principal Ca(II) ion binding sites known as CD and EF. At low pH where only the CD and EF sites are occupied, Eu(III)-substituted parvalbumin exhibits two barely resolvable bands centered at the positions indicated in Table III. The calculated charge for the EF site (4.2-) is in accord with the presence of four carboxylates in this binding loop. The charge obtained from the laser excitation spectrum for the CD site (2.2-) is less negative than expected since four carboxylate ligands are also available at this site. Whether this discrepancy is due to the inherent uncertainty in the method owing to the influence of factors other than net charge on the band position or indicates that one of the protein carboxylates remains uncoordinated cannot be ascertained. Data are also presented for parvalbumin isotypes isolated from codfish and rabbit in Table III. In the former case the spectral bands due to the two sites are not resolved and the peak position suggests that not all five of the carboxylate moieties present in the CD loop are coordinated to the Eu(III) ion in this site. The results for rabbit parvalbumin are similar to those observed in the carp protein with a suggestion of a somewhat less negative net charge, again within the uncertainty of the method.

Phospholipase A$_2$. This lipolytic protein[54] contains a single Ca(II) ion at the active site which is required for activity. The

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X-ray structure[55] reveals coordination of the Ca(II) ion by a single carboxylate group from Asp-49, plus three peptide carbonyl groups and two water molecules. The charge prediction from our laser measurements (1.1–) is in excellent agreement with that expected on the basis of the known Ca(II) structure.

X-ray structural data is not available for the remaining proteins for which we have $^7F_0 \rightarrow {}^5D_0$ excitation spectra (Table III). In the case of calmodulin,[56] where separate peaks for Eu(III) in the four distinct sites (I–IV) are not resolved, a charge of 2.7– is predicted from the center of the observed band. This finding is consistent with the results obtained on parvalbumin, with which calmodulin is highly homologous. From the primary sequence[52] it appears that either three or four carboxylates are available in each of the binding sites. The constitution of the metal ion binding sites in prothrombin, human factor Xa, and transferrin are not precisely known from X-ray or primary sequence data, and the predictions presented here (Table III) represent a tentative guide.

While the correlation presented in this paper does not allow us to determine the metal ion coordinating groups in proteins with exact certainty, it does provide a useful guide and, in cases where

changes are observed as a function of various biochemical parameters (pH, ionic strength, inhibitor concentration, etc.), should allow a choice to be made between alternative possibilities. The utility of the present method is by no means limited to macromolecular systems but is proving useful in eluidating the solution coordination chemistry of complexes of smaller molecules such as the crown ethers.[57]

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Comparison of the Self-Reactivity of [CpCo(P⌒P)I]⁺ Complexes, P⌒P = Ph₂P(CH₂)ₙPPh₂ (n = 1–4), Leading to Bridged and Oxidized "Dangling" P⌒P Reactions and Spectral Studies and the Structures of [CpCo(dppm)I]I·CHCl₃, CpCo[dppm(O)]I₂, and Cp₂Co₂I₄(μ-dpppent)

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The intrinsic stability of bis(tertiary phosphine) chelate complexes of the type [CpCo(P⌒P)I]I, where P⌒P = Ph₂P(CH₂)ₙPPh₂ (n = 1–4), was investigated in CH₂Cl₂ and CDCl₃ solution by ¹H and ³¹P{¹H} NMR spectroscopy and product isolation. Depending on P⌒P, the complexes range from indefinitely stable (n = 2, 3, ≥90 h) to reactive (n = 4, <2 h). The organometallic products of these rearrangements were identified as CpCo[P⌒P(O)]I₂, containing an oxidized "dangling" phosphine, and Cp₂Co₂I₄(μ-P⌒P), containing a bridged phosphine. The stability (n = 4 << 1 < 2, 3) originates in the chelate ring strain, which in turn controls the ease of formation of the neutral complex, CpCoI₂(P⌒P), containing monodentate P⌒P. The importance of this species in the stability of the chelate salts of interest, [CpCo(P⌒P)I]I, is evidenced by the fact that these salts are destabilized by the presence of excess I⁻. Moreover, the BF₄⁻ and PF₆⁻ salts of [CpCo(P⌒P)I]⁺ are stable in situations where the I⁻ salt is not. Dissociation constants, K_D, are in the range of (2–7) × 10⁻⁴ M for the ion pairs [CpCo(P⌒P)I]X, X = I⁻, BF₄⁻, PF₆⁻. These relatively small values along with evidence from the ¹H NMR spectra of various contact ion pairs suggest that the [CpCo(P⌒P)I]I contact ion pair is responsible for the formation of CpCoI₂(P⌒P). The formation of CpCo[P⌒P(O)]I₂ requires the presence of light and at least adventitious O₂/H₂O. These observations, although not conclusive, are most consistent with oxidation initiated by photolysis of the Co–I bond. H₂O₂ oxidizes [CpCo(P⌒P)I]I to CpCo[P⌒P(O)]I₂ to a greater extent in complexes where the chelate ring is strained. X-ray crystal structure determinations were conducted on [CpCo(dppm)I]I·CHCl₃, (I4̄, a = 18.271 (6) Å, c = 25.496 (8) Å, Z = 8), CpCo[dppm(O)]I₂ (P2₁/n, a = 8.383 (4) Å, b = 10.388 (3) Å, c = 33.16 (1) Å, β = 91.43 (3)°, Z = 4), and Cp₂Co₂I₄(μ-dpppent) (P2₁/c, a = 14.978 (5) Å, b = 18.556 (5) Å, c = 15.011 (5) Å, β = 103.03 (2)°, Z = 4).

Introduction

The synthesis of metal–halide complexes in which bis(tertiary phosphine) ligands are present in the coordination sphere has been important to us for parameterizing the steric and electronic factors that bias the Michaelis–Arbuzov transformations involving these complexes.[1–3] In most instances, the desired complexes, [CpCo(P⌒P)I]I, where P⌒P is a bis(tertiary phosphine) ligand,[4]

could be prepared by reaction 1 in relatively high yield with clean

$$CpCoI_2(CO) + P⌒P \rightarrow$$
$$[CpCo(\underset{1}{P⌒P})I]I + CO + \text{"other products"} \quad (1)$$
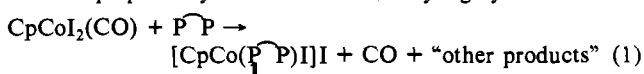
product separation.[2] However, **1** may undergo further chemistry, and variables such as reactant stoichiometry, time, solvent, and temperature decidedly influence the products obtained from reaction 1. To the extent that these reactions and other products might intervene in the Arbuzov transformation of **1**, we felt obliged to characterize them.

The present study contributes to the understanding of factors that affect the modes of bonding of bis(tertiary phosphine) ligands in transition-metal chemistry. Chelated, bridged, and monodentate or "dangling" bis(tertiary phosphine) ligands have been isolated

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