166. Interaction between the Buffer *Tris* and the Eu(III) Ion: Luminescence and Potentiometric Investigation[1])

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The interaction between the buffer 2-amino-2-(hydroxymethyl)propane-1,3-diol (*Tris*) and the Eu(III) ion has been studied by luminescence spectroscopy in D_2O. Emission and excitation spectra ($^5D_0 \leftarrow {}^7F_0$ transition) indicate an interaction with both [*Tris*H]$^+$ and neutral *Tris* species. The former is weak and probably of the outer-sphere type. The latter is of inner-sphere type and corresponds to the formation of the [Eu(*Tris*)]$^{3+}$ species (estimated $\log K_1 = 2.3 \pm 0.3$). Buffer *Tris* is also demonstrated to prevent the formation of an Eu-hydroxo species in the pD range of 7–8. Potentiometric measurements in H_2O allowed a more precise calculation of the stability constant: $\log K_1 = 2.44 \pm 0.07$. Comparison with the data for aliphatic amines and other metal ions lead to the conclusion that the Eu/*Tris* interaction is mainly achieved through the amino group. ^{1}H-NMR spectra in presence of Tb(III) ions confirmed both this assumption and the presence of a weak interaction with *Tris*H$^+$. Quantitative determinations of association constants between lanthanide ions and macromolecules of biological interest performed in presence of *Tris* should, therefore, be corrected for the Eu/*Tris* interaction.

1. Introduction. – The aminoalcohol 2-amino-2-(hydroxymethyl)propane-1,3-diol (*Tris*) is one of the most commonly used buffers in biochemical studies, since its effect occurs in a pH range often encountered in natural systems (*ca.* 7–9). However, *Tris* interferes with some enzymatic reactions [1], and this action can be related either to the presence of the aliphatic amine moiety or to the ability of the buffer to coordinate metal ions. Indeed, complexes with several transition metal ions, *e.g.* Mn(II), Fe(III), Co(III), Ni(II), Cu(II), have been isolated [2] [3], and the crystal structures of two Cu(II) complexes have been established [3].

Increasing interest for the role of metal ions in biochemical processes is stimulating numerous investigations. When spectroscopically silent metal ions such as Ca(II) or Zn(II) are involved, it is common practice to substitute them by metal ions having similar chemical properties and specific magnetic and/or spectroscopic properties. In this respect, the trivalent lanthanide ion Eu(III) is finding widespread application as luminescent substitution probe, especially in the study of Ca-binding proteins [4] [5]. The use of ions, which do not naturally occur in biochemical systems, necessitates a study of their side-effects on the biological materials, as well as on the other chemical species present in the investigated systems. In this communication, therefore, we report a luminescence and potentiometric study on the interaction of Eu(III) with the buffer *Tris*.

2. Experimental. – *Tris* · HCl (*Fluka, puriss. biochem.*) was used without further purification after drying 20 h/120°. Hydrated $EuCl_3$ was synthesized from the oxide (*Fluka*, puriss.); stock solns. were standardized by titration with EDTA at pH 6.0 using xylenol orange as indicator and urotropin as buffer. Solns. for luminescence measurements were prepared by addition of the required volume of *Tris* and $EuCl_3$ stock solns. (micropipette

[1]) Abstracted, in part, from the Ph. D. Thesis of *J.-M. P.*

Socorex 841) into 1-cm quartz cells filled with 3.00 ml of D_2O (99.8%, *Stable Isotopes Inc.*) and containing 0.01M NaCl (*Merck, p.a.*) to ensure reliable pD measurements. The pD was adjusted to a constant value with NaOD and DCl (*Merck, p.a.*). The pD value was obtained by adding 0.41 to the pH-meter reading [6], while the pK_a value of *Tris* in such a medium is assumed to be equal to 8.6 [7]. Solns. for potentiometric measurements were prepared with quartz-bidistilled H_2O degassed with N_2.

Luminescence measurements were performed on a previously described instrumental setup [8]. Emission spectra were excited at 395 nm ($^5L_6$ level, bandwidth 10 nm) by a 450-W Xe lamp and a *Zeiss M20* monochromator. Spectra were corrected for instrumental response. Excitation spectra were recorded between 577 and 581 nm using a tunable *Rhodamin-6G* dye laser (*Coherent CR-599*, band-pass 0.03 nm). Curve-fitting calculations were performed on spectra defined by 300 points and using *Gaussian* functions. The quality of the curve fitting is expressed by the residual index $R_i = \Sigma\| Y_{obs}|-|Y_{calc}\|/\Sigma|Y_{obs}|$.

The stability constant was determined by titrating solns. containing [*Tris*H]$^+$ and $EuCl_3$ by NaOH dispensed from a *Metrohm E 457* microburet. The pH measurements were performed with a *Metrohm 6.0204* micro-electrode and a *Metrohm E-632* pH-meter. The ionic strength was set at 0.15M with NaCl, while the temp. was kept constant at 25 ± 0.03° using a *Haake-FK* water bath. Under such experimental conditions, the pK_a of *Tris* is 8.110 ± 0.003 [9], and the ionic product of H_2O to 13.755 [9]. To avoid hydroxide precipitation [10], the pH was kept below 7. Calculation of the stability constant was performed with the program SUPERQUAD [11] running on a *VAX 8550* computer.

^{1}H-NMR spectra were recorded in D_2O solutions with a *Bruker AC-200* spectrometer, using (D_6)acetone as reference.

3. Luminescence Study. – Measurements were performed in D_2O to avoid quenching of the $^5D_0$(Eu) level by the OH oscillators of coordinated water molecules [4]. Also, the pD was kept below 7, namely at 6.56 ± 0.02, to evade the formation of $Eu(OH)_3$. Under these experimental conditions, two potentially ligating species are in equilibrium, *Tris* and [*Tris*H]$^+$, in ratio 1:124, the concentration of deprotonated *Tris* being negligible. The emission spectra obtained after adding various amounts of buffer to an Eu(III) solution in D_2O do not display striking differences, but a noticeable quenching. The variation of the total luminescence intensity (calculated as the sum of the integrated $^5D_0 \rightarrow {}^7F_j$ transitions, with J = 1,2,4) *vs.* the concentration of both [*Tris*H]$^+$ and *Tris* is presented in *Fig. 1*[2]). At low concentration of buffer (*Fig. 1*, right), the total luminescence decreases sharply up to *ca.* 2 equiv. of added [*Tris*H]$^+$ and then stabilizes slowly down to a 35% decrease for 13.5 equiv. of [*Tris*H]$^+$. At this point, the [*Tris*]/[Eu(III)]$_t$ ratio is only *ca.* 0.1 so that the observed quenching is indicative of an interaction between the Eu(III) ions and the [*Tris*H]$^+$ species. More quantitative information, *i.e.* the stoichiometry and the stability constant of the adduct, are not easily deduced from *Fig. 1*, since the data are influenced by the interaction with neutral *Tris*. The emission spectrum of the Eu(III)-aquoion is characteristic of a species possessing D_{3h} symmetry, *i.e.* the $^5D_0 \rightarrow {}^7F_0$ transition is very weak and the spectrum is dominated by a strong $^5D_0 \rightarrow {}^7F_1$ transition. Inner-sphere complexation by a ligand usually results in a lowering of symmetry and expulsion of coordinated H_2O molecules, henceforth, in an increase in the transition probability of the hypersensitive $^5D_0 \rightarrow {}^7F_2$ transition, with respect to the magnetic dipole transition $^5D_0 \rightarrow {}^7F_1$. In the present case, no significant change in the $^5D_0 \rightarrow {}^7F_2/{}^5D_0 \rightarrow {}^7F_1$ ratio occurs, which indicates a weak, probably outer-sphere, interaction betwen Eu(III) and [*Tris*H]$^+$. Upon adding more buffer to increase the concentration of the neutral *Tris* species, the total luminescence intensity remains constant in the [*Tris*]/[Eu(III)] molar-ratio range of 0.1–1 and then linearly decreases by about 20% for *ca.* 8 equiv. of added *Tris* (*Fig. 1*,

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[2]) The total luminescence has been corrected for the isotopic effect of the H^+ ions introduced into the solution by the addition of *Tris* [12] [13].

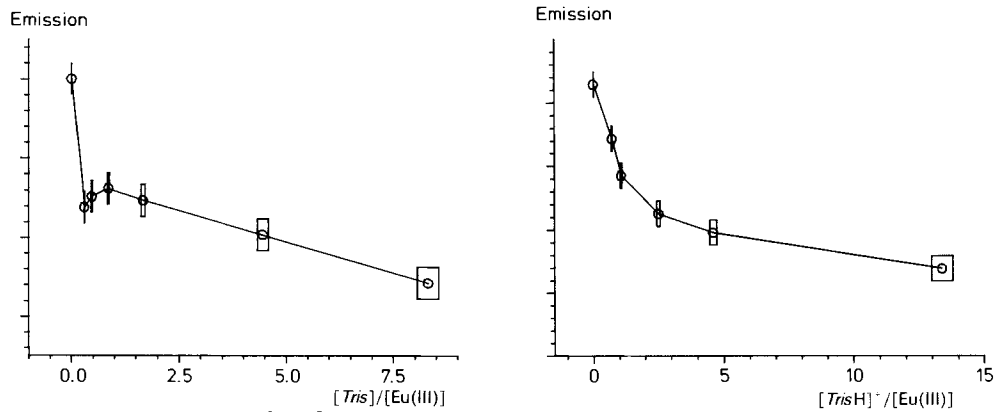

Fig. 1. *Sum of the integrated* $^5D_0 \rightarrow {}^7F_J$ *transitions* (J = 1,2,4) vs. *the molar ratios* $[[\text{Tris}H]^+]/[Eu(III)]_t$ (right) *and* $[\text{Tris}]/[Eu(III)]_t$ (left). [Eu(III)] = $1.09 \cdot 10^{-3}$ M in D_2O, $[Tris]_t$ = 0–0.0134M (right) and 0–0.0083M (left), [NaCl] = 0.01M, pD = 6.56(2), λ_{exc} = 395 nm, vertical scales: arbitrary units.

left). The $^5D_0 \rightarrow {}^7F_2/{}^5D_0 \rightarrow {}^7F_1$ ratio increases by *ca.* 12% in the same range. This points to the formation of an inner-sphere complex between *Tris* and Eu(III) having a lower symmetry than the aquoion.

To gain more information on the interactions evidenced by the emission spectra, $^5D_0 \leftarrow {}^7F_0$ excitation spectra were recorded under high resolution. The latter transition being unique for a given chemical environment of the Eu(III) ion, the number of its components indicates the number of Eu-containing species in solution [4]. Up to a $[[Tris\text{H}]^+]/[\text{Eu(III)}]$ ratio of 25, the $^5D_0 \leftarrow {}^7F_0$ transition is comprised of a single, broad band with *Gaussian* shape and centered at 17279.2 ± 0.8 cm^{-1} (full width at half height (fwhh) = 19.4 ± 0.9 cm^{-1}). These parameters are close to those reported for the aquoion [5], pointing to a very weak $\text{Eu}/[Tris\text{H}]^+$ interaction. At larger concentrations of the buffer, important modifications of the $^5D_0 \leftarrow {}^7F_0$ excitation spectra occur (*Fig. 2*). The

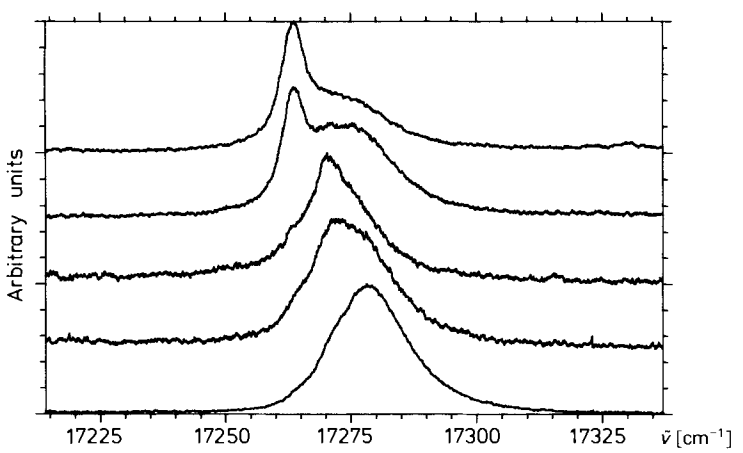

Fig. 2. $^5D_0 \leftarrow {}^7F_0$ *excitation spectra for different concentrations of the buffer* Tris. [Eu(III)] = $1.0 \cdot 10^{-3}$ M in D_2O, pD = 6.60(2), λ_{an} = 615 nm, $[Tris]_t$ = 27, 53.4, 90.3, 249, 500 mM (from bottom to top).

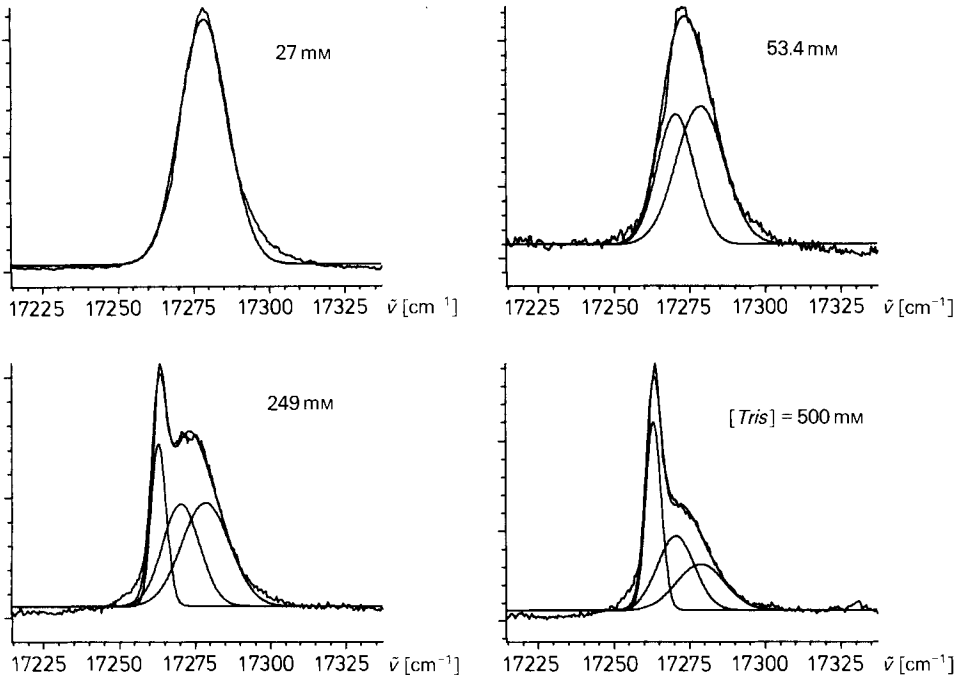

Fig. 3. *Decomposition into* Gaussian *components of the 4 top spectra of* Fig. 2. Residual indices (see text) $R_I = 0.0733$, 0.0746, 0.0716, d 0.0851 (from bottom to top).

excitation band shifts towards lower energies, broadens, and another, narrower component appears. Decomposition of the spectra into *Gaussian* components [14] (*Fig. 3*) evidences the presence of two additional excitation bands. One is close to the band assigned to the aquoion; it is centered at 17270.50 cm^{-1} (fwhh = 14.4 ± 0.3 cm^{-1}) and appears at high concentration of $[TrisH]^+$ ($[[TrisH]^+]/[Eu(III)] = 50$) but at low concentration of the neutral species ($[Tris]/[Eu(III)] = 1.25$). We assume that this component reflects the weak $Eu/[TrisH]^+$ interaction discussed above. The other band is centered at 17263.5 ± 0.5 cm^{-1} and is quite narrow (fwhh = 6.0 ± 0.1 cm^{-1}). We consider it as arising from the formation of a well-defined $[Eu(Tris)]^{3+}$ complex with a relatively small stability, since it appears only at $[Tris]/[Eu(III)]$ ratios > 6. The intensity of this band is proportional to the concentration of the $[Eu(Tris)]^{3+}$ complex, allowing an estimation of its formation constant: $\log K_1 = 2.3 \pm 0.3$.

The *Eu/Tris* interaction is better evidenced at higher pD. The $^5D_0 \leftarrow {}^7F_0$ excitation spectra of Eu(III) in absence and in presence of *Tris* at pD 7.7–7.8 are shown in *Fig. 4* (top). In absence of buffer, the spectrum displays at least two very broad components arising from the species $[Eu(OH)]^{2+}$ and $[Eu(OH)_2]^+$. In presence of 5 equiv. of *Tris*, the spectrum is considerably simplified and reduced to the narrow component assigned above to the $[Eu(Tris)]^{3+}$. This feature revealed useful in studies of Eu(III) bound to a macromolecule in presence of *Tris* buffer: free Eu(III), that is not bound to the macromolecule, gives rise to one symmetrical component in the $^5D_0 \leftarrow {}^7F_0$ excitation spectrum

even at relatively high pD, hence facilitating the decomposition of the spectrum into *Gaussian* components [5] [14].

Finally, as the Eu/*Tris* interaction is weak, we have checked for interference from the Cl^- ions used to set the ionic strength of the solutions, since the buffer was always added as [*Tris*H]$^+Cl^-$. Seven $^5D_0 \leftarrow {}^7F_0$ excitation spectra recorded in presence of different concentration of Cl^- up to a [Cl^-]/[Eu(III)] ratio of 204 are presented in *Fig. 4* (bottom). Only minor differences can be observed in the region 17250–

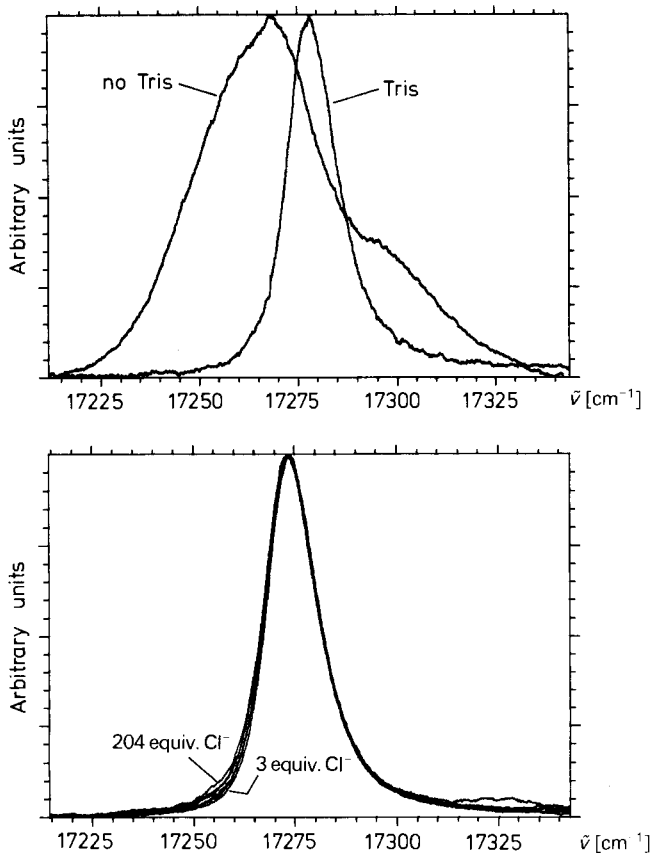

Fig. 4. *$^5D_0 \leftarrow {}^7F_0$ excitation spectra of Eu(III).* Top: effect of *Tris*; [Eu(III)] = $1.0 \cdot 10^{-4}$ M in D_2O, λ_{an} = 615 nm, pD = 7.77(2), a) no *Tris*, b) [*Tris*] = $5.0 \cdot 10^{-3}$ M. Bottom: effect of NaCl; [Eu(III)] = $1.0 \cdot 10^{-4}$ M in D_2O, λ_{an} = 615 nm, pD = 7.43(2); [Cl^-] = 3.0, 7.0, 19.0, 31.0, 83.0, 123.0, and 204 mM (from bottom to top).

17270 cm^{-1} which may be traced back to the formation of the $[Eu(H_2O)_n(Cl)]^{2+}$ and $[Eu(H_2O)_n(Cl)_2]^+$ species: the energy of the $^5D_0 \leftarrow {}^7F_0$ transitions for these species have been reported to occur at 17270 and 17263 cm^{-1}, respectively [15]. This experiment demonstrates that Cl^- ions do not affect substantially the $^5D_0 \leftarrow {}^7D_0$ excitation spectra, thereby the features we observed in the presence of *Tris* are solely due to the Eu/*Tris* interaction.

4. Potentiometric Measurements. – These measurements were performed to better quantify the Eu/*Tris* interaction evidenced in the luminescence study, in particular with respect to the stoichiometry of the complex and its stability constant. Potentiometric data were fit with several models including $[Eu(Tris)_n]^{(3-n)+}$ and hydroxo species. The best agreement was achieved with the simplest model taking into account the formation of $[Eu(Tris)]^{3+}$ only (*Table 1*). The mean log K_1 obtained, 2.44 ± 0.07, is in excellent agreement with the estimation from the luminescence data and in the range of values obtained for other transition-metal ions under identical experimental conditions : 3.14 [16], 2.63, 4.11, and 2.27 [9] for Ag(I), Ni(II), Cu(II), and Zn(II), respectively. The binding abilities of the buffer *Tris* and several aliphatic primary amines, including ammonia, are compared in *Table 2* [16] [17]. Despite some differences in experimental conditions, mainly in the ionic strength, the log K_1 values for the transition-metal ions mentioned above are quite similar for all the amines studied, despite large differences in their pK_a values. The

Table 1. *Potentiometric Determination of the Formation Constant of [Eu(*Tris*)]$^{3+}$ at 25° and* I = *0.15* M *(NaCl).* Standard deviations are given in parentheses and concentrations are expressed in M.

log K_1	W[a])	N[b])	[Eu(III)]	$[Tris]_t$
2.38(4)	1	23	$5.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-3}$
2.42(3)	0	23	$5.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-3}$
2.47(3)	1	45	$5.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-3}$
2.52(2)	0	45	$5.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-3}$
2.34(3)	1	50	$5.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-3}$
2.45(2)	0	50	$5.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-3}$
2.35(1)	1	76	$5.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-2}$
2.40(1)	0	76	$5.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-2}$
2.53(5)	1	56	$5.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-2}$
2.51(3)	0	56	$5.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-2}$

[a]) Weighting scheme; 0 = proportional to $1/\sigma^2$, 1 = unit weights.
[b]) Number of points.

Table 2. *log* K_1 *at 25° for the Interaction of Different Metal Ions and the Proton with Some Aliphatic Primary Amines.* Data from [16] [17].

Ion	Amine				
	NH_3	$MeNH_2$	$EtNH_2$	$PrNH_2$	$(i\text{-}Pr)NH_2$
Ag(I)	3.35[a])	3.15[d])	3.38[a])	3.40[a])	3.19[a])
Cu(II)	4.14[b])	4.11[c])			
Ni(II)	2.61[a])		2.81[a])		2.71[a])
Zn(II)	2.136[c])		2.30[d])	2.42[a])	2.37[a])
H^+	9.43[b])	10.72[d])	10.66[d])	10.74[d])	10.61[d])

[a]) NH_4NO_3, 2M. [b]) NH_4NO_3, 1M. [c]) KNO_3, 1M. [d]) $I = 0.5$. [e]) $I = 0$.

pK_a value of a base being often related to its metal-binding ability, it is noteworthy that the pK_a of the *Tris* buffer (8.11) is 1.3–2.6 units smaller than the pK_a values of the other amines, while the stability constants of its complexes are comparable to those of the other amines. Additional coordination of one of the CH_2OH groups, making *Tris* a bidentate ligand, has been evoked to explain this behaviour [18] [19]. However, this assumption

does not explain why differences of more than one log unit in the pK_a values between ammonia and ethyl- or isopropylamines lead to such small differences in the corresponding log K_1 values. In fact, the data reported in *Table 2* indicate that the amino groups of both the aliphatic amines and *Tris* behave in the same way towards metal ions. This leads to the conclusion that *Tris* coordinates metal ions mainly by its amino group, but a participation of one or several CH_2OH groups in the coordination cannot be *a priori* excluded.

5. Solution Structure of the [Eu(*Tris*)]$^{3+}$ Complex. – Attempts to isolate crystalline [Eu(*Tris*)]$^{3+}$ complexes following the procedure described for transition-metal complexes [2] [3] failed, preventing a structural study of the coordination around the Eu(III) ion. To clarify the question of the OH group participation in the binding of Eu(III), we have recorded ^{1}H-NMR spectra of *Tris* solutions containing Tb(III) used as a shift reagent producing large shifts but no broadening. If the coordination is exclusively achieved by the amino group, a single resonance is expected, the protons of the CH_2OH groups being equivalent. If one or several CH_2OH groups participate in the coordination, their protons get closer to the Tb(III) ion, giving rise to another resonance. The spectra are shown in *Fig. 5*. In absence of Tb(III) a single band appears at 3.68 ppm (fwhh = 3.3 Hz). In

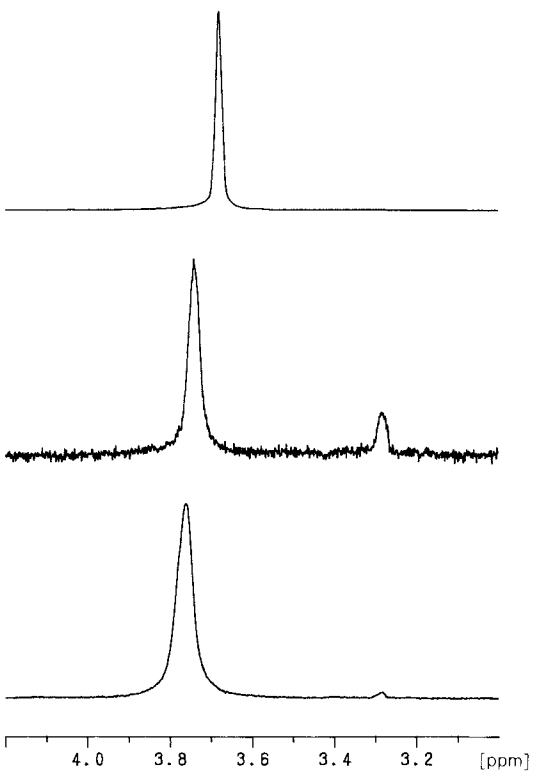

Fig. 5. *^{1}H-NMR spectra of* Tris *in presence of various amounts of Tb(III) ion, at pD = 6.91(2).* Top: *Tris* alone, 0.01M in D_2O. Middle: $[Tris]_t = 5.35 \cdot 10^{-3}$ M, [Tb(III)] = $1.05 \cdot 10^{-2}$ M. Bottom: $[Tris]_t = 4.3 \cdot 10^{-2}$ M, [Tb(III)] = $1.05 \cdot 10^{-2}$ M.

presence of 0.25 equiv. of Tb(III), a second, weaker resonance appears at 3.29 ppm (fwhh = 5.6 Hz), while the main resonance is shifted to 3.74 ppm and becomes broader (fwhh = 6.7 Hz). When the [Tb(III)]/[*Tris*] ratio is increased, this second resonance becomes weaker: the ratio of the integrated areas is equal to *ca* 7:1 and 145:1 for [Tb(III)]/[*Tris*] = 0.25 and 2, respectively. In view of this behaviour, we assign this supplementary band as arising from the interaction with [*Tris*H]$^+$, in which some of the CH_2OH protons are closer to the metal center. An increase in the total concentration of the buffer allows the deprotonated *Tris* to interact with Tb(III) ions so that the additional resonance becomes less intense. This experiment demonstrates that the buffer *Tris* interacts mainly through its neutral NH_2 group. Indeed, if the CH_2OH groups were participating in the coordination, the ratio of the integrated areas of the two resonances would be $\leqslant 3$, each of the three CH_2OH groups bearing two non-labile protons.

6. Conclusion. – This study shows the existence of a weak Ln/[*Tris*H]$^+$ interaction, probably of the outer-sphere type, and of a more substantial, inner-sphere Ln/*Tris* interaction (Ln = Eu, Tb). The latter interaction occurs mainly through the amino group of the buffer. Quantitative determinations of association constants between lanthanide ions and macromolecules in presence of *Tris* buffer should, therefore, be corrected for this interaction.

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REFERENCES

[1] H. Neumann, F. Kezdy, J. Hsu, I. H. Rosenberg, *Biochim. Biophys. Acta* **1975,** *391,* 292.
[2] R. L. Dotson, *J. Inorg. Nucl. Chem.* **1972,** *34,* 3131.
[3] D. Masi, C. Mealli, M. Sabat, A. Sabatini, A. Vacca, F. Zanobini, *Helv. Chim. Acta* **1984,** *67,* 1818.
[4] J.-C. G. Bünzli, in 'Lanthanide Probes in Life, Chemical and Earth Sciences', Eds. J.-C. G. Bünzli and G. R. Choppin, Elsevier Science Publ. B. V., Amsterdam, in press; J.-C. C. Bünzli, *Inorg. Chim. Acta* **1987,** *139,* 219; W. De W. Horrocks, Jr., M. Albin, *Prog. Inorg. Chem.* **1984,** *31,* 1.
[5] J.-M. Pfefferlé, J.-C. G. Bünzli, submitted to *Inorg. Chem.*
[6] A. K. Convington, M. Paabo, R. A. Robinson, A. G. Bates, *Anal. Chem.* **1968,** *40,* 700.
[7] E. W. Baumann, *Anal. Chem.* **1966,** 1255.
[8] J.-C. G. Bünzli, G.-O. Pradervand, *J. Chem. Phys.* **1986,** *85,* 2489; D. Plancherel, L. Jin, R. Massara, J.-C. G. Bünzli, *Helv. Chim. Acta* **1987,** *70,* 1807.
[9] L. Bologni, A. Sabatini, A. Vacca, *Inorg. Chim. Acta* **1983,** *69,* 71.
[10] J. Kragten, 'Atlas of Metal-Ligand Equilibria in Aqueous Solutions', Ellis Horwood Ltd., Chichester, 1978.
[11] P. Gans, A. Sabatini, A. Vacca, *J. Chem. Soc., Dalton Trans.* **1985,** 1195.
[12] J. L. Kropp, M. W. Windsor, *J. Chem. Phys.* **1965,** *42,* 1599.
[13] Y. Haas, G. Stein, *J. Phys. Chem.* **1971,** *75,* 3677.
[14] J.-M. Pfefferlé, Ph. D. Dissertation, Université de Lausanne, 1989.
[15] M. Albin, W. de W. Horrocks, Jr., *Inorg. Chem.* **1985,** *24,* 895.
[16] P. M. Smith, A. E. Martell, 'Critical Stability Constants', Plenum Press, New York, 1975.
[17] E. Högfeldt (part A), D. D. Perrin (part B), 'Stability Constants of Metal-Ion Complexes', IUPAC Chemical Data Series *21, 22,* Pergamon Press, Oxford, 1982.
[18] B. E. Fischer, U. K. Häring, R. Tribolet, H. Sigel, *Eur. J. Biochem.* **1979,** *94,* 523.
[19] R. L. Dotson, *Inorg. Nucl. Chem. Lett.* **1973,** *9,* 215.