

Dissociation kinetics of europium(III) cryptate complexes in aqueous buffers

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Dissociation of the $[\text{EuL}^1]^{3+}$ complex ($\text{L}^1 = 4,7,13,16,21$ -penta-oxa-1,10-diazabicyclo[8.8.5]tricosane) in aqueous buffer solutions of pH 7.0–9.0 was studied by monitoring the absorbance change of its charge-transfer (c.t.) band. While the dissociation rate is linearly dependent on complex concentration, the rate constant (k_d) is dependent on both the concentration and the type of buffer employed. In tris(hydroxymethyl)aminomethane (Tris) the measured rate constant is composed of a concentration-independent term and another term based on the square of the concentration of the basic form of Tris, *i.e.* $k_d = k_0 + k_2[\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3]^2$, which indicates that a general base mechanism is dominant at high buffer concentrations. The dissociation of the analogous complex of 4,7,13,16,21,24-hexa-oxa-1,10-diazabicyclo[8.8.8]hexacosane is more rapid. The unique c.t. band of the europium complexes was utilized to elucidate their thermodynamic behaviour in aqueous buffers. No absorbance was observed even at relatively high concentrations of the europium ion and the cryptands ($0.100 \text{ mol dm}^{-3}$). This enables an upper limit of the formation constants of $0.50 \text{ dm}^3 \text{ mol}^{-1}$ to be set in aqueous buffer.

Recently lanthanide ions and their complexes have been shown to exhibit high catalytic activity toward hydrolysis of phosphate esters, especially DNA, RNA, and their analogues.¹ In particular, the design and synthesis of compounds which are able to co-ordinate labile metal ions and form stable complexes without losing their reactivity are of great importance because the sequence-specific hydrolytic cleavage of DNA and RNA can be achieved by tethering molecular catalysts to a sequence-specific director.² In order fully to utilize the desirable properties of the lanthanide ions, neutral polydentate compounds seem to be most appropriate. There are many polydentate anions such as edta (ethylenedinitrilotetraacetate) which can form stable complexes, but the reactivity of the latter toward hydrolysis is reduced due to neutralization of the charge of the central metal ion. Among various neutral polydentate compounds, a hexadentate Schiff base,³ 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (tcmc),^{1e} and cryptand 221 (4,7,13,16,21-penta-oxa-1,10-diazabicyclo[8.8.5]tricosane, L^1)^{1f,4} have been applied satisfactorily.

The detailed thermodynamic and dissociation properties of these well defined lanthanide macrocyclic complexes not only provide valuable information on their equilibrium behaviour and stability in solution, but also permit prediction of the corresponding properties of other complexes for which data are lacking.⁵ Especially, the nature of the complexes in aqueous buffer solutions of neutral pH is informative, because most of the above-mentioned hydrolyses are observed under physiological conditions.

While the thermodynamic properties of the lanthanide cryptates in non-aqueous solutions have been studied in detail,^{5,6} in aqueous solution studies have been focused on the dissociation kinetics due to their pronounced instability in water. Weaver and co-workers⁷ successfully applied electrochemical methods to elucidate the kinetic stability of lanthanide cryptates in neutral and acidic aqueous solutions. Torres and Baisden⁸ used proton NMR spectroscopy to monitor the dissociation of the diamagnetic lanthanum(III) cryptate $[\text{LaL}^1]^{3+}$ in neutral and basic aqueous solutions.

We report here the dissociation kinetics of the europium(III) complexes of cryptands 221 and 222 (4,7,13,16,21,24-hexa-oxa-1,10-diazabicyclo[8.8.8]hexacosane, L^2) in aqueous buffer solu-

tions of pH 7.0–9.0. By observing the absorbance changes in their charge-transfer bands various kinetic parameters have been obtained. Also, the equilibrium behaviour of the complexes in the medium has been elucidated by a spectroscopic method.

Experimental

The lanthanide cryptates were prepared as previously described.⁴ These hygroscopic compounds were handled and weighed in an N_2 -filled glove-box. Kinetic traces were recorded on a Hewlett-Packard 8452A diode-array UV/VIS spectrophotometer. Buffers were made up from tris(hydroxymethyl)aminomethane (Tris, Aldrich), 1,3-bis[tris(hydroxymethyl)methylamino]propane (bis-tris propane, Sigma), *N'*-(2-hydroxymethyl)piperazine-*N*-ethanesulfonic acid (hepes, Sigma), and sodium hydrogenphosphate (Aldrich), and standardized HCl and NaOH in deionized water ($18 \text{ M}\Omega \text{ cm}^{-1}$). The buffer concentration was fixed at $0.200 \text{ mol dm}^{-3}$ for the dissociation kinetics, and $0.010 \text{ mol dm}^{-3}$ for thermodynamic studies, unless otherwise mentioned, and the ionic strength was maintained at $0.500 \text{ mol dm}^{-3}$ (NaCl) for the dissociation kinetics and $0.100 \text{ mol dm}^{-3}$ (NaCl) for measuring the formation constants.

For dissociation kinetic studies, the recrystallized europium(III) cryptate chloride $[\text{EuL}^1]\text{Cl}_3$ was dissolved in a buffer solution at room temperature. The pH of the reaction medium was finely adjusted by titrating with dilute NaOH solution. The temperature of the stirred reaction mixture was kept constant with a constant-temperature water-bath. Typically, the concentration of the europium complex was $5.00 \times 10^{-3} \text{ mol dm}^{-3}$. After certain periods of time aliquots of the solution were withdrawn, and the UV/VIS spectra recorded. A white precipitate of europium hydroxide was formed as the dissociation proceeded, and was filtered off prior to spectroscopic measurement.

In an attempt to measure the formation constants between europium(III) ion and the cryptands, an aqueous solution of $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ and a buffer solution of the cryptand were mixed, and the UV/VIS spectrum of the resulting mixture recorded at room temperature after fine adjustment of the pH.

Results and Discussion

Charge-transfer band

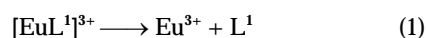
It has been reported that some lanthanide macrocyclic com-

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plexes absorb UV light through a ligand-to-metal charge transfer (l.m.c.t.). Only the most reducible metal ions (Sm^{3+} , Eu^{3+} and Yb^{3+}) exhibit this phenomenon.⁹ In particular, $[\text{EuL}^{13+}]$ shows two distinct bands (248 and 298 nm) which were assigned to oxygen-to-metal and nitrogen-to-metal charge transfer.^{9c} We have observed that the shape and wavelength of the maximum of the c.t. bands are not sensitive to the pH of the medium. Also, the absorption coefficient of the band is almost invariant with pH (Table 1).

Dissociation kinetics

By observing the decrease in absorbance of the c.t. band, the dissociation kinetics of the complexes can be directly monitored under various conditions. Typically, the absorbance was recorded until more than 85% of the complex had dissociated to metal ion and the free macrocycle [equation (1)]. The strict



linearity ($r > 0.990$) obtained on plotting \log (absorbance, A) against the reaction time (t) indicates that the dissociation rate exhibits a first-order dependence on the concentration of the metal complex. Therefore, the dissociation rate constant (k_d) could be obtained from the slope [equations (2) and (3)].

$$\text{Rate} = k_d[\text{EuL}^1] \quad (2)$$

$$\ln A = -k_d t \quad (3)$$

The rate constants thus measured in Tris buffer at pH 7.0–9.0 are summarized in Table 2. Relatively high buffer concentrations were employed in order to avoid pH changes which typically accompany the dissociation. As seen in Table 2, the rate constant regularly increases with the pH of the medium. The half-life of the europium complex is 57 h at pH 7.00, which is reduced to 11 h at pH 9.00.

A plot of k_d against pH gives a sigmoidal curve. When k_d is plotted against the concentration of the basic form of Tris a second-order dependence is evident as shown in Fig. 1 ($r = 0.9894$). The measured rate constant is composed of a concentration-independent term and a second term proportional to the square of the concentration of the basic form of Tris, $k_d = k_0 + k_2[\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3]^2$, where k_0 and k_2 are $0.33 \times 10^{-5} \text{ s}^{-1}$ and $0.42 \times 10^{-3} \text{ dm}^6 \text{ mol}^{-2} \text{ s}^{-1}$, respectively. At low concentration of the general base, such as $0.015 \text{ mol dm}^{-3}$, the k_0 term is dominant, but the second term becomes important as the concentration increases.

Complications from the effect of hydroxide can be totally excluded by varying the buffer concentration at fixed pH. When the buffer concentration was increased from 0.040 to 0.15 mol

dm^{-3} at pH 8.00 the apparent rate constant increased from 3.9×10^{-6} to $5.6 \times 10^{-6} \text{ s}^{-1}$ (Table 3). A plot of $\ln(k_d - k_0)$ against $\ln[B]$ was linear with a slope of 1.74 ($r = 0.9880$). This plot also supports the second-order dependence on the concentration of the basic form.

As well as the above observations, the strong dependence on the type of buffer (see below) indicates that the dissociation is general base catalysed. The rate law is a sharp contrast to the previously observed dependence on the oxonium-ion^{7a} or the hydroxide-ion concentration⁸ in the absence of buffers.

Since the chemical structures of the buffers are very different from each other, their efficiency as a general base should be widely variable. As seen in Table 4, phosphate buffer most effectively facilitates the dissociation, bis-tris propane the least. The effectiveness of phosphate buffer must reflect the hard nature of the phosphate anion and europium(III) ion. It is noteworthy that the dissociation rate constant in unbuffered solution is comparable to those in buffered solutions. However, direct comparison is not straightforward, because the pH of the medium decreases as the dissociation proceeds in the absence of buffers.

Since $[\text{SmL}^{13+}]$ shows a similar dissociation rate constant to that of the europium complex in Tris buffer ($k_d = 1.42 \times 10^{-5} \text{ s}^{-1}$ at 50°C and pH 8.00), it can be said that the other lanthanide cryptate complexes manifest similar dissociation behaviour. In contrast to the cryptand 221 complex, $[\text{EuL}^{23+}]$ dissociates very rapidly, $k_d = 1.7 \times 10^{-3} \text{ s}^{-1}$ ($\tau_{1/2} = 6.8 \text{ min}$) in Tris buffer of pH 7.00. This reflects the lower stability of the L^2 complex.

The reaction temperature of the solutions at pH 8.50 has been varied to determine the sensitivity of the rate constants. An increase from 30 to 60°C resulted in a 5.9 times enhancement of the overall dissociation rate constants (Table 5). The half-lives of the metal complexes are 66 and 11 h at 30 and 60°C , respectively, at the particular pH and buffer condition.

Mechanism

As previously concluded from time-resolved luminescence spectral and X-ray crystallographic data,⁴ the metal complex has

Table 1 Absorption coefficient of the lower-energy c.t. band of $[\text{EuL}^{13+}]$ at various pH^a

| pH | $\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ |
|------|---|
| 7.00 | 128 |
| 7.50 | 130 |
| 8.00 | 142 |
| 8.50 | 139 |

^a In Tris buffer. ^b Measured at 298 nm.

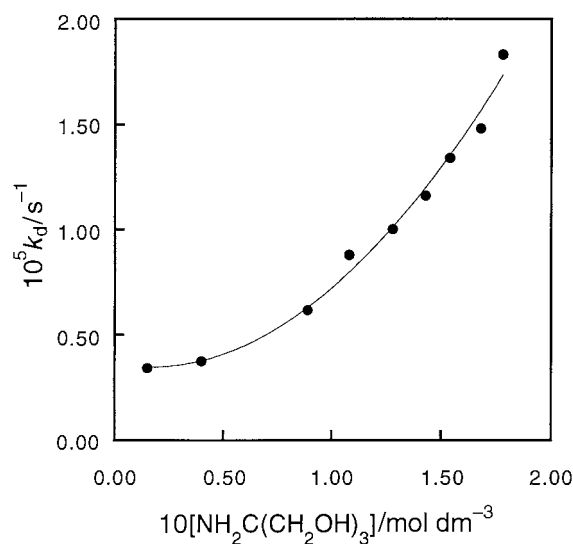


Fig. 1 Plot of the dissociation rate constant (k_d) versus the concentration of the basic form of Tris at 50°C

Table 2 Dissociation rate constants of $[\text{EuL}^{13+}]$ ($5.0 \times 10^{-3} \text{ mol dm}^{-3}$) measured at 50°C in Tris buffer ($0.200 \text{ mol dm}^{-3}$), $I = 0.500 \text{ mol dm}^{-3}$

| pH | 7.00 | 7.50 | 8.00 | 8.17 | 8.35 | 8.50 | 8.63 | 8.82 | 9.00 |
|-------------------------------------|------|------|------|------|------|------|------|------|------|
| $10^5 k_d / \text{s}^{-1}$ | 0.34 | 0.38 | 0.62 | 0.88 | 1.00 | 1.15 | 1.34 | 1.48 | 1.83 |
| $10[\text{B}] / \text{mol dm}^{-3}$ | 0.15 | 0.40 | 0.89 | 1.08 | 1.28 | 1.43 | 1.54 | 1.68 | 1.78 |

* $[\text{B}]$ represents the concentration of Tris in its basic form calculated from its $\text{p}K_a$ value (8.10).

Table 3 Dissociation rate constants of $[\text{EuL}^1]^{3+}$ ($1.0 \times 10^{-3} \text{ mol dm}^{-3}$)^a at pH 8.00 at various Tris buffer concentrations (50°C , $I = 0.500 \text{ mol dm}^{-3}$)

| | $10 [\text{Tris}]^b / \text{mol dm}^{-3}$ | | | | | |
|--|---|------|------|------|------|------|
| | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.50 |
| $10^2 [\text{B}]^c / \text{mol dm}^{-3}$ | 1.9 | 2.9 | 3.8 | 4.8 | 5.7 | 7.1 |
| $10^6 k_d / \text{s}^{-1}$ | 3.9 | 4.0 | 4.2 | 4.5 | 5.0 | 5.6 |

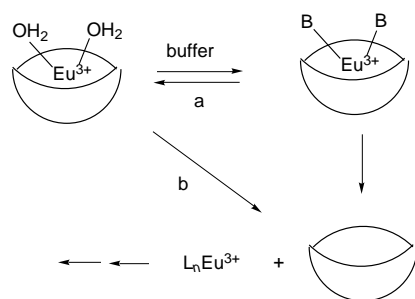
^a A lower concentration of the complex was employed in this case in order to avoid pH changes during the dissociation, which are typically observed at the relatively low buffer concentrations. ^b The total concentration of Tris and Tris·HCl. ^c The concentration of Tris in its basic form.

Table 4 Dissociation rate constants of $[\text{EuL}^1]^{3+}$ ($5.0 \times 10^{-3} \text{ mol dm}^{-3}$) in various buffer solutions (0.20 mol dm^{-3}) at 50°C , pH 8.00 and $I = 0.500 \text{ mol dm}^{-3}$

| Buffer | $10^5 k_d / \text{s}^{-1}$ |
|------------------|----------------------------|
| Tris | 0.62 |
| Phosphate | 16.0 |
| hepes | 0.85 |
| bis-tris propane | 0.55 |
| None | 0.53 |

Table 5 Dissociation rates of $[\text{EuL}^1]^{3+}$ ($5.0 \times 10^{-3} \text{ mol dm}^{-3}$) in Tris buffer solution (0.20 mol dm^{-3}) of pH 8.50 ($I = 0.500 \text{ mol dm}^{-3}$)

| $T / ^\circ\text{C}$ | $10^5 k_d / \text{s}^{-1}$ |
|----------------------|----------------------------|
| 30.0 | 0.29 |
| 40.0 | 0.50 |
| 50.0 | 1.15 |
| 60.0 | 1.71 |



Scheme 1 Proposed mechanism

binding sites for extra ligand molecules. Two are located on the open face with a *cis* geometry as in Scheme 1. In the absence of buffers the sites are normally occupied by water molecules. The aqua ligands can be replaced by the basic form (B) of the buffer employed, and the metal complex with two B molecules can be dissociated into the cryptand and the metal ion. Considering the lability of the lanthanide complexes for ligand exchange, the first step should be a fast equilibrium. The last step, the dissociation of the cryptate, must be the rate-determining step. Pathway a is consistent with the second-order dependence of the dissociation rate on the buffer concentration and its dependence on the chemical nature of the buffer. At the same time, the metal complex can be dissociated without interaction with B (pathway b). The latter pathway is dominant at low buffer concentration.

Thermodynamics

After europium(III) chloride hexahydrate had been mixed with cryptand 221 or 222 in aqueous solutions at room temperature UV/VIS spectra were recorded. Various buffers were employed as the medium. No charge-transfer band was observed even at higher concentration of the europium ion and the cryptands ($0.100 \text{ mol dm}^{-3}$) and longer reaction times. This observation enables us to set an upper limit on the formation constants as $0.50 \text{ dm}^3 \text{ mol}^{-1}$ assuming that 5% of the complex, at best, could be formed under such conditions.

Conclusion

The dissociation rate of $[\text{EuL}^1]^{3+}$ in aqueous buffer solutions of pH 7.0–9.0 is linearly dependent on the concentration of the metal complex, and the apparent rate constant is dependent on both the concentration of Tris buffer and the type of buffer employed. A general base mechanism is dominant at high buffer concentration. Faster dissociation of the analogous L^2 complex is observed. Complex formation between europium(III) ion and cryptand 221 or 222 is thermodynamically unfavourable under these conditions.

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