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Complexes of Uracil (2,4-Dihydroxypyrimidine) Derivatives. Part II. Potentiometric and Luminescence Studies with Eu(III)

by A. Kufelnicki[1*], I. Kupińska[1], S. Lis[2] and Z. Hnatejko[2]

[1]*Laboratory of Physical and Biocoordination Chemistry, Medical University of Łódź, 1 Muszyński Str., 90-151 Łódź, Poland*
[2]*Department of Rare Earths, Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*

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Luminescence spectroscopy studies were carried out on europium(III) complexation with HL = uracil, thymine (5-methyluracil), 6-chloromethyluracil, 5-hydroxymethyluracil and 6-methyluracil in acidic perchlorate solutions. In acidic medium the decrease of the hydration number with increasing the ligand excess indicates a consecutive complexation *via* metal – O4 coordination. The potentiometric method confirmed the possibility of coordination by N3 at higher pH for thymine, 6-chloromethyluracil and 6-methyluracil. The formation of hydroxo species has been ascertained for all the ligands.

Key words: europium(III), uracil ligands, luminescence spectroscopy, coordination modes

As it was evidenced up to now, Ca(II) as well as Mg(II) ions, the most abundant metals in living systems, form complexes with uracil and some derivatives [1,2]. These complexes, however, of such colorless and diamagnetic ions are undetectable by commonly used spectroscopic methods. Moreover, in the acidic medium also the potentiometric technique is rather ineffective. Therefore, in order to extend our studies to low pH we have applied the laser induced luminescence spectroscopy of europium(III) – the Eu(III) ion used, instead of calcium(II), has an ionic radius (112 pm) very similar to Ca(II) – 106 pm [3]. This spectroscopic technique enables to measure the luminescence lifetime of Eu(III), which is proportional to the hydration number (number of H_2O molecules in the inner coordination sphere, n_{H_2O}) of the Eu(III) ion [4,5,6].

In a neutral and more basic medium the N3H nitrogen of the pyrimidine ring becomes accessible, owing to proton release, which may be followed potentiometrically. Up to now only some experiments on complexing reactions of nucleosides were described with Eu(III), indicating the coordination not only by phosphoric groups but also by the N3 nitrogen [7].

*Author for correspondence.

EXPERIMENTAL

Reagents: Uracil and thymine, both pure, were purchased from Sigma; 6-chloromethyluracil, 5-hydroxymethyluracil and 6-methyluracil, also pure, were obtained from Aldrich. The purity of the ligands was checked potentiometrically. The stock europium(III) perchlorate solution (3×10^{-2} mol dm^{-3}) was standardized complexometrically, using disodium salt of ethylenediaminetetraacetic acid in presence of murexide. Sodium hydroxide, Merck, 0.1 M solution was p.a. – prior to the titrations involved in calculations of formation constants. The content of carbonates was controlled by the Gran method [8], incorporating the data of EMF = f(–log [H^+]) acid – base calibrations. Potassium perchlorate (Merck) and perchloric acid, 60% (Apolda) were p.a.

Luminescence spectroscopy: The experiments were carried out in perchlorate solutions within a wide pH range (2–8) at various metal to ligand molar ratios. At higher pH the results were overlapped by hydrolysis. The total ligand concentration was 1.6×10^{-2} mol dm^{-3}. Various metal to ligand concentration ratios (from 1:1 up to 1:10) could be prepared by adding appropriate amounts of europium(III) perchlorate. pH was adjusted by 0.5 M $HClO_4$ and 0.1 M NaOH to *ca* 2.5; 5.5; 7; 9 and 11, respectively. The luminescence lifetimes of Eu^{3+} excited states were measured using a laser detection system, consisting of a nitrogen laser and a tunable dye laser, described earlier [9,10]. The Eu^{3+} ion in the samples was excited to the higher electronic level by a pulsed laser beam at the wavelength of 394 nm. Subsequently, the emission from the lowest luminescent level to the ground state manifold was measured to obtain the luminescence lifetime. The 394 nm laser beam of spectral width, $\Delta\lambda$ = 0.1 Å, was obtained by a pulsed 337 nm output of a nitrogen laser (KB6211, Cobrabid, Poznań), pumping a methanolic solution of the LD390 dye (Exciton Chemicals) in a tunable dye laser, in a biprism configuration, equipped with a computer controlled step motor. This allows a scanning of the dye laser emission wavelength through the whole dye emission range with a precision of 0.1 Å. The beam from the dye laser with a typical pulse energy ~10 μJ and a duration of 5 ns, passed through the sample cell. The samples were placed in a dark box and the experiments were conducted at room temperature. The emitted light of 617 nm by a sample was observed at 90° to direction of the exciting beam, collected on the entrance slit of monochromator (Triax180) and detected by a M12 FCV 51 photomultiplier. The scattered light was cut off using an interference filter. An electric signal from PM tube was fed into a transient recorder MC 101 (Mescomp) interfaced to a PC computer. The luminescence decay curves observed were fitted to single-exponential curves with correlation coefficients of 0.99–0.999.

Potentiometric studies: The total concentration of the metal in each sample (initial volume V_0 = 3 cm^3) amounted to 4.0×10^{-3} mol dm^{-3} and the ligand to metal ratios were 2:1 and 5:1. The ionic strength was adjusted to 0.1 mol dm^{-3} with $KClO_4$. The titrations were carried out in a double-walled thermostated vessel, starting from pH ~2 with a carbonate-free NaOH solution of known concentration (0.1 mol dm^{-3}). The value of pK_w = 13.41, resulting from our acid – base calibrations was lower by 0.36 than the one reported in [2,11] for the same ionic strength, but using KNO_3 medium.

The pH was measured with a Molspin Ltd (Newcastle upon Tyne, England) automatic titration set and a combined CMAWL/4/5/S7 electrode (Russell pH Limited, Auchtermuchty, Scotland). The total volume of the Hamilton microsyringe in the autoburette was 250 μl, the volume increments amounted to 0.0025 ml. The titrations were performed by using MOLSPIN.EXE software. The electrode was calibrated in the –log [H^+] scale by titration of a 0.005 M $HClO_4$ (adjusted to I = 0.1 M by $KClO_4$) with 0.1 M NaOH, temp. 25°C. The concentration overall stability constants β_{mlh} = $[M_m L_l H_h]/[M]^m[L]^l[H]^h$ were calculated by the SUPERQUAD computer program [12].

RESULTS AND DISCUSSION

Eu(III) luminescence spectroscopy: The exponential decay constants k_{H2O} of luminescence intensity from Eu(III) were used to calculate the hydration numbers n_{H_2O}, that is to evaluate the numbers of water molecules in the inner coordination sphere of Eu(III). According to [6,13], if there is no contribution from the ligand to

Table 1. Luminescence lifetimes of Eu(III) and hydration numbers in Eu(III) – pyrimidine base aqueous solution at various M:L molar ratio. $C_L = 1.6\times10^{-2}$ mol dm^{-3}. $I = 0.1$ ($KClO_4$). Temp. 25°C.

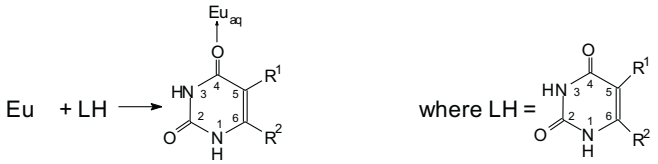
1:1			1:2			1:4			1:5			1:10		
pH	$\tau[\mu s]$	n_{H_2O}	pH	$\tau[\mu s]$	n_{H_2O}	pH	$\tau[\mu s]$	n_{H_2O}	pH	$\tau[\mu s]$	n_{H_2O}	pH	$\tau[\mu s]$	n_{H_2O}
uracil														
2.63	121.2	8.0	2.74	121.9	7.9	2.75	127.4	7.5	2.74	130.5	7.3	2.74	143.1	6.6
5.50	119.6	8.1	5.50	121.1	8.0	5.25	127.2	7.6	5.32	133.2	7.2	5.70	145.3	6.5
thymine														
2.64	124.9	7.7	2.64	125.3	7.7	2.60	134.8	7.1	2.58	140.1	6.8	2.66	148.7	6.4
5.48	123.6	7.8	5.58	126.9	7.6	5.50	138.2	6.9	5.54	140.4	6.8	5.63	152.9	6.2
7.25	125.2	7.7	7.24	127.4	7.5	7.30	136.7	7.0	7.38	141.8	6.7	7.17	151.6	6.2
6-chloromethyluracil														
2.60	133.6	7.1	2.57	135.4	7.1	2.56	139.8	6.8	2.56	142.5	6.7	2.54	146.7	6.5
5.65	133.9	7.1	5.66	135.0	7.1	5.72	139.7	6.8	5.74	142.4	6.7	5.80	144.2	6.6
6.86	140.0	6.8	6.87	140.2	6.8	7.00	139.9	6.8	6.91	138.8	6.8	6.97	139.5	6.8
5-hydroxomethyluracil														
2.63	142.8	6.8	2.61	145.5	6.5	2.59	146.5	6.5	2.58	148.2	6.4	2.58	152.4	6.2
5.46	141.6	6.7	5.51	145.5	6.5	5.56	147.9	6.4	5.55	152.2	6.2	5.54	158.5	5.9
6-methyluracil														
2.54	130.8	7.3	2.58	133.4	7.2	2.62	144.9	6.5	2.63	148.5	6.4	2.60	152.6	6.2
5.49	130.7	7.3	5.59	137.6	6.9	5.58	144.5	6.6	5.54	149.2	6.3	5.49	153.3	6.1
6.82	133.2	7.2	6.83	140.6	6.8	6.86	149.0	6.3	6.79	149.1	6.3	6.83	154.5	6.1

the de-excitation of the luminescent excited state, the hydration number follows from $n_{H_2O} = 1.05\, k_{H_2O} - 0.70$, where k_{H_2O} is the reciprocal lifetime, τ^{-1} (ms^{-1}). The inherent uncertainty of the luminescent method is about ±0.5 water molecule. On the other hand, k for uncomplexed $Eu(III)_{aq}$ in $Eu(ClO_4)_3$ solution was measured to be 9.00 ± 0.05 ms^{-1} [13]. Thus, the hydration number amounts to *ca* 8.75. The luminescence lifetimes and hydration numbers are listed in Table 1.

The n_{H_2O} values were almost independent on pH for all the ligands, but the lowering of hydration numbers with the rise in L:M is clearly visible. Starting already from pH ≈ 2.5, a decrease in 0.7 up to 2.8 units has been observed. At pH lower than 8, the solutions became turbid (opalizing), due to the hydrolysis of $Eu(III)_{aq}$. Europium(III) occurs in aqueous solution mainly as a nanoaquo-ion: $Eu(H_2O)_9^{3+}$. Hence, the decrease in hydration number may be interpreted in terms of consecutive substitution of water molecules by one of the uracil functions, thus

$$Eu(H_2O)_9^{3+} + nHL \rightarrow Eu(HL)_n(H_2O)_{9-n}^{3+} + nH_2O \qquad (1)$$

According to Table 1 and the uncertainity of the luminescent method, it may be concluded that complexes with $n = 1$ predominate at low ligand excess, whereas at higher L:M the species with $n = 2$ are the most abundant. As it follows, *e.g.* from the data for 5-hydroxymethyluracil, even the species with 3 uracil ligands should not be neglected in the equilibrium mixture. As the log of N3 protonation constants in uracil derivatives are of order 9, under acidic conditions a deprotonation of the N3H site is impeded. Hence, the available binding sites in the pyrimidine derivatives are restricted to carbonyl oxygens – most probably O4, as it follows from calculations [1]. For instance the first step of reaction (1) may be shown as in Scheme 1, thus, explaining the lack of dependence on pH.



EuLH

Scheme 1. $R^1 = H$; $R^2 = H$: uracil (2,4 dihydroxypyrimidine)
$R^1 = Me$; $R^2 = H$: thymine (2,4 dihydroxy-5-methylpyrymidine)
$R^1 = H$; $R^2 = CH_2Cl$: 6-chloromethyluracil
$R^1 = CH_2OH$; $R^2 = H$: 5-hydroxymethyluracil
$R^1 = H$; $R^2 = Me$: 6-methyluracil

On the other hand, as the N3 nitrogens become accessible at higher pH, the coordination mode may change towards the new donor function. Moreover, it may be predicted that the observed decrease in number of water molecules in the inner coordination sphere could also follow a deprotonation to at least mono-hydroxo complexes.

Potentiometric data: The equilibrium model under discussion consisted of the following complexing reactions:

$$M + L \rightleftarrows ML \qquad \beta_{110} = [ML][M]^{-1}[L]^{-1}$$

$$M + 2L \rightleftarrows ML_2 \qquad \beta_{120} = [ML_2][M]^{-1}[L]^{-2}$$

$$M + L + H \rightleftarrows MLH \qquad \beta_{111} = [MLH][M]^{-1}[L]^{-1}[H]^{-1}$$

$$M + L \rightleftarrows MLH_{-1} + H \qquad \beta_{11-1} = [MLH_{-1}][H][M]^{-1}[L]^{-1}$$

$$2M + 2L \rightleftarrows M_2L_2 \qquad \beta_{220} = [M_2L_2][L]^2[M]^2$$

$$M \rightleftarrows MH_{-1} + H \qquad \beta_{10-1} = [MH_{-1}][H][M]^{-1}$$

The formation constant of the Eu(III) hydroxo aquo-complex in the last equilibrium was described by literature data under similar conditions [14]: $\log\beta_{10-1} = -8.31$, temp. 25°C; $I = 0.3$; $NaClO_4$. Protonation constants were determined here under conditions close to those in [2], differing only in the electrolyte ($KClO_4$ instead of KNO_3).

As it follows from Table 2, the 110 species were probable in three cases, *i.e*. thymine, 6-chloromethyluracil and 6-methyluracil (an exemplary distribution diagram in Figure 1).

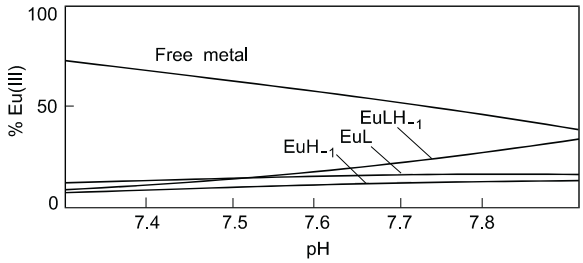


Figure 1. Species distribution diagram for the Eu(III)–thymine system. $C_{Eu} = 0.004$ mol dm^{-3}, $C_{ligand} = 0.008$ mol dm^{-3}; $\sigma = 2.61$, $\chi^2 = 6.00$.

The 11-1 hydroxo-complex, however, was confirmed for every ligand, just as it was already the case for some other metals [2,15]. On the contrary, in spite of starting the titrations from low pH, the protonated 111 complex, corresponding to the first one justified by luminescence spectroscopy, could not be evidenced by potentiometry – due to low fidelity of the glass electrode under acidic conditions. Hence, instead of interactions *via* O4, within pH 6–8, *i.e*. just below the range of Eu(III) hydroxide precipitation, an alternative coordination mode was taken into consideration (Scheme 2) as a result of the growing share of the N3H deprotonated ligand, $L = L^-$.

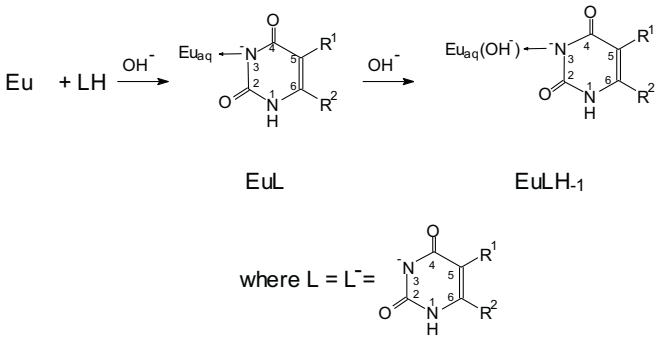
The values of deprotonation constants $pK^{ML}_{MLH_{-1}}$ ranged within 7–7.5, that is to say they were only one order of magnitude lower than the hydroxo aqua-ion formation constant. Hence, the titrations could not be carried out at higher pH, the formation of consecutive, *e.g*. ML_2, complexes was not confirmed. For two of the ligands (uracil

and 5-hydroxomethyluracil) even the share of the ML species was too low to be detected by the fitting procedure (also at a high excess of the ligand).

Table 2. Refined formation constants in the Eu(III)–ligand-proton system. Solvent: H_2O; I = 0.1 ($KClO_4$); temp. 25°C. $C_{Eu} = 4.0\times10^{-3}$ mol dm^{-3}, $C_L = 8.0\times10^{-3}$ as well as 2.0×10^{-2} mol dm^{-3}. Standard deviations in parentheses.

Ligand	Number of points[a)]	$\log\beta_{110}$	$\log\beta_{11-1}$	$pK^{ML}_{MLH_{-1}}$ [b)]	χ^2 [c)] σ
uracil	43	–	–4.14(1)	–	5.85 3.92
thymine	43	3.37(14)	–4.15(4)	7.52	3.60 4.20
6-chloromethyluracil	33	2.95(6)	–4.25(4)	7.20	9.42 5.96
5-hydroxomethyluracil	44	–	–2.79(5)	–	10.55 5.34
6-methyluracil	46	3.33(16)	–3.80(4)	7.13	5.83 4.72

[a)]Comprehensive files used in refinements of formation constants consisted of points from 5 single titrations (limitation of SUPERQUAD). [b)] $K^{ML}_{MLH_{-1}} = [MLH_{-1}][H][ML]^{-1}$, $pK^{ML}_{MLH_{-1}} = \log\beta_{110} - \log\beta_{11-1}$. [c)]The statistical fitting parameters should be lower than: $\chi^2 < 12.60$ at significance level 0.05, $\sigma < 3$. In the case of weak complexes compliance with both requirements is hardly attainable.



Scheme 2. R^1 = H; R^2 = H : uracil (2,4 dihydroxypyrimidine)
R^1 = Me; R^2 = H : thymine (2,4 dihydroxy-5-methylpyrymidine)
R^1 = H; R^2 = CH_2Cl : 6-chloromethyluracil
R^1 = CH_2OH; R^2 = H : 5-hydroxymethyluracil
R^1 = H; R^2 = Me : 6-methyluracil

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