# Chemiluminescent Oxidation Reaction of $Eu^{2+}$ Ions with $H_2O_2$

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A series of chemiluminescence (CL) investigations of rare earth ions in redox reactions has been initiated. The study of oxidation reactions of $Eu^{2+}$ – uncomplexed and complexed by ethylenediaminetetraacetic acid (EDTA) – with $H_2O_2$ has revealed a distinct CL of $Eu^{3+}$ with two-phasic kinetics, controlled by pH and the concentration of $H_2O_2$.

## Introduction

Lanthanide ions, mainly europium and terbium ions, are used as luminescent probes in structural investigations of biological systems [1–4]. Less numerous, however, are studies concerning chemiluminescence (CL) involving lanthanide ions [5–8] and connected with biological molecules [9, 10]. In this paper investigations on a chemiluminescent system containing $Eu^{2+}$ ions and $H_2O_2$ are presented.

## Experimental

The experiments were carried out with systems containing $Eu^{2+}$ ions, uncomplexed or complexed by ethylenediaminetetraacetic acid (EDTA) in a phosphate buffer solution of different pH values. Because of the hydrolysis of $EuCl_2$ and $EuCl_3$, in the uncomplexed systems the pH value was limited to $pH \leqq 5.3$. In the complexed systems we used the range $3 < pH < 8.5$. $Eu^{2+}$ was obtained by reducing $Eu^{3+}$ with McCoy's method [11].

Immediately after the reduction of $Eu^{3+}$ the $Eu^{2+}$ was complexed with EDTA.

The chemiluminescence begins when the $Eu^{2+}$ solution is put into a cell containing $H_2O_2$ and the phosphate buffer solution. The temperature was 295 K and inert gas passed the cell to provide anaerobic conditions during the whole reaction time.

The spectral distribution of the CL was measured combining the single-photon counting and the cut-off filter method [12]. An EMI 9558QB photomultiplier, sensitive in the range 180–800 nm, was used. Details of the method are given elsewhere [13].

## Results and Discussion

In the systems with uncomplexed $Eu^{2+}$ ions, at pH = 1.5 or 5.3, immediately after the mixing of the $Eu^{2+}$ solution with $H_2O_2$ a weak short lived CL appears (duration time about 45 s). The decay time of the CL is comparable with the oxidation reaction time.

The integral intensities and decay times of this short lived CL of uncomplexed solutions with different $Eu^{2+}$ and $H_2O_2$ concentrations are shown in Table 1. The CL intensity increases with growing $Eu^{2+}$ concentration whereas the decay time decreases with growing $H_2O_2$ concentration.

Table 1. Integral intensity $\sum I \Delta t$ and half-decay time $T_{1/2}$, s (in brackets), of a short-lived chemiluminescence in the oxidation reaction of uncomplexed $Eu^{2+}$ with $H_2O_2$ under various conditions.

| $[H_2O_2]$ mmole/dm$^3$ / $[Eu^{2+}]$ mmole/dm$^3$ | 20 | 40 | 60 |
|---|---|---|---|
| 4 | 0.15 (8) *<br>0.75 (8) | | |
| 10 | 0.55 (8) * | | 1.0 (3) * |
| 20 | 1.9 (6) *<br>2.7 (9) | | 2.0 (3) * |
| 40 | | 4.0 (5.5) * | 4.4 (4) |
| 60 | | 5.2 (5) *<br>5.9 (7.5) | 5.6 (4) |

* Results obtained at pH = 1.5; without asterisk at pH = 5.3.

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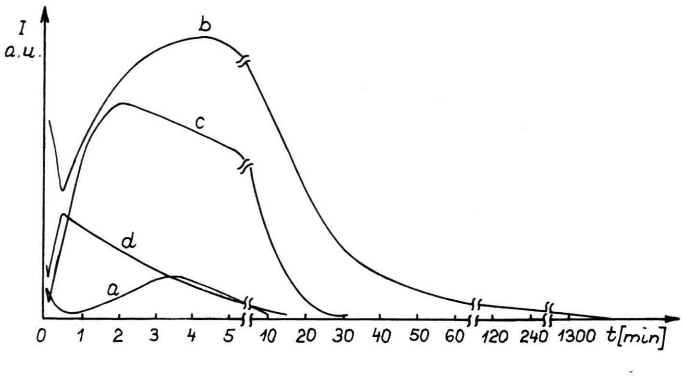

Fig. 1. Total chemiluminescence (CL) intensity as a function of time during oxidation of $Eu^{2+}$ by $H_2O_2$: a) uncomplexed $Eu^{2+}$ (16 mmole/dm$^3$) and $H_2O_2$ (20 mmole/dm$^3$), pH = 5.3 (the intensity of CL multiplied $\times 10^2$); b) complexed $Eu^{2+}$ (4 mmole/dm$^3$) by EDTA (40 mmole/dm$^3$), $H_2O_2$ (20 mmole/dm$^3$) and phosphate buffer (30 mmole/dm$^3$), pH = 7.5; c) complexed $Eu^{2+}$ (4 mmole/dm$^3$) by EDTA (40 mmole/dm$^3$), $H_2O_2$ (20 mmole/dm$^3$) and phosphate buffer (30 mmole/dm$^3$) and additionally 1% n-butanol, pH = 7.5; d) complexed $Eu^{2+}$ (4 mmole/dm$^3$) by EDTA (40 mmole/dm$^3$) and phosphate buffer (30 mmole/dm$^3$) in the presence of $O_2$, pH = 7.5.

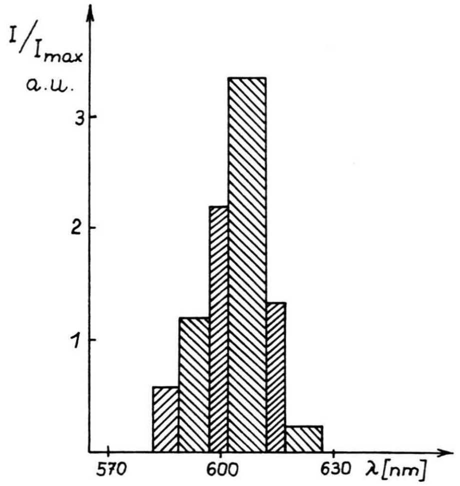

Fig. 2. Spectral distribution of $Eu^{3+}$ chemiluminescence generated in the oxidation of $Eu^{2+}$ by $H_2O_2$. The reaction mixture contains: $Eu^{2+}$ (4 mmole/dm$^3$), EDTA (40 mmole/dm$^3$), $H_2O_2$ (20 mmole/dm$^3$) and phosphate buffer (30 mmole/dm$^3$), pH = 7.5.

In solutions of pH 5 and at an excess of $H_2O_2$, 1 min after the start of the reaction also a weak long lived emission appears, which decays during about 10 min (Figure 1a).

In solutions of $Eu^{2+}$ complexed with EDTA, at pH = 3 the short lived CL appears immediately after the mixing with $H_2O_2$, just as before.

At higher pH values (5–8.5) also a long lived component occurs, analogous to the uncomplexed solutions, but with much higher intensity and much longer decay time (Figure 1b).

The spectral distribution of the CL of the complexed system is shown in Figure 2. The strong maximum at about 610 nm indicates that the observed CL is emitted by the $Eu^{3+}$ ions.

The short lived CL observed in the case of uncomplexed and complexed $Eu^{2+}$ systems evidently originates from the two following reactions giving excited $Eu^{3+}$ ions:

$$Eu^{2+} + H_2O_2 \rightarrow Eu^{3+} + OH^- + OH^{\bullet}, \qquad (1)$$

$$OH^{\bullet} + Eu^{2+} \rightarrow Eu^{3+} + OH^-. \qquad (2)$$

Reaction (1) is analogous to the Fenton reaction [14] as a source of $OH^{\bullet}$ radicals. Reaction (1) has 1.6 eV of free enthalpy ($\Delta G°$). The $\Delta G°$ value of reaction (2) is 2.6 eV. The $\Delta G°$ values are calculated from the redox potentials of the radical reactions given for pH = 0 [15] and from the $E^0$ value −0.43 V for $Eu^{3+}/Eu^{2+}$ [16]. Taking into account the CL spectrum as well as the calculated values of $\Delta G^0$, one may conclude that the excitation of $Eu^{3+}$ to the luminescent level $^5D_0$ (2.10 eV) is possible. In the redox system of $Ru^{2+}/Ru^{3+}$ with 2,2′-bipyridine, the excited product was identified as a metal ($Ru^{2+}$) – to ligand charge-transfer triplet [17].

The long lived CL is a result of consecutive reactions initiated by the $OH^{\bullet}$ free radicals. This ascertainment is justified by the observed dependence of the intensity and the decay time of the CL on the added n-butanol as a scavenger of $OH^{\bullet}$ radicals (Figure 1c). When the complexed system was not deaerated, the intensity and the decay time of the CL were markedly decreased. This may be connected with the direct oxidation of $Eu^{2+}$ ions by atmospheric oxygen and by oxygen from the decomposition of $H_2O_2$ (Figure 1d). The mechanism of the long lived CL, including also the role of $OH^{\bullet}$ radicals, is at present under study.

The strong dependence of the CL intensity on the experimental conditions observed in this work indicates the applicability of lanthanides in biological investigations. In the studies which have been carried out till now, $Eu^{3+}$ ions have served as acceptors of electronic excitation energy generated chemically and/or biochemically [9, 10].

The results of this work show that the europium ions may be excited in the oxidation reaction with the participation of free radicals.

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