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The Europium(III) Ion as Spectroscopic Probe in Bioinorganic Chemistry*

JEAN-CLAUDE G. BÜNZLI

Institut de chimie minérale et analytique, Université de Lausanne, Place du Château 3, CH-1005 Lausanne, Switzerland

Abstract

The Eu(III) ion easily substitutes other metal ions in materials of biological importance. It is particularly well suited for the replacement of divalent calcium ions. Both Ca(II) and Eu(III) are spherical ions with non-directional chemical binding; their ionic radius and hydration numbers are similar. The chemical and structural properties of the calcium ion sites may therefore be probed by analysing the spectroscopic response of the Eu(III) ions imbedded in these sites.

In particular, the Eu(III) ion may be used as a sensitive luminescent probe. It displays an intense luminescence from a long-lived excited state, $^5D_0$, which along with its ground-state $^7F_0$, is not split by ligand field effects. High resolution, laser-excited excitation and emission spectra allow the following information to be extracted from a detailed group-theoretical analysis: (i) the number of chemically different metal-ion sites in the material; (ii) the total formal charge of the ligating groups directly bonded onto the Eu(III) ion; and (iii) the symmetry of these sites. Moreover, energy transfer experiments can be performed to determine the distance between two metal-ion sites or between an organic chromophore and a metal-ion site.

The paper is divided into three sections. The first deals with the chemical and spectroscopic properties of the Eu(III) ion. In the second part, the sensitivity of the luminescence method is illustrated, describing a probe experiment on model compounds, *i.e.* crown ether complexes. Finally, the investigation of the Ca(II) metal sites of proteins is presented and discussed.

Introduction

The trivalent lanthanide ions have very specific spectroscopic and magnetic properties which make them ideal as probes in studies of biological systems [1]. This is particularly true in the investigation of metal-containing macromolecules in which the metal ion is spectroscopically silent, e.g. Ca(II), Mg(II), or Zn(II). Lanthanide probes are provided for solving analytical problems (trace analysis procedures for biological material, with the help of macrocyclic ligands [2]) or for gaining insight into the structure of metal-ion sites. We focus this paper on the latter aspect, especially in the case of calcium-containing material.

The use of Ln(III) or, more specifically Eu(III), as a replacement probe for Ca(II) is made possible by the analogy between the chemical and physico-chemical properties of these two ions:

(i) Both ions are spherical with approximately the same radius, *ca.* 1.1 Å for a coordination number of 8 to 9.

(ii) Both are hard cations with a preference for O- and N-donors, with essentially non-directional and ionic bonding.

(iii) Their aqua ions have the same coordination number (9–10 for Ca(II) [3] and probably 9 for Eu(III) [4]) and are kinetically highly labile.

Most importantly, the replacement of Ca(II) by Ln(III) ions does not perturb the biological system too much [2, 5], although there might be some exceptions [6].

Eu(III) Ions as Luminescent Probe

The electronic configuration of the Eu(III) ion is $4f^6$. It is 3003 times degenerate. The interelectronic repulsion and the spin-orbit coupling generate 119 spectroscopic terms and 295 spectroscopic levels. Upon insertion of the Eu(III) ion into a molecular structure, the ligand-field potential further lifts the degeneracy of the levels into a number of Stark or J sublevels which depend upon the local symmetry of the ion. The ground state is $^7F_0$ and there are long-lived luminescent excited states such as $^5D_0$ and $^5D_1$ (*cf.* Fig. 1). Transitions between the sublevels are in principle forbidden by an electric dipole mechanism. However, mixing of vibronic or charge-transfer states in the electronic states, as well as the J-mixing arising from the spin-orbit coupling, make the wavefunctions composite so that the selection rules do not apply strictly. The transitions may be observed with a very

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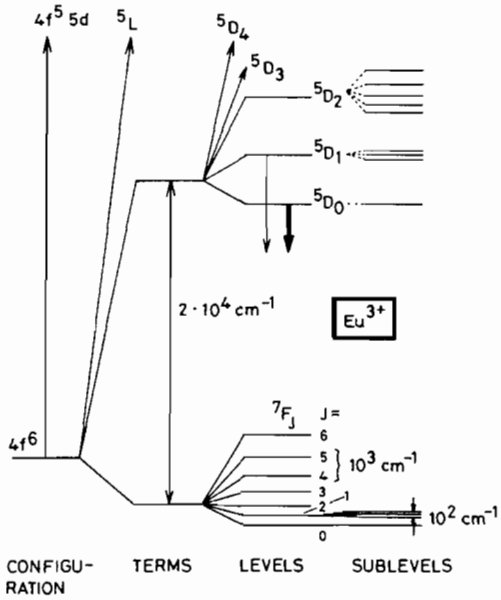

Fig. 1. Part of the energy diagram for the Eu(III) ion.

weak intensity, comparable to that of the allowed magnetic dipole transitions. Since the 4f orbitals are shielded by the $5s^2\ 5p^6$ sub-shell, all these transitions appear as narrow bands and their energy is not much affected by the chemical environment of the ion. In order to have some intensity however, a transition between two given sublevels must obey the symmetry-related selection rule: the irreducible representation of the dipolar operator must be contained in the product of the irreducible representations of the wavefunctions for the initial and final states.

In view of the weakness of the transitions, it is best to use sensitive luminescent techniques, rather than absorption measurements. The Eu(III) ion may be selectively excited by tuning a dye laser to the energy of the $^5D_0 \leftarrow {}^7F_0$ transition. This transition occurs around 580 nm, that is in a spectral range in which the biological materials are usually transparent. The following information may be gathered by means of luminescent experiments.

Number of Metal-ion Sites

By luminescent titration

Here the total Eu(III) luminescence is monitored *versus* the amount of metal ion added to the biological material. For this type of experiment, Tb(III) is often used and the excitation of the lanthanide ion may take place through energy transfer from chromophoric groups (e.g. tryptophan or tyrosine) that absorb in the UV.

By monitoring the $^5D_0 \leftarrow {}^7F_0$ transition under high resolution (<0.1 Å, excitation spectroscopy)

Indeed, neither the initial nor the final states can be split by ligand-field effects since $J = 0$ for both states. One specific chemical environment therefore generates only one 0–0 transition. Some care has to be exercised. When the local symmetry is high or when an inversion center is present, the 0–0 transition is forbidden by the symmetry-related selection rule; the transition is nevertheless sometimes observed, but is extremely faint. Such a situation rarely prevails in biological molecules, the local symmetry of the metal sites being usually quite low. Another difficulty arises when two (or several) metal ion sites generate 0–0 transitions too close in energy to be resolved into distinct components.

Sum of the Ligand Formal Charges

The nephelauxetic effect [7] shifts the energy of the 0–0 transition according to the following experimental equation [8]:

$$\nu\,(\mathrm{cm}^{-1}) = 17\,273 + 2.29q - 0.76q^2$$

in which q is the sum of the formal charges of the ligands bonded to the metal ion. This relationship is accurate to only ±1 unit of charge and must be used with care. It provides, however, useful information for the assignment of the 0–0 transition to a given chemical environment.

Site Symmetry of the Metal-ion Sites

The local symmetry of each of the metal-ion sites is determined by analysis of the $^5D_0 \rightarrow {}^7F_J$ transitions according to group-theoretical principles, after selective excitation of each site. There may be complications arising from vibronic transitions, from the interaction between the phonon density of states and electronic sublevels [9–11], and from energy migration from one site to another one. In this latter situation, time-resolved spectroscopy might be needed.

The presence of an inversion center is particularly easy to recognize since in this case magnetic dipole transitions only are allowed and the emission spectrum is dominated by the $^5D_0 \rightarrow {}^7F_1$ transition.

Number of Bonded Water Molecules

High energy vibrations, e.g. O–H or N–H stretches, provide an efficient pathway for the radiationless de-excitation of Ln(III) ions [12]. Comparison between the lifetime τ of the excited $^5D_0$ state in the presence of H_2O and D_2O allows the determination of the number n of water molecules directly bonded onto the Eu(III) ion, after suitable calibration [13]:

$$n = 1.05\,[1/\tau(\mathrm{H_2O}) - 1/\tau(\mathrm{D_2O})]$$

A similar relationship may also be established

for Tb(III) and for other quenchers, e.g. NO_3^- [14].

Distances Between Two Metal-ion Sites

If two metal sites are populated by two different and properly chosen Ln(III) ions, part of the excitation energy of one ion is transferred to the other ion, resulting in a decrease of the life-time of the excited state. The yield of the energy transfer η is related to both the lifetimes of the donor ion in the absence (τ_0) and in the presence (τ) of the acceptor, and the distance r between the donor and the acceptor:

$$\eta = 1 - \tau/\tau_0 = 1/(1 + r^6/R_0^6),$$

where R_0 is the critical distance for 50% transfer; R_0 depends upon an orientation factor, the quantum yield of the donor, the refractive index, and the overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor. For an application to a calcium-containing protein, calmodulin, see ref. 15.

Applications

An example of the sensitivity of the Eu(III) luminescent probe is provided by the study of nitrato complexes with the ionophore 18-crown-6 ether (18C6). Their general formula is $[Ln(NO_3)_2(18C6)]_3[Ln(NO_3)_6]$ [16] and it was shown [17] that the Nd complex contains three different complex cations, one with local C_{2h} symmetry and the other ones with C_s symmetry, while the hexakis(nitrato) anion possesses a C_{2h} local symmetry. The Eu complex has been probed at low temperature and the 0–0 transition displays three main components. The high-resolution study of both polycrystalline samples and of monocrystals of the Eu-doped Gd complex reveals that each metal site generates a series of very similar spectra arising from molecules experiencing slightly different ligand-field effects, that is having slightly different conformations [18].

The use of Eu(III) as a replacement probe for Ca(II) is described in the study of the metal sites of bovine α-lactalbumine (BLA) [19]. This protein, of molecular weight 14 200, belongs to the lactose synthetase enzymatic complex. Luminescent titration (Fig. 2) points to the binding of more than two lanthanide ions per molecule of protein. High-resolution excitation spectra of the 0–0 transition (Fig. 3) indicate the population of at least three metal-ion sites. Two of these sites (0–0 transition at 17 254 and 17 256 cm^{-1}) seem to be relatively well defined within the protein. The third one (0–0 transition at 17 267 cm^{-1}, width at half height: 14 cm^{-1}) is less well defined and its population is extremely pH-dependent. It could correspond to the

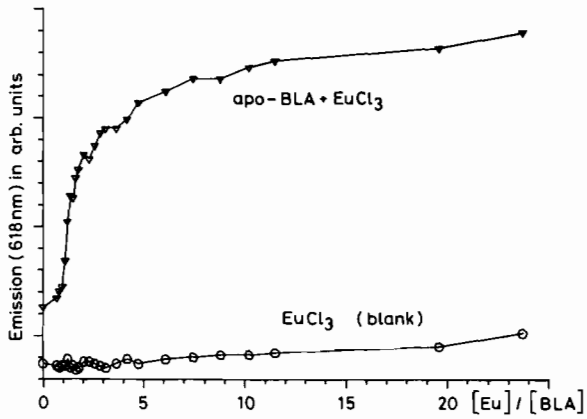

Fig. 2. Luminescent titration of apo-BLA by $EuCl_3$: [BLA] = 4×10^{-6} M in D_2O, pH = 6.3 (buffer: TRIS). Top curve: titration; bottom curve: blank (Eu alone).

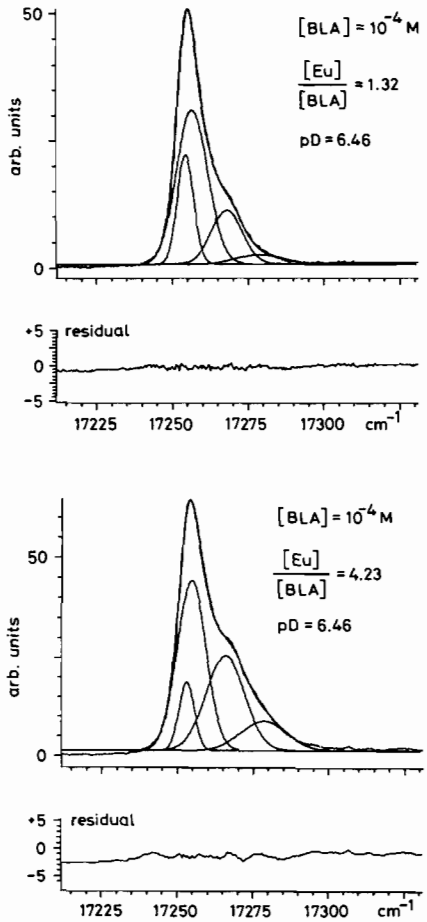

Fig. 3. Excitation spectra (0–0 transitions) of solutions containing BLA and $EuCl_3$ in D_2O: [BLA] = 9×10^{-5} M, pH = 6.05 (buffer: TRIS), KCl 0.01 M. The spectra have been resolved by means of Gaussian curves; the residual is shown at the bottom.

fixation of the metal ion on the outskirts of the protein. Possible assignments to potential metal-ion sites within BLA will be discussed in a subsequent paper.

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