SPECIATION OF RADIONUCLIDES WITH BIOLIGANDS USING TIME-RESOLVED LASER-INDUCED FLUORESCENCE (TRLIF) AND ELECTROSPRAY MASS SPECTROMETRY (ES-MS)

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1 INTRODUCTION

There is still a great interest concerning environmental and biochemical behaviour of radionuclides, in particular with respect to nuclear waste repository and to their removal (especially actinides) from contaminated workers. Nevertheless, studies on radionuclides complexation and uptake mechanisms at the cellular and molecular level are very scarce. Consequently, the chelating properties of these elements in biological media are not fully understood. As a result, this work was directed toward the study of the interactions between several bioligands of interest and the following radionuclides : uranium(VI) and several lanthanides(III), the latter being chosen as analogues of trivalent actinides.

A literature survey has been carried out upon several sequestering agents of heavy metals, present at the cellular scale as well as in the cytoplasm to select the most biologically relevant. This research revealed important discrepancies among the few existing complexation constants and lead us to the choice of the following bioligands : amino acids (especially those constitutive of the binding sites of proteins) and some oligopeptides such as N-2-mercaptopropionyl glycine (MPG).

The selected systems were investigated by two major analytical techniques, namely electrospray mass spectrometry (ES-MS) as well as time-resolved laser-induced fluorescence (TRLIF). These two techniques are very powerful for such studies since, aside their speciation capabilities, it is also possible to evaluate interaction constants as well as entropy and enthalpy by experiments (for TRLIF) with temperature. Moreover, these data acquisition can contribute to the improvement of thermodynamical data banks.

This work is intended to quantify the interactions between uranyl and rare earths and previously quoted bioligands as a function of temperature (only for TRLIF) and pH, at negligible and biological ionic strength. In this paper, only results between europium and cysteine at pH 3 and negligible ionic strength will be developed[1].

2 INTERACTIONS BETWEEN EUROPIUM AND CYSTEINE

The ligand, cysteine, is a thiol-containing amino acid. The complex chemical significance of cysteine is determined by the mercaptosulfur donor atom, which is known to have a soft character. Via the mercapto group, this compound may thus participate in both redox and acid-base reactions. That is why cysteine is often constitutive of the binding sites of proteins and why such a great biological importance is attached to the study of its interaction with metals. Indeed, cysteine especially has the capacity to form stable complexes with heavy metals and proteins having a heavy metal-scavenging role are mainly cysteine-rich protein such as metallothioneins or phytochelatins in which up to one third of all amino acids are cysteine residue[2]. As a consequence, studying interactions between radionuclides ans cysteine is of prime importance.

Europium, as an analogue of trivalent actinides, was chosen not only because of its fluorescence properties but also for its isotopic pattern, easily recognizable with ES-MS. Cysteine contains three dissociable protons, with macroscopic protonation constants[3] $pK_{a\text{-}COOH} = 1.91$, $pK_{a\text{-}SH} = 8.14$ and $pK_{a\text{-}NH3^+} = 10.28$.

The coordination chemistry of trivalent lanthanides as a group is similar to that of calcium or strontium, and more dependent upon electrostatic interactions and ionic size than upon electronic interactions. Thus, the strongest binding site on the cysteine ligand is the carboxylate group. The amino group even in the deprotonated form, is not expected to interact strongly with the metal ion.

The complexation equilibrium reaction and the global mononuclear complexation constants ßi are defined in Equation (1).

$$Eu^{3+} + i\,(H_2Cys)^0 \xleftrightarrow{\beta_i} Eu(H_2Cys)_i^{3+} \qquad \text{with} \qquad \beta_i = \frac{[Eu(H_2Cys)_i^{3+}]}{[Eu^{3+}].[(H_2Cys)^0]^i} \qquad (1)$$

3 EXPERIMENTAL

All chemicals used were reagent grade and Millipore deionised water (Alpha-Q, 18.2 MΩ cm) was used troughout the procedure. The mass spectrometric measurements were recorded in positive ion mode using a Quattro II tandem quadrupole mass spectrometer (Micromass, Manchester, England) equipped with an electrospray ionization source. The resolution of the quadrupole mass selector easily allows the observation of the isotopic mass distribution of Eu (i.e. ~48% ^{151}Eu and ~52% ^{153}Eu). TRLIF recordings were carried out using our "FLUO 2001" experimental set-up that has been described elsewhere[4]. The excitation wavelength of the laser (266 nm quadrupled Brilliant Nd-YAG laser, coupled to an optical parametric oscillator system from Quantel, France) was tuned to 395 nm.

4 RESULTS AND DISCUSSION

4.1 TRLIF results

The measurements of the variations of europium emission transition at 593 nm and the hypersensitive one at 618 nm, enabled the determination of the fluorescence lifetime, the complex stoichiometry and the complexation constant by using non linear regression.

The complexation constant value found is consistent with that of calcium, whose charge/ionic radius ratio is comparable to that of trivalent europium[5].

4.2 ES-MS results

It has previously been observed that the ion abundance profile measured in ES-MS experiments is in good agreement with the aqueous solution speciation[6-8].

To reach Eu speciation from mass spectrometry experiments, europium species have been divided into two categories: complexed Eu species (with the ligand) and "free" Eu species (solvated europium and inorganic complex (hydroxo and nitrate)). Relative amounts of complexed ligand can be measured by assuming equal signal responses for charged complexes containing the same ligand and metal (as already observed for other similar systems investigated by ES-MS) as well as for inorganic and organic species.

Aside species linked to "free" europium, the main complexes observed are presented in Table 1.

Table 1 *ES-MS main peaks identification*

Type	m/z	Species
(1:1) complex	365-7	$Eu(H_2Cys)(NO_3)(MeO)^+$
(1:2) complex	454-6	$Eu(HCys)_2(HNO_3)^+$

Quantisation of the "free" Eu species was based on a calibration curve whose linear interpolation correlation coefficient R^2 was 0.997. The linearity range of the curve was $[Eu]_{free} = 1.10^{-5}M - 2.10^{-4}M$. Thanks to peaks identification and to quantisation of the "free" Eu, it was possible to determine the formation constants of each complex. The complexation constant value found is in agreement with the one obtained by TRLIF.

5 CONCLUSION

This study of the europium-cysteine system illustrates the complementarity of the two techniques used (TRLIF and ES-MS) for stoichiometry and complexation constants determinations, especially at very low metal concentration.

Work is under progress for measuring complexation constants at higher pH.

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