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Complexation of Th (IV) with the siderophore pyoverdine A

M. Bouby, I. Billard*, J. MacCordick

IReS, CNRS/IN2P3-ULP, Chimie Nucléaire, Bât 35, B.P. 28, 67037 Strasbourg Cedex 2, France

Abstract

The complexation of thorium (IV) ions in aqueous solutions by pyoverdine A has been studied. On the basis of a simple model, the thermodynamical equilibrium constant was determined and the value $K_{\mathrm{Th}}=(0.15\pm0.03)$ M was compared with those previously obtained with uranyl ions. However, it is shown that the set of data obtained does not give definite preference to this model with respect to another that involves complexation with 2+ charged thorium ions.

Keywords: Thorium; Siderophore; Complexation; Equilibrium constant

1. Introduction

A more extensive knowledge of the interactions which occur between radionuclides and the biosphere is necessary in order to predict the possible migration of these elements. Following our previous work with uranyl ions [1], we present here new results on the complexation of thorium ions by the siderophore pyoverdine A, a molecule extracted from a widespread bacterium (*Pseudomonas aeruginosa*). Because of the possible hydrolysis of the cation, special care was taken to prepare the thorium solutions. These experiments in aqueous solution were conducted using common spectrophotometric methods. Data analysis consisted of a first approach by a graphical method and a comprehensive model that included the effect of ionic strength.

2. Materials and methods

2.1. Solution preparations

2.1.1. Pyoverdine solution

Pyoverdine A (succinic form) was obtained by growth of *Pseudomonas aeruginosa* ATCC 15692 strain PAO1, in a chemically defined medium. Solvent extraction and purification were carried out according to described procedures [2,3]. The concentration of the solution, $C=1.6\times10^{-4}$ M, was controlled by titration with a freshly prepared solution of iron (III) (Merck–Schuchardt, iron (III) chloride, 98%) [2,3]. The pH of the solution was 3.3.

2.1.2. Thorium (IV) solution

Thorium (IV) triflate was prepared by reaction of trifluoromethane sulfonic acid (Aldrich®, 99+%) with [$Th(NO_3)_4$, 5 H_2O] (Merck) in stoichiometric proportions [4]. The content of thorium, as well as the Th:F ratio in the salt [$Th(CF_3SO_3)_4$, 2 CF_3SO_3H] were verified by neutron activation analysis (NAA) and the crystalline state checked by X-ray powder diffraction. The synthesized thorium triflate salt was stored in solid form until the beginning of the experiments. To prevent hydrolysis, an acid medium was prepared by adding perchloric acid (Acros, PA) to freshly distilled ultra-pure water (Millipore, Milli-Q PLUS 185). The pH was 0.02. The thorium solution, $C=4\times10^{-3}$ M, was then prepared in a glovebox under a nitrogen atmosphere.

The sodium hydroxide solutions were prepared using standard solutions (Merck, Titrisol®) and ultra-pure distilled water.

2.2. Experimental procedures

UV-visible spectra were recorded on a UVIKON 930 spectrophotometer. The sample compartment was thermoregulated at (298±0.5) K.

In our previous study [1], it was checked that pyoverdine was unaffected by basic or acidic conditions. The interaction of pyoverdine with thorium ions was followed

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*Corresponding author. Tel.: +33 3 88106401; fax: +33 3 88106431; e-mail: fastrade@in2p3.fr

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by measurements of the absorption spectra of 2 ml of the pyoverdine solution (C=1.6×10^{-4} M, pH=3.3) to which 5 μl portions of a solution of thorium triflate (C=4×10^{-3} M, pH=0.02) were successively added. After each addition the pH was measured with a standard pH-meter (Knick 765, Calimatic electrode type U402-57 INGOLD). The final addition of thorium corresponded to a total solution volume of 2.1 ml, for which the ratio pyoverdine: thorium was 1:1.25 and the pH was 1.38. At this point, the pH was changed by addition of increasing amounts of sodium hydroxide and the corresponding absorption spectra were again recorded.

It should be noted that the reproducibility of spectrophotometric measurements is generally very satisfactory. In fact, the repeated spectra of solutions in the course of several weeks remained virtually identical.

3. Results and discussion

3.1. Experimental results

Fig. 1 shows the spectra recorded between 250 and 550 nm for the various additions of thorium and NaOH. The complexation is clearly pH-dependent and the formation of the complex can be best followed in the range 390–440 nm.

Pyoverdine, as a biological molecule, is somewhat intricate [3,5,6]. However, it is known that this molecule contains four labile hydrogen atoms at the complexing site [7] and it will thus be hereafter denoted as PYH_4. In a first approach to the problem, a very simple model of complexation was considered.

$$Th^{4+} + PYH_4 \Leftrightarrow nH^+ + PYH_{(4-n)}Th^{(4-n)+} \qquad (I, n)$$

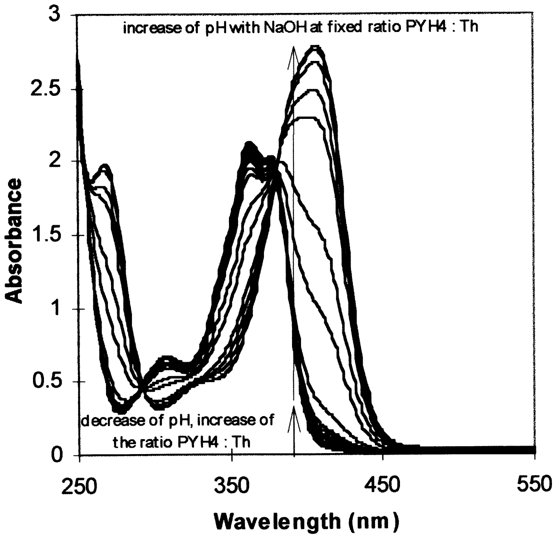

Fig. 1. Variation of the absorption spectra of pyoverdine by addition of thorium and NaOH.

where n, possibly ranging from 0 to 4, is the number of labile hydrogens liberated in the complexation process. The unknown parameters of the problem are n, the equilibrium constant, K_{Th}, and the molar absorption coefficient of the complex formed; the molar absorption coefficients of both thorium and pyoverdine can be determined directly from two independent measurements. It was therefore checked that thorium does not significantly absorb in the range 390–440 nm, for all pH values (ranging from 0.15 to 3.5), and the measured molar absorption coefficient of pyoverdine appeared to be in very good agreement with previous determinations [1].

3.2. Graphical approach

In a first attempt to determine the value of n, this very simple model can be readily solved:

$$K_{Th} = \frac{(PYH_{(4-n)}Th^{(4-n)+})(H^+)^n}{(PYH_4)(Th^{4+})} \qquad (1)$$

where the brackets () represent is the concentration of the species X. It should be noted that, in this case, K_{Th} includes the correction for ionic strength, supposing that it is constant throughout the experimental process. In fact, this approximation is correct in the second part of the experiment only, which thus will be considered alone in the following discussions.

At a given wavelength, the absorbance $A(\lambda)$ can be written as:

$$A(\lambda) = \varepsilon_1 \cdot (PYH_4) + \varepsilon_2 \cdot (Th^{4+}) + \varepsilon_3 \cdot (PYH_{(4-n)}Th^{(4-n)+}), \qquad (2)$$

where the terms in ε_i (i=1,2,3) are the molar absorption coefficients for PYH_4, Th^{4+} and $PYH_{(4-n)}Th^{(4-n)+}$, respectively, at wavelength λ.

From $\alpha = (A(\lambda) - \varepsilon_1 P_0 - \varepsilon_2 T_0)/(\varepsilon_3 - \varepsilon_2 - \varepsilon_1)$, where P_0 and T_0 are the initial concentrations of pyoverdine and thorium, it follows that:

$$\log_{10}(\alpha - P_0 - T_0 + P_0T_0/\alpha) = pK_{Th} - n\text{pH}\,. \qquad (3)$$

Eq. (3) is derived assuming that the experimental absorbance value obtained for the last addition of NaOH is solely due to the complex. The attainment of the experimentally observed maximum absorbance, and the very low molar absorption coefficient of thorium indicate that this hypothesis is reasonable. The plot of $\log_{10}(\alpha - P_0 - T_0 + P_0T_0/\alpha)$ as a function of pH is a straight line, with slope n and the intercept with the y axis is equal to pK_{Th}.

As an example of the validity and interest of this graphical method, Fig. 2 shows the variation of $\log_{10}(\alpha - P_0 - T_0 + P_0T_0/\alpha)$ with pH at λ=420 nm. Owing to the approximations made, the straight line is effective only in a somewhat limited region of pH, to which our plot was restricted. The best fit to the data gives a slope of 2.17. The

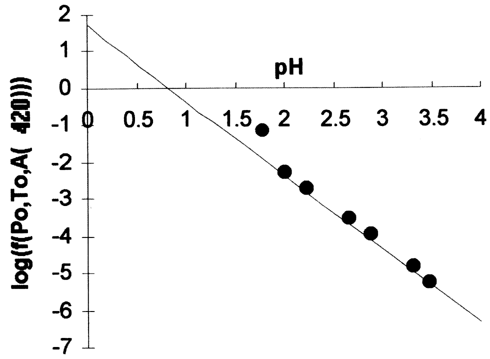

Fig. 2. Results of graphical data treatment, ●: exp; —: fit.

best straight line through the points, for a fixed integer value of 2 for the slope, is represented as a solid line in Fig. 2. The derived value of K_{Th} is then equal to 1.82 10^{-2} M. In the region of interest, the value of the ionic strength is almost constant at 0.048 M, a value mainly due to the presence of ClO_4^- and Na^+ ions, while thorium and pyoverdine moieties have minor contributions, owing to their low concentrations. This leads to a thermodynamic value of the equilibrium constant of 0.13 M, using the Davies equation to account for the ionic strength [8].

3.3. Comprehensive model

For a more accurate description of the experiment, a MINUIT subroutine (CERN library) was written on the basis of the chemical scheme (I, *n*), which includes the effect of dilution and the effect of the ionic strength, using the Davies equation [8].

In order to fit the absorption data, the five possible options of scheme (I, *n*) (with *n* varying between 0 and 4) were tested for 23 wavelengths (in the range 390–440 nm), using the adapted MINUIT subroutine. The quality of the fit was assessed from the value of σ defined as:

$$\sigma = \frac{\sum_{i=1}^{N_p} \frac{(A(\lambda) - S(\lambda))^2}{S(\lambda)}}{N_p},$$

where N_p is the number of experimental points and $S(\lambda)$ the calculated value of the absorbance at a given wavelength. σ is to be compared to the experimental error on absorbance, here equal to 2×10^{-3}.

In the fitting procedures, the molar absorption coefficient of pyoverdine at each wavelength was left free, while that of thorium was fixed, because its low value precluded a precise determination in a multi-parameter fit. It was therefore checked that the corresponding value for pyoverdine derived from the fits was in good agreement with the experimental one. Choosing $\lambda = 420$ nm as a typical case, Fig. 3 presents, for each option, the best fit obtained. The model assuming $n=0$ is, by far, not representative of the data ($\sigma=0.2$); this result was foreseen from the direct observation of Fig. 1. Even if models including $n=3$ ($\sigma=2\times10^{-3}$) or $n=4$ ($\sigma=3\times10^{-3}$) are not incoherent, the best description of the data is clearly obtained for $n=2$ ($\sigma=2\times10^{-4}$), in agreement with the graphical method. The average value, over the 23 wavelengths, of the thermodynamic equilibrium constant K_{Th}, derived from the best fit to the data of model (*I*, 2) is: $K_{Th}=(0.15\pm0.03)$ M. This value is very close to that determined with the help of the graphical method.

In our previous study on the complexation of pyoverdine with UO_2^{2+} [1], it was shown that in this case also, the complexation is accompanied by the liberation of two hydrogen atoms. Since the complexing site of pyoverdine presents two kinds of labile hydrogens, i.e. two on the hydroxamic groups and two others on the chromophore group (the latter being much more basic than the former), it is suggested that complexation might occur via the liberation of two hydrogens for cations of various charges. Considering that the complexation of either Th^{4+} or UO_2^{2+} proceeds via the liberation of the same number of hydrogens, the complexing affinities of pyoverdine towards the two cations may be easily compared. This is done in Fig. 4, using $C=1.6\times10^{-4}$ M for pyoverdine and either cation. The percentage of metal complexed by pyoverdine is shown as a function of pH in the range 1–5, considering $K_U=(8.3\pm0.5)\times10^{-4}$ M [1]. Under the conditions chosen here, a neat selectivity appears at pH 3, where more than 80% of thorium is complexed as compared to 10% of uranium (VI).

3.4. Testing other models

The hydrolysis scheme of thorium [9–11] is still under discussion and therefore many chemical models could be tested in order to check the possible uniqueness of the description. It is not within the scope of this work to analyze all the possibilities, such as the introduction of various hydrolysis equilibria of thorium or the effect of carbonates. Considering that model (I, 2) appears to be very satisfactory, it is clear that additional equilibrium parameters will not drastically change the results of the fit. Rather, it appeared desirable to test the hypothesis of the two-hydrogen liberation versus complexation of a cation with charge +4. In fact, it has been suggested that, owing to very slow backward reactions of some of the hydrolysis equilibria, thorium is only present in the form $Th[OH]_2^{2+}$, at least for pH values below 4 [10,12]. In this case, the model to be tested is:

$$Th[OH]_2^{2+} + PYH_4 \Leftrightarrow 2H^+ + PYH_2Th[OH]_2 \qquad (II)$$

this leads to the question of a possible distinction between model (I, 2) and (II), on the basis of this work. Under the hypothesis of model (II), the only difference in the mathematical treatment appears in the correction term of ionic strength resulting from the change in the charges of

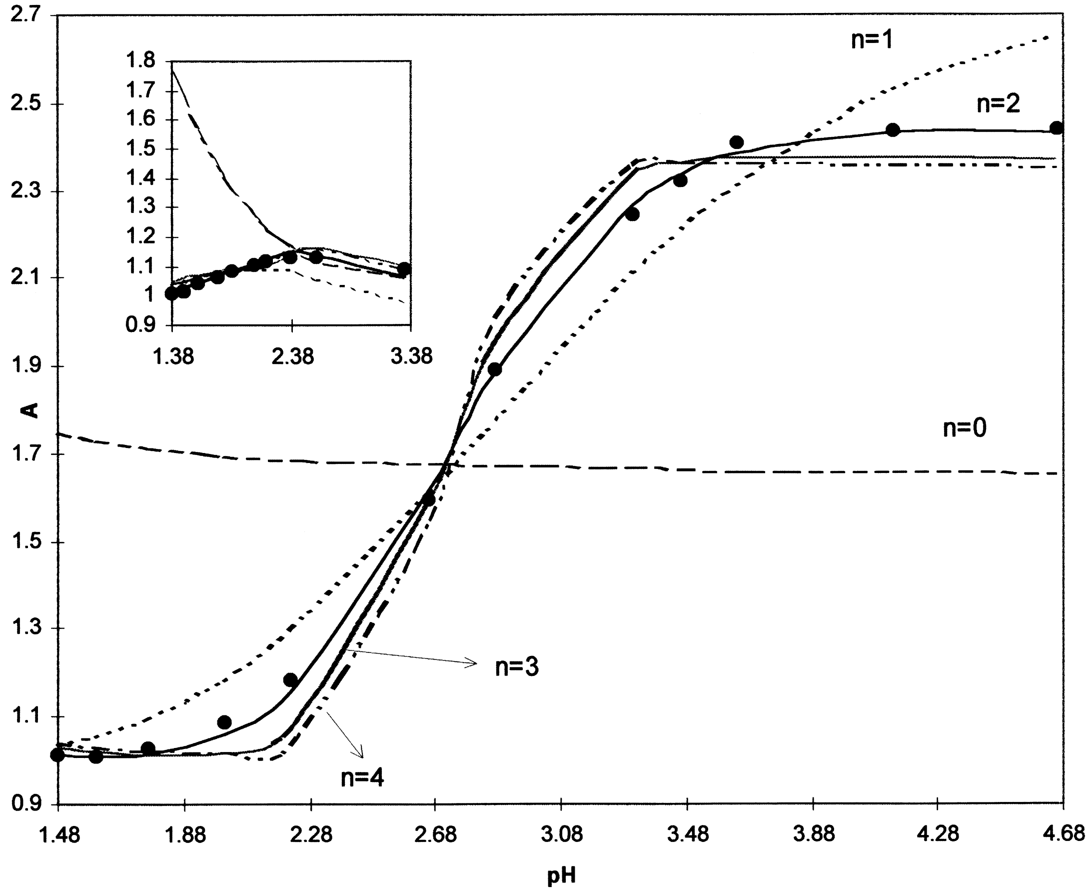

Fig. 3. Comparison between the different possibilities for model I, ●: exp.

the cation and the complex formed. Applying the same fitting procedure as above to the data and using a consequently modified program, it appears that no distinction, on the basis of the quality of the fit, can be made between the two models and the result of this second fit ($\sigma = 2\times10^{-4}$) did not visibly differ from that of the calculations of model (I, 2), in Fig. 3. This conclusion is generally valid for any thorium moiety with charge +2. However, the fitted value of the thermodynamical equilibrium constant is 0.05 M, a value slightly different from that of the graphical determination which gives in this model 0.03 M. This latter fact favors model (I,2) rather than model (II) but a definitive conclusion can not yet be stated. Note that our results, here favoring model (I,2) do not preclude the possibility of model (II) in the case of the previous work cited [12]. The different procedure required for preparation of the thorium solution may be partly responsible for the observed tendencies.

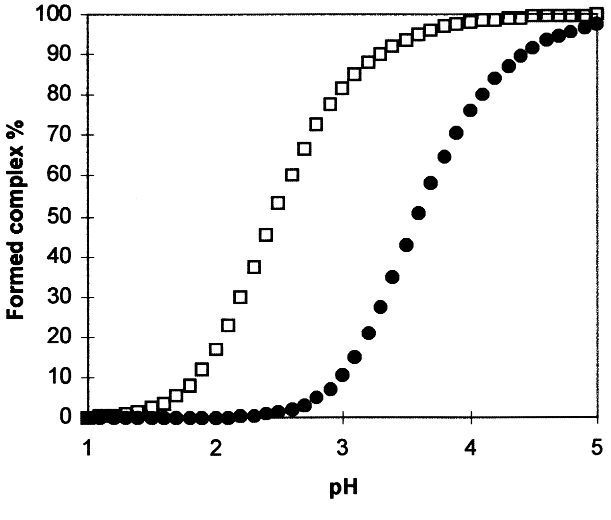

Fig. 4. Selective behavior of pyoverdine towards Th(IV): □ and U (VI): ●.

4. Conclusion

We have performed complexation experiments between pyoverdine A and thorium, in aqueous solutions, in the pH range 1–5. On the basis of a graphical method and fitting procedure, we propose a model to account for the data, involving the liberation of two, out of four, hydrogens of pyoverdine. The exact charge of the thorium moiety could not be definitely assessed, even though there are some indications that it is Th^{4+} instead of any +2 charged entity. On this basis, the thermodynamic equilibrium constant was determined and compared to that previously found for uranyl ions. Work in progress concerning the complexing affinities of pyoverdine towards ions of various charges (U(IV), Fe(III)...) may provide new insight into the proposed model.

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References

[1] M. Bouby., I. Billard, J. MacCordick, I. Rossini, Radiochimica Acta, accepted.
[2] H. Zähner, W. Keller–Schierlein, R. Hutter, K. Hess–Leisinger, A. Deer, Arch. für Mikrobiologie 45 (1963) 119–135.
[3] J.M. Meyer, M.A. Abdallah, J. Gen. Microbiol. 107 (1978) 319–328.
[4] A.I. Skoblo, D.N. Suglobov, Radiokhimya 16(4) (1974) 518.
[5] J.-M. Meyer, A. Stintzi, D. De Vos, P. Cornelis, R. Tappe, K. Taraz, H. Budzikiewicz, Microbiology 143 (1997) 35–43.
[6] G. Briskot, K. Taraz, H. Budzikiewicz, Liebigs. Ann. Chem., (1989) 375–384.
[7] A.M. Albrecht-Gary, S. Blanc, N. Rochel, A.Z. Ocaktan, M.A. Abdallah, Inorg. Chem. 33 (1994) 6391–6402.
[8] C.W. Davies, Ion Association, 1st ed., Butterworths, London, 1962, p. 39.
[9] C.F. Baes, R.E. Mesmer, The Hydrolysis of Cations, Wiley, Chichester, New York, 1976.
[10] P.L. Brown, J. Ellis, J. Chem. Soc. Dalton Trans., (1983) 31.
[11] I. Engkvist, Y. Albinsson, Radiochimica Acta 58/59 (1992) 109.
[12] G. Montavon, thesis, Université Paris XI, Orsay, n° 4523, 1996.