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Cation Complexation Properties of Hexakis(2-*O*-methyl-3,6-anhydro)-α-cyclodextrin: A ^{1}H NMR Study

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The affinity of hexakis(2-*O*-methyl-3,6-anhydro)-α-cyclodextrin (3,6-α-CDM) for Ba^{2+}, Pb^{2+}, Ca^{2+} and Sr^{2+} has been tested by ^{1}H NMR. It was shown that 3,6-α-CDM forms strong complexes in water with Pb^{2+} and Ba^{2+}. The comparison with the parent hexakis(3,6-anhydro)-α-cyclodextrin bearing hydroxyl groups instead of methoxy groups reveals that the *O*-CH_3 substitution significantly improves the anhydro-cyclodextrin selectivity.

Keywords: Cyclodextrins, decontamination, lead, NMR

INTRODUCTION

Natural cyclodextrins (CDs) are cyclic oligosaccharides composed of α-(1–4) linked *D*-(+)-glucopyranose units (six for the α-CD), having the molecular shape of truncated cone. This structure confers them the ability to include in their cavity a variety of poorly water soluble substrates. This property, widely described in the past two decades [1], has found numerous applications in industrial fields -pharmaceutic, agrochemical, cosmetic for example. One of the derivatization of parent cyclodextrins has produced the group of 3,6-anhydro-cyclodextrins [2], which gross structure and complexation properties are dramatically modified: besides the absence of any hydrophobic cavity, such structures result in a particular affinity for cationic species [3]. Such properties lead to consider their biological use for decontamination of toxic cations: for example, chronic ingestion of lead -even at very low levels- is still a public health problem nowadays, especially for children [4]. Cyclodextrins could be suitable candidates for biological decontamination due to their low toxicity and to their high biodisponibility. Previous works showed the affinity of hexakis(3,6-anhydro)-α-cyclodextrin (3,6-α-CD) for a wide number of cations [5], specially for Pb^{2+}. Hexakis(2-*O*-methyl-3,6-anhydro)-α-cyclodextrin (3,6-α-CDM) has been prepared [6] in order to enhance this selectivity. Classical methods [7] and ^{1}H-NMR technics

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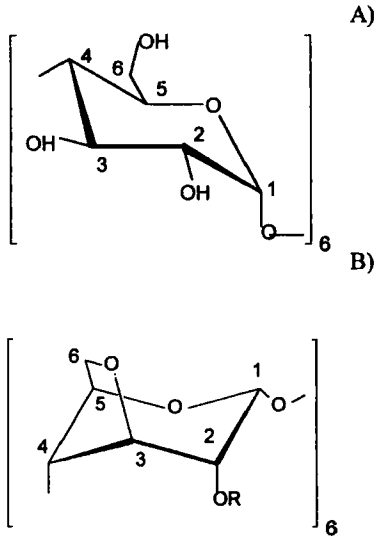

FIGURE 1 (A) Natural α-cyclodextrin; (B) R = H: Hexakis (3,6-anhydro)-α-cyclodextrin; R = CH_3: Hexakis(2-*O*-methyl-3,6-anhydro)-α-cyclodextrin. The labelling corresponds to 1H-NMR résonance attribution.

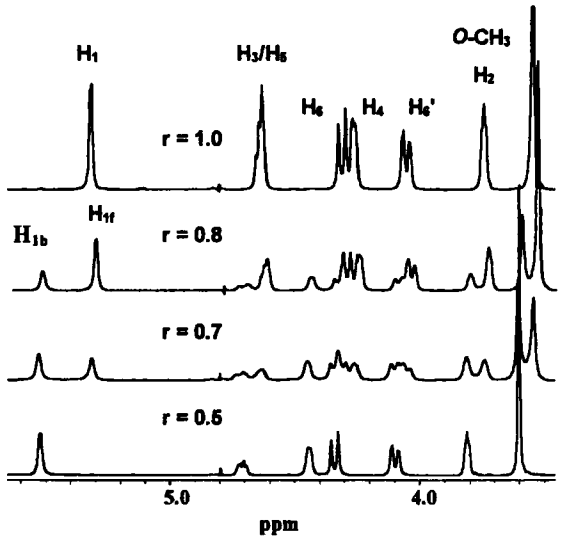

FIGURE 2 400 MHz ^{1}H NMR spectra of 3,6-α-CDM in D_2O with various amounts of $Pb(NO_3)_2$. r = [3,6-α-CDM]/[total concentration], the total concentration being maintained to 5 mM for all samples. The relative intensities of each spectrum have been rescaled for better legibility, but it is clear that H_{1b} (bound) resonance increases with lead amount while H_{1f} (free) resonance decreases. ^{1}H-NMR assignement: H_1(*d*, 5.31 ppm), $H_{3/5}$(*c*, 4.64 ppm), H_6(*d*, 4.304 ppm), H_4 (*dd*, 4.26 ppm), $H_{6'}$(*dd*, 4.05 ppm), H_2(*t*, 3.74 ppm), *O*-CH_3 (*s*, 3.54 ppm). *d* doublet, *dd* doublet of doublet, *s* single line, *t* triple, *c* composite peak.

have been used to test the affinity of 3,6-α-CDM for Pb^{2+}, Ba^{2+}, Sr^{2+} and Ca^{2+}. A comparison with 3,6-α-CD, represented Figure 1B, was finally realized.

RESULTS

Hexakis(2-*O*-methyl-3,6-anhydro)-α-cyclodextrin

Stoechiometry and Association Constant (Ka) Determination

The upper part of Figure 2 shows the 3,6-α-CDM ^{1}H-NMR spectrum, using the proton labelling of Figure 1B. The lower traces of Figure 2 were obtained after lead addition: the result was the detection of a new peak for each resonance, low field shifted from the original peak (see Tab. II for chemical shift differences). The corresponding intensity proportionally increased with lead amount. Such spectral variations are characteristic of a slow exchange between bound and free cyclodextrin at the NMR timescale used, *i.e.*, at 400 MHz. The same feature was also observed with Ba^{2+}, Sr^{2+} and Ca^{2+}. 1 : 1 complex stoechiometries were deduced from direct integration of cyclodextrin resonances for all cations tested. Furthermore, the apparent association constant was also directly calculated at stoechiometric proportions using the area of bound and free resonances and the relationship:

$Ka = [complex]/[3,6\text{-CDM}]_f \cdot [cation]_f \cdot$ with f for free. As the cation concentration was not measurable by ^{1}H NMR, it was deduced from cyclodextrin concentration.

Ka values for Ca^{2+} and Sr^{2+} complexes calculated with this method are respectively 140 M^{-1} and 1600 M^{-1}. Conversely, such a determination was not possible with Ba^{2+} and Pb^{2+} complexes due to the lack of free cyclodextrin detection. (see Fig. 2, $r = 0.5$). Several experiments were then performed to obtain an estimation of these Ka values.

Ba^{2+} and Pb^{2+}

Increasing temperature up to 368 K neither dissociated the complexes nor changed the apparent exchange rate from slow to intermediate or rapid conditions. However, the dilution of the 1:1 Pb^{2+}: 3,6-α-CDM complex from 2.5 mM to 0.1 mM allowed the observation of free cyclodextrin (Fig. 3). Thus, the upper concentration limit for free cyclodextrin detection was 0.5 mM. The association constant was then calculated for 0.1, 0.15, 0.2 and 0.5 mM complex concentrations, and the Ka values were graphically reported *versus* these concentrations. A good fit of the experimental data was obtained with a second order equation (see Fig. 4), providing an estimation of $\log Ka \approx 6.55$ (for a 2.5 mM complex concentration).

Conversely, similar dilution of Ba^{2+} complex did not result in any dissociation. Competition experiments were then undertaken using classical chelating agent like EDTA and EGTA. These experiments showed that cyclodextrin and EDTA/EGTA have similar affinity for Ba^{2+}, *i.e.*, free cyclodextrin resonance was detected in presence of these chelators (Fig. 5B); However, a precise determination of Ka was not possible since the proportion of free and bound cyclodextrin was varying with time. It was concluded that the Ka value of Ba^{2+}: 3,6-α-CDM complex is close to EDTA and EGTA values, respectively $\log Ka = 7.8$ and 8.4 [8] at 0.1 ionic strength.

Hexakis(3,6-anhydro)-α-cyclodextrin

The affinity of 3,6-α-CD for Ba^{2+} and Ca^{2+} was similarly tested (results for Pb^{2+} and Sr^{2+} have already been published elsewhere [5]). Upon barium addition, progressive chemical shift variations were observed for each cyclodextrin resonance, indicating a fast exchange process at the NMR timescale, *i.e.*, peaks are detected at an intermediate chemical shift between free and bound cyclodextrin resonances. Rapid exchange are classically observed in NMR studies of cyclodextrin complexes, and the methods extensively used for stoechiometry and Ka determination in the fast exchange regim have been described elsewhere [5]. The Job plot construction [7]

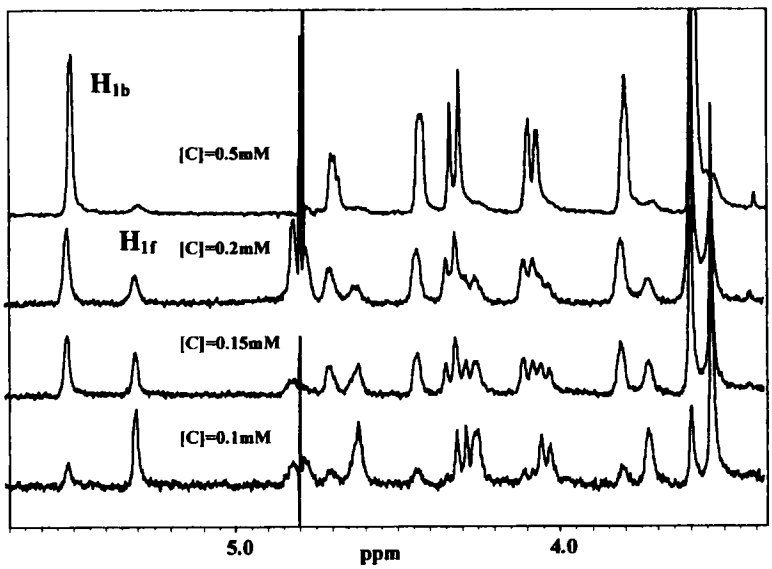

FIGURE 3 Dilution of the 1:1 Pb^{2+}: 3,6-α-CDM complex, at concentrations [C] indicated on each spectrum. One can observe that H_{1f}(free) significantly increases with decreasing complex concentration.

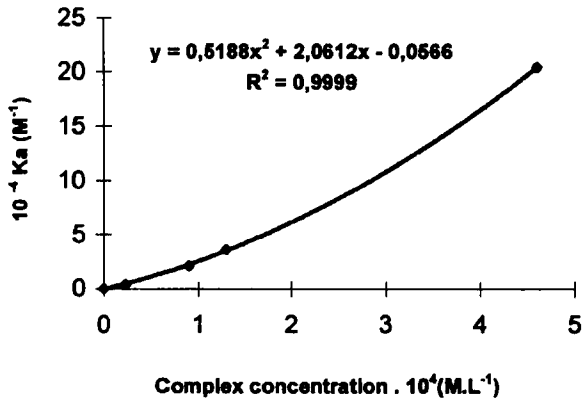

FIGURE 4 Fitting of experimental ($10^{-4} \cdot$ Ka) in M^{-1}, calculated after dilution of the 2.5 mM Pb^{2+}: 3,6-α-CDM complex, *versus* ($10^4 \cdot$ complex concentration).

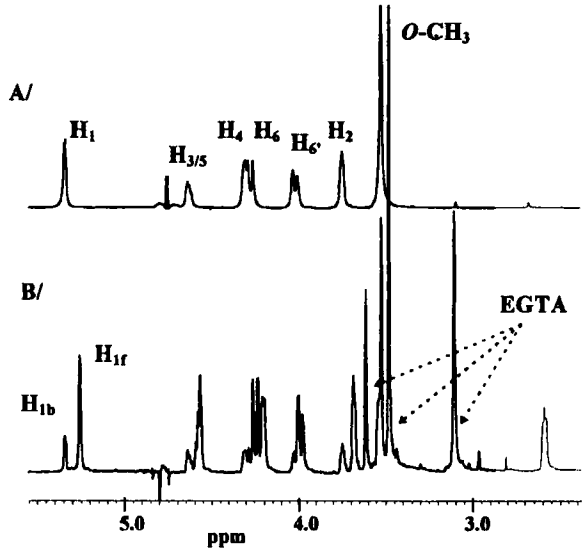

FIGURE 5 A/ ^1H NMR spectrum of 3,6-α-CDM: Ba^{2+} 1:1 complex 2.5 mM in D_2O; B/2.5 mM EGTA(4Na) was added to the A/solution.

gave a 1:1 stoechiometry for Ba^{2+}:3,6-α-CD complex. Ka was estimated around 2000 M^{-1} using a computer assisted programm [5].

Besides, no spectral variations were observed upon calcium addition, indicating that no complex formation can be detected by ^1H NMR in the experimental conditions used for this study. The same feature was also noted upon UO_2^{2+} addition to 3,6-α-CD or 3,6-α-CDM solutions.

TABLE I Comparison of log Ka values for 3,6-α-CDM and 3,6-α-CD complexes. EDTA: log Ka=7.8 and EGTA: log Ka=8.4 at 0.1 ionic strength

	3,6-α-CD	3,6-α-CDM
Ba^{2+}	3.30	EDTA ≤ log Ka ≤ EGTA
Pb^{2+}	3.40^5	log Ka ≈ 6.55
Sr^{2+}	2.60^5	3.20
Ca^{2+}	-	2.15

All Ka values obtained in this study are recapitulated in Table I.

DISCUSSION

Comparison Between 3,6-α-CDM and 3,6-α-CD

It is noteworthy (see Tab. II) that H_1 and H_4 resonances of 3,6-α-CDM undergo the most important chemical shift variations upon cation complexation, while H_6 and $H_{6'}$ resonances are the least affected. In the case of 3,6-α-CD, these methylene protons are similarly poorly affected, and H_2 is mostly shifted upon complexation (0.05 ppm). The six hydroxyl groups (see Fig. 1: $OH_{(2)}$) could be involved in the coordination of cations. In 3,6-α-CDM molecule, hydroxyl groups have been replaced by methoxy groups; the cations could be coordinated by the six glycosidic link oxygens ($CH_{(1)}$—O—$CH_{(4)}$). The methoxy group steric hindrance could then stabilize the complexes: this feature is in great agreement with the slow exchange process exclusively observed for 3,6-α-CDM complexes. Ionic radii and charge are probably key parameters for the complex setting.

TABLE II Chemical shift difference between bound and free ^1H NMR resonances of 3,6-α-CDM in presence of various cations in 1:1 solutions, measured at 400.13 MHz. Positive signs are conventionally assigned to low field shifts

	H_1	H_3/H_5	H_6	H_4	$H_{6'}$	H_2	CH_3
Ba^{2+}	**0.091**	0.055	0.029	**0.092**	0.032	0.075	0.049
Pb^{2+}	**0.21**	0.081	0.032	**0.18**	0.049	0.082	0.062
Sr^{2+}	**0.11**	0.054	0.025	**0.14**	0.024	0.070	0.042
Ca^{2+}	**0.12**	0.055	0.028	**0.15**	0.028	0.090	0.064

In conclusion, the selectivity of 3,6-α-CDM has been largely improved by substitution of the methoxy group on the $OH_{(2)}$ position (see Fig. 1 for the proton labelling). Crystallisation of the two cyclodextrins and of their lead complexes are now performed and will give important data for the future synthesis of selective cyclodextrins. The biological properties of 3,6-α-CDM are also under investigation.

MATERIEL AND METHODS

Nitrate salts of Pb^{2+}, Sr^{2+} and Ca^{2+} and choride salt of Ba^{2+} (Sigma, La Verpillère France) were weighted to prepare stock solutions in D_2O 99.9% (Euriso-top, Gif-Sur-Yvette, France). 3,6-α-CD [2a] and 3,6-α-CDM [6] were prepared as described elsewhere. Lyophilised powder of cyclodextrin was weighted to prepare 5 mM solutions in D_2O 99.9%. The complexes were prepared according to Job [7] experiment conditions, *i.e.*, keeping a total concentration of 5 mM in all samples.

NMR experiments were undertaken on a Bruker AM-400WB spectrometer (^1H frequency: 400.13 MHz), with a multinuclear 5 mm probehead. Usually, 32 scans were acquired with presaturation of solvant using the Bruker library programm PRESAT. For quantitative measurements, all integrals were compared to an external standard corresponding to a cyclodextrin spectrum at the same concentration. For dilute solutions, 256 scans were acquired. Chemical shifts are given relative to external 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP, 0 ppm), with calibration having been done using the residual HDO resonance (4.8 ppm at 297 K).

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