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Acidic Derivative of Per(3,6-anhydro)-α-cyclodextrin: Preparation and a First Evaluation of Its Affinity for Lanthanides by ^{1}H NMR

F. FAUVELLE[1]*, A. GADELLE[2], Y. PAILLER[1], S. AOUS[1] and J.C. DEBOUZY[1]
[1]*CRSSA, laboratoire de Biophysique, 24 Avenue des Maquis du Grésivaudan, 38702 La Tronche cedex, France;* [2]*CEA-Grenoble, DRF/MC/SCIB/RI2M, 17 rue des Martyrs, 38054 Grenoble cedex 9, France (Fax : (33)47663694; E-mail : jcdebouzy@compuserve.com)*

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Abstract

We report on the first synthesis of hexakis(2-*O*-carboxymethyl-3,6-anhydro)-α-cyclodextrin, an acidic derivative of per(3,6-anhydro)-α-cyclodextrin. Preliminary qualitative tests showed that this new compound would have greater affinity for lanthanides, cobalt and uranyl cations, than for sodium, potassium and calcium physiological ions.

Introduction

Among the numerous branched cyclodextrins which have been synthesized to date, the per-3,6-anhydro-derivatives exhibit quite original properties (Figure 1B). Hence, these molecules have the ability to complex cationic species [1], whereas classical cyclodextrins, with an apolar cavity (Figure 1A), are known to complex hydrophobic molecules [2].

Per(3,6-anhydro)-α-cyclodextrin itself (Figure 1B, R=H) [3] showed a preferred affinity for lead [4]. Further persubstitutions of the free hydroxyl, numbered 2 on Figure 1, were then undertaken in order to increase the complexing properties and to improve the selectivity of these cyclodextrins. So the per-2-O-methyl-3,6-anhydro-α-cyclodextrin derivative (Figure 1B, R=CH_3) has a much higher affinity for Pb^{2+} and Ba^{2+}, while weakly complexing the physiologic cations, especially Ca^{2+} [5]. Generally, most chelating agents also strongly complex calcium [6]. Hence a poor affinity for this cation is a good and original property if the non toxicity and biodisposibility of cyclodextrins are required, in biological use for example.

Now the first synthesis of hexakis(2-*O*-carboxymethyl-3,6-anhydro)-α-cyclodextrin (ACX) (Figure 1B, R=CH_2CO_2H) is described [7]. Lanthanide cations could be complexed by this acidic cyclodextrin: it is indeed well known that oxygen atoms of carboxylic groups can coordinate lanthanide ions [8].

* Author for correspondence.

Experimental

Materials and methods

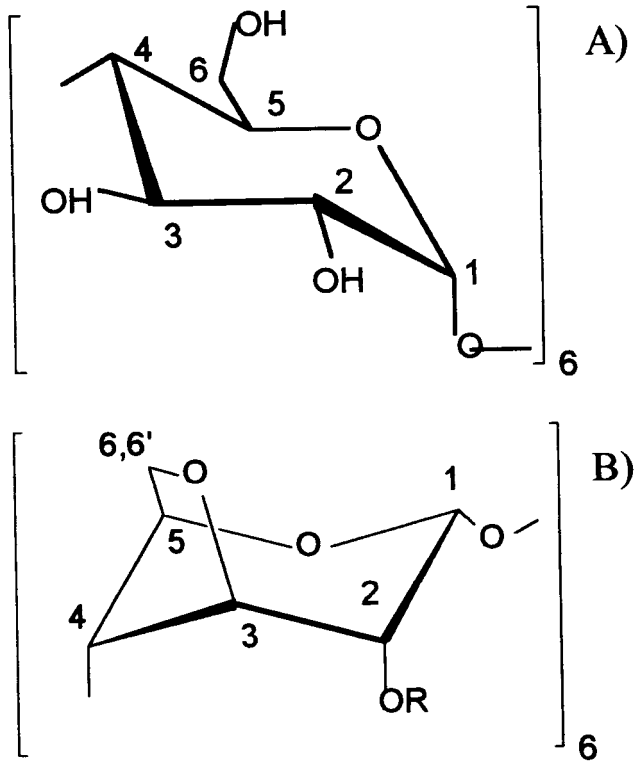
Metal salts were from Sigma (La Verpillère, France). Nitrate salts were used except for cobalt which was used as the chloride. D_2O was purchased from Euriso-Top (France).

For complexation experiments, the following cations have been tested: Eu^{3+}, Gd^{3+}, Dy^{3+}, Yb^{3+}, Lu^{3+}, La^{3+}, UO_2^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Ca^{2+}, Cs^+, Na^+ and K^+. Concentrated solutions of metal salts and ACX were mixed to 500 μl, giving final concentrations of metal/ACX of either 4 mM/1 mM or 2.5 mM/2.5 mM. ^{1}H NMR spectra were recorded immediately after sample preparation. Control spectra recorded a few days later showed no changes.

^{1}H NMR experiments were performed using a Bruker Avance DMX 400 spectrometer operating at 400.13 MHz. One-dimensional spectra were collected with suppression of the residual water signal using the presaturation program of the Bruker library. Chemical shifts were measured in ppm downfield from external tetramethylsilane (TMS), and using the internal HDO residual signal at 4.8 ppm at 297 K.

Synthesis of hexakis(2-O-carboxymethyl-3,6-anhydro)-α-cyclodextrin [7]

A solution of 1 g (1.15 mmol) of dry per(3,6-anhydro)-α-cyclodextrin [3] in 10 mL anhydrous dimethylsulfoxide (DMSO) was stirred for 3 hours with 10 mL of a 2N solution of NaH in DMSO at ambient temperature under an argon atmosphere. Sodium monochloroacetate (1.6 g, 14 mmol) was added and the mixture was maintained at ambient temperature under an argon atmosphere for 24 hours. 10 mL of methyl alcohol was then added, and the crude product was dried, dissolved in acetone and filtered. The powder obtained



1/R=H : parent per (3,6-anhydro)-α-cyclodextrin.

2/R=CH_3 : hexakis (2-*O*-methyl-3,6-anhydro)-α-cyclodextrin.

3/R=CH_3–CO_2H: hexakis (2-*O*-carboxymethyl-3,6-anhydro)-α-cyclodextrin.

Figure 1. (A) Natural α-cyclodextrin. (B) per(3,6-anhydro)-α-cyclodextrin with different substituents R, in the 2 position. 1/R=H: parent per(3,6-anhydro)-α-cyclodextrin. 2/R=CH_3: hexakis(2-*O*-methyl-3,6-anhydro)-α-cyclodextrin. 3/R=CH_2–CO_2H: hexakis(2-*O*-carboxymethyl-3,6-anhydro)-α-cyclodextrin.

was dissolved in water at neutral pH (hydrochloric acid 1N), and dialysed against water for 24 hours (Spectra/Port®CE Sterile DispoDialysers®-cellulose ester membrane-MWCO 500). The final product (yield 100%) was lyophilised and characterized by ^{1}H NMR. The complete proton assignment was performed using a classical 2D NMR COSY [9] experiment. δ(ppm): 5.37 (H-1, d, $J_{1,2}$ 2.9 Hz), 4.8 (H-3, under residual HDO resonance), 4.72 (H-5, b), 4.36 (H-4, dd, 2.2 Hz and 5.3 Hz), 4.33 (H-6, d, $J_{6,6'}$ 11 Hz), 4.12 (O–CH_2–CO2H, m), 4.06 (H-6', dd, $J_{6'-6}$ 11 Hz and $J_{6'-5}$ 2.5 Hz), 3.84 (H-2, t, J_{2-3} 3.5 Hz).

In order to have a rapid evaluation of the complexing properties of the new compound, ^{1}H-NMR spectra of ACX with selected cations in aqueous solutions were recorded.

Results

Among the toxic cations tested, three groups could be identified regarding their effect on the ^{1}H NMR spectrum of ACX. Representative examples of these groups are presented in Figure 2.

First, Pb^{2+}, Cd^{2+} and Cs^+ did not induce significant modifications of the ACX ^{1}H NMR spectrum, indicating no or poor affinity of ACX for these metals.

A second group of ions – Eu^{3+}, Gd^{3+}, Dy^{3+} and Co^{2+} – produced a complete extinction of the ^{1}H NMR spectrum of ACX, even in 1/1 proportions, as represented at the bottom of Figure 2.

Finally, the third group of cations, i.e., Yb^{3+}, Lu^{3+}, La^{3+} and UO_2^{2+}, allowed the detection of the ^{1}H NMR spectra of ACX which was however dramatically modified: the addition of lanthanide and of uranyl cations resulted in a significant broadening of all resonances. In contrast, the addition of ytterbium produced a splitting of all ACX resonances (Figure 3): the ^{1}H ACX spectrum spread between 65 and −50 ppm, consisted of 30 or 35 resonances instead of 6 for uncomplexed ACX. The same increase in resonance number was observed with lutetium in excess (minimum ratio Lu^{3+}/ACX = 3) but the peaks remained observable in the classical 0–10 ppm domain (Figure 2).

In the case of the physiologic cations, minor chemical shift variations were observed with Na^+ and K^+ (Figure 4). For Ca^{2+} in excess, chemical shift variations of most of the ACX resonances were observed (bottom of Figure 4); however, in the 1/1 ratio experiment (not shown), a much more complicated pattern was observed, and no direct conclusion could be deduced.

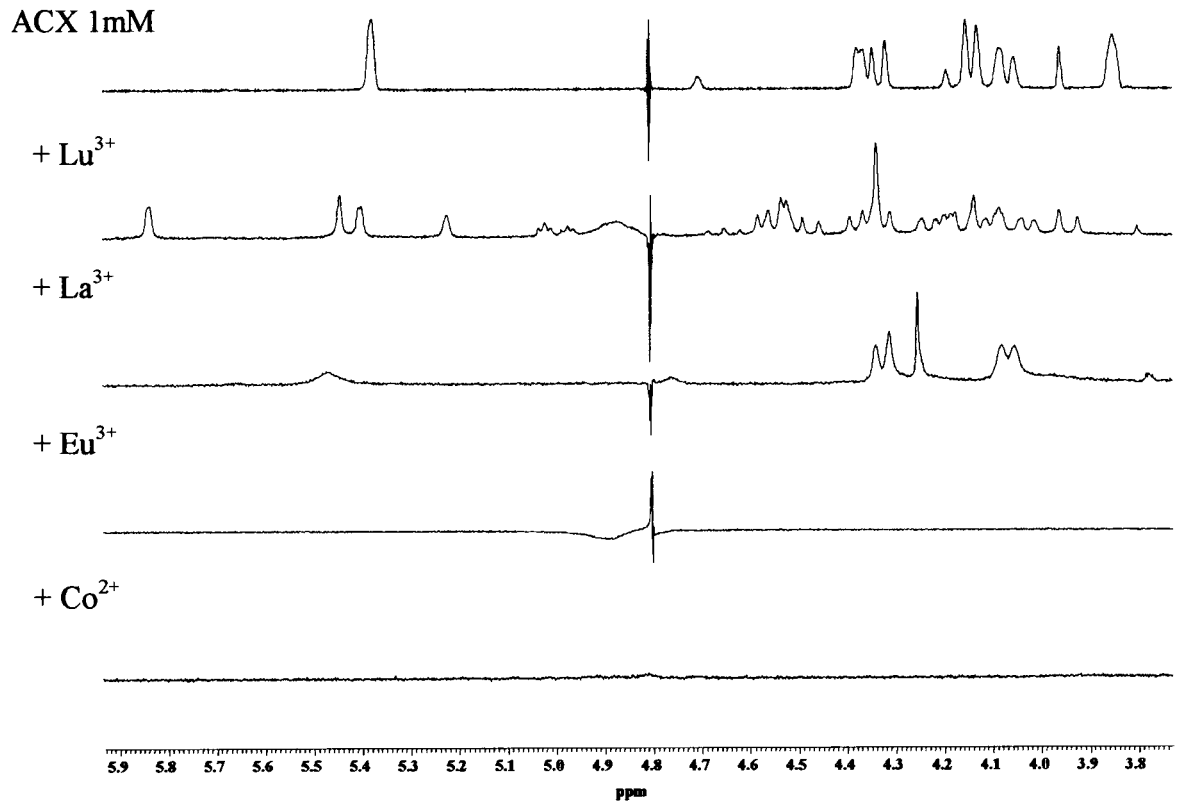


Figure 2. ^{1}H-NMR spectra of ACX 1 mM (top), and with 4mM of lanthanide cations (bottom).

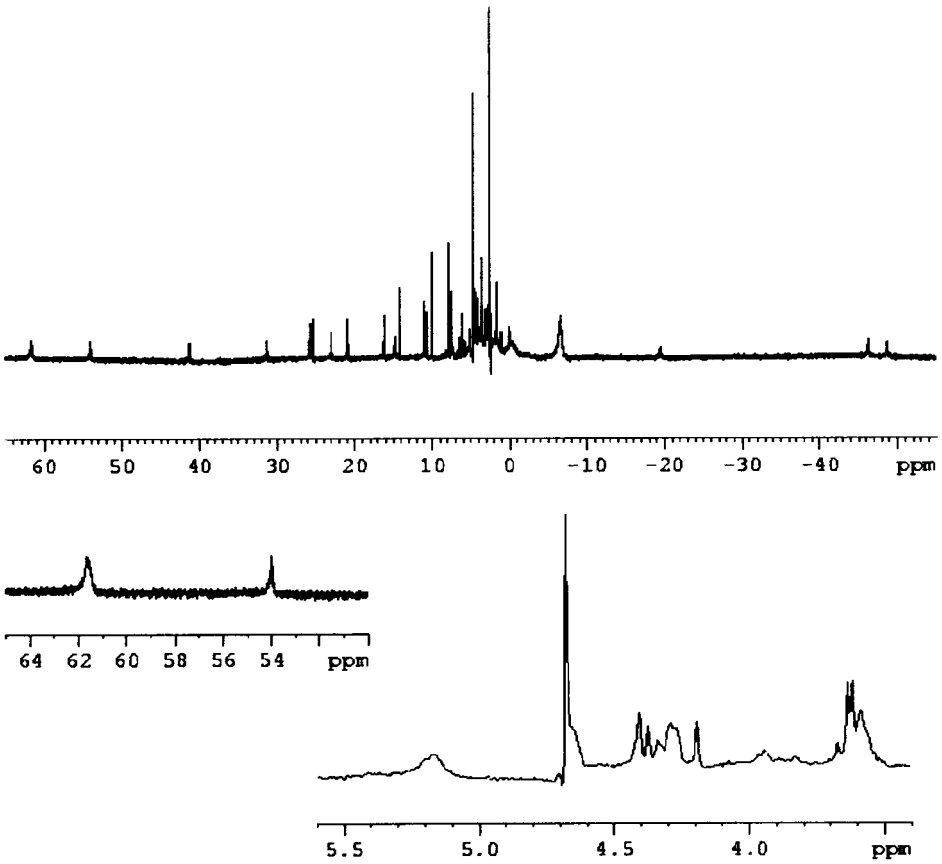


Figure 3. Full ^{1}H NMR spectrum of AXC/Yb^{3+} 2.5 mM/2.5 mM (top); two most shifted resonances (middle); the frequency domain of the ACX spectrum (bottom).

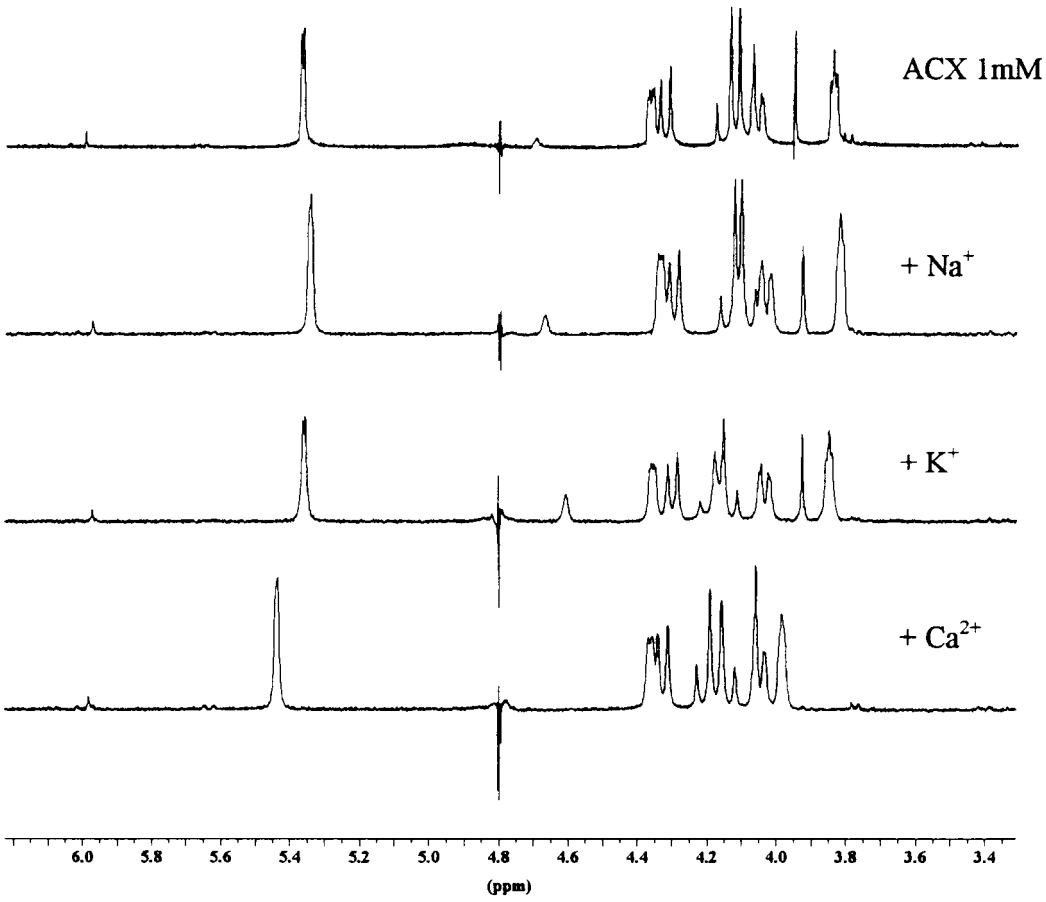


Figure 4. ^{1}H-NMR spectra of ACX 1 mM (top trace), and with 4 mM of physiologic cations (bottom).

Discussion and conclusions

The complexation process usually induces spectral modifications of the host (cyclodextrin in our case) and of the guest (cations). NMR spectroscopy is one of the more powerful methods [10] to deduce thermodynamic and stoichiometric parameters from these spectral variations, generally from chemical shift variations. Although most of the cations tested gave no or not exploitable NMR signals, the ^{1}H NMR spectra of cyclodextrin can allow the determination of the stoichiometry and association constant [5]. In the ACX case, these fundamental parameters cannot be directly deduced from spectral variations since they are very complicated: the unpaired electrons of paramagnetic compounds produce shifts and relaxation rate enhancements which are very specific to each nucleus [11]. So, if the general conclusion of the spectra reported in this paper is that all lanthanide ions are complexed by ACX, each case has to be discussed separately.

First, the extinction of the ACX NMR signal in the presence of Gd^{3+}, Co^{2+}, Dy^{3+} and Eu^{3+}, indicates that these cations are complexed by ACX, but gives no indication of the relative complex strength. Indeed, it is well established that the long electronic relaxation time of the gadolinium ion results in a severe induced line broadening of the resonances which prevent the detection of high resolution spectra. So europium and ytterbium are often used to gain insight to the solution structure of lanthanide complexes [12]. Such a broadening effect is then more surprising with europium which generally produces moderate shifts and broadening [11]. Concerning ytterbium, strong ^{1}H resonance shifts are often observed with other complexing structures [12]. As shown in Figure 3, the resonances are also broadened, between 20 and 150 Hz. A dynamical study is now necessary to separate exchange effects from structural effects.

For non paramagnetic lanthanides Lu^{3+} and La^{3+}, the complex formation results in different effects which have now to be rationalized. Thus, the ACX spectrum recorded in the presence of an excess of lutetium is very complex. The appearance of numerous new resonances may be due to the superposition of dynamical processes – the coexistence of different complexes and slow exchanges between free and bound cyclodextrin for some complexes – and structural process: it could be indicative of an assymetrical supramolecular structure related itself to a non-centered position of the ion among the six carboxyl functions. Concerning lanthanide, chemical exchange could be responsible for the broadening observed.

Under the scope of biodecontamination, a crucial restriction is the absence of complexation of the physiologic cations. This is apparently true for Na^{+} and K^{+}; calcium is probably complexed, but the strength of the complex cannot be directly determined following chemical shift variations. It is expected that the poor affinity of the other anhydro-

cyclodextrins for calcium is preserved in the case of this acidic derivative.

However, these early experiments have shown that hexakis(2-*O*-carboxymethyl-3,6-anhydro)-α-cyclodextrin has affinity for toxic lanthanides, uranyl, and cobalt in aqueous solution: this was the first step of our project. Now the complete study of this new compound will be undertaken. Fortunately, the ytterbium/ACX and lutetium/ACX complexes are observable by NMR and will give key points for elucidation of the lanthanide/ACX supramolecular structure in solution. Further NMR experiments at different temperatures/B_0 field and at different ion/ACX ratios are required. Competition experiments with another chelating agent – EDTA for example – could also be interesting. However, for a complete evaluation of the complexes strength and stoichiometry, other methods like potentiometry or conductimetry will be necessary.

Acknowledgements

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