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Phosphodiester cleavage by trivalent lanthanides in the presence of native cyclodextrins

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ABSTRACT

Testing phosphodiesterase activity of Eu(III) in the presence of native cyclodextrins revealed capacity of β-cyclodextrin (β-CD) to stabilize catalytically active metal hydroxocomplexes in mildly basic solutions. Kinetics of the hydrolysis of bis(4-nitrophenyl) phosphate (BNPP) and transesterification of 2-hydroxypropyl 4-nitrophenyl phosphate (HPNP) as models of DNA and RNA respectively has been studied with La(III), Pr(III), Nd(III), Eu(III), Gd(III) and Dy(III) cations in the presence of β-CD in the range of pH 7.0–9.0. The overall catalytic effect with 2 mM lanthanide–β-CD complexes was up to 10^5 for HPNP and 10^8 for BNPP at pH 8 demonstrating the highest catalytic activity among so far reported artificial phosphodiesterases. Analysis of concentration and pH-dependences of observed rate constants for different lanthanides showed that active species are binuclear polyhydroxocomplexes of general type $[M_2(\beta\text{-CD})(OH)_n]^{6-n}$ with $n = 3–5$. The metal–β-CD and phosphodiester–β-CD interactions were studied by ^1H NMR spectroscopy. Mechanistic implications of much higher catalytic efficiency in BNPP hydrolysis as compared to HPNP transesterification are discussed.

1. Introduction

Native cyclodextrins form complexes in water with metal ions of different types including lanthanides [1]. Ability of cyclodextrins to stabilize trivalent lanthanide ions against precipitation in basic media was demonstrated long time ago by Komiyama and co-workers [2]. The authors proposed formation of both 1:1 and 2:1 metal cyclodextrin (CD) complexes on basis of solubility data, demonstrated catalytic activity of the complexes in the hydrolysis of a phosphate monoester but did not determine the stability constants and did not extend the reactivity studies beyond testing of just one phosphate monoester as a substrate. Fatin-Rouge and Bünzli [3] reported a detailed study of lanthanide complexation by different CDs both in acid and basic solutions. Stability constants for aqua-ions were in the range $10^2–10^4\ M^{-1}$ and did not vary significantly for α-, β- and γ-CD. Spectroscopic data confirmed inclusion of cations into CDs, which behaved like crown ethers with anomeric oxygen atoms being possible binding sites. In strongly basic solutions at pH > 12 complexation with deprotonated secondary OH groups was the predominant form of binding. In a more recent study lanthanides were found to form equally stable complexes with anhydrocyclodextrins lacking OH groups [4], which are principal binding groups for transition metal ions [1]. This confirms the original idea [3] that cyclodextrins behave towards lanthanides essentially as crown ethers affording inclusion complexes via ion–dipole contacts with neutral oxygen atoms.

Hydroxocomplexes of trivalent lanthanides constitute an important group of most efficient catalysts for the hydrolysis of phosphate esters including DNA, RNA and model compounds [5]. Development of these catalysts depends critically on the use of appropriate stabilizing ligands, which must prevent precipitation of metal hydroxide and at the same time allow for formation of active hydroxocomplexes. Results of cited above studies point to a possibility that CDs may act as such ligands. Metal-free cyclodextrins [6] as well as metal complexes of chemically modified cyclodextrins [7] were studied as artificial phosphodiesterases, but the potential of lanthanides stabilized by native CDs was not explored yet. Previously we reported successful catalysis by lanthanides stabilized by neutral polyol ligands like bis-Tris propane or Tris [8,9]. Like CDs these compounds act as neutral O-donor ligands using non-dissociated hydroxyl groups as metal binding sites [9,10]. They form rather weak complexes with lanthanide aqua-ions with stability constants in the same range as with CDs, but stabilize active binuclear hydroxocomplexes providing much higher phosphoesterolytic activity than that observed with free metal ions.

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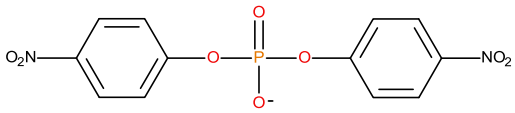
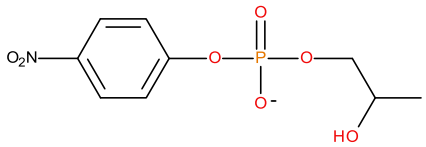
In this paper we demonstrate that similar effect is observed with CDs, which act as somewhat less powerful stabilizing ligands, but induce formation of significantly more active lanthanide species at lower pH. The catalytic activity was studied with two model phosphate diesters: bis(4-nitrophenyl) phosphate (BNPP), which is considered as a model for DNA hydrolysis, and 2-hydroxypropyl 4-nitrophenyl phosphate (HPNP), which is considered as a model for RNA transesterification.



BNPP



HPNP

Comparison of results of this study with previously reported results for other lanthanide-based artificial phosphodiesterases shows that CD-stabilized complexes in mildly basic solution at pH 8 reach the level of catalytic activity typical for the most active systems and in some cases even surpass their activity making lanthanide/β-CD system a simple practically useful artificial phosphodiesterase catalyst.

2. Results and discussion

In preliminary experiments we observed that the catalytic activity of lanthanides is significantly higher with β-CD than with α- or γ-CD (Fig. S1, Supplementary Material). All studies therefore were performed with β-CD. In the concentration range of lanthanides from 0.5 to 2.5 mM addition of 1 equivalent of β-CD was sufficient to prevent precipitation of metal hydroxide on increase in pH approximately up to pK_a value of the respective cation, e.g. up to pH 9 with less acidic La(III) and up to pH 8 with most acidic Dy(III). In the absence of CD precipitation with 1 mM lanthanides starts already at pH 7. An excess of CD did not improve the stability. To control the reaction pH the 50 mM Tris/HCl buffer was employed. The buffer by itself has some stabilizing effect but the rate constants measured in the absence of β-CD were at least one order of magnitude smaller than those in the presence of β-CD.

Attempts to characterize the interactions of lanthanides with β-CD in more details as compared to those reported by Fatin-Rouge and Bünzli [3], in particular establish the type of hydroxo complexes, provided limited information. Lanthanides affect very little the ^{1}H NMR spectra of β-CD in acid solutions. Fig. 1 shows the ^{1}H NMR spectra of β-CD alone and in the presence of some lanthanide cations at pH 5.5. A single noticeable metal-induced change is a down-field shift of the signal of H-5 proton located in the interior of the CD cavity [11]. The shift is very small for La(III), but larger for more acidic Eu(III) and Nd(III) cations for which the signal overlaps with more intense signal of H-6 protons and with increased concentration of Nd(III) it is shifted further to a more down-field position (upper spectre in Fig. 1). These results confirm inclusion of lanthanide cations into β-CD cavity.

More importantly the interaction between a lanthanide cation and β-CD becomes stronger at higher pH values. Fig. 2 shows ^{1}H NMR spectra of β-CD in the presence of Nd(III) recorded at increased pH, which demonstrates that at pH 7 besides the signal of H-5 the cation shifts also the position of H-3, another interior proton of β-CD, and at pH 8 one observes a strong broadening of all β-CD signals indicating stronger although less specific interaction with hydroxo complexes of Nd(III). These observations confirm ability of β-CD to interact with potentially catalytically active hydroxo complexes of lanthanides [5], which explains both the stabilizing effect of β-CD and observed catalytic activity.

Potentiometric titrations of lanthanide salts (Eu(III), Nd(III)) and β-CD were reproducible only at pH below 8 where the fraction of hydroxo complexes was still very low and species assignment was very uncertain. Therefore a possible composition of active species was inferred from kinetic results obtained at variable pH.

Besides interactions with metal ions β-CD may form inclusion complexes with substrates possessing hydrophobic nitrophenyl groups, which also may affect the observed reactivity. A complexation of diphenyl phosphate with β-CD with the binding constant $K = 200\ M^{-1}$ determined by fluorescence titration was reported previously [12]. Fig. 3(A) shows the course of ^{1}H NMR titration of β-CD by BNPP in D_2O. No shifts of the signals of external H-2 and H-4 protons is observed, but the signals of internal H-3 and H-5 protons undergo strong up-field shifts consistent with inclusion of aromatic groups of BNPP inside the CD cavity. The complexation induced shifts in the signals of H-5 and H-3, which sit at opposite sides of the cyclodextrin cavity, are similar by their magnitude which means that BNPP can enter the cyclodextrin cavity from both sides. The profile of the signal of H-3 proton vs. guest concentration is shown in Fig. 3(B) and the fitting of this profile to a 1:1 binding isotherm gives the binding constant $K = 320 \pm 20\ M^{-1}$ for BNPP. Similar experiment with HPNP showed much smaller complexation-induced shifts in the signals and a linear profile (Fig. 3 (B)), which does not allow one to estimate a significantly smaller binding constant in this case.

Curiously, recent theoretical calculations predict external BNPP binding to β-CD via H-bonding of the host OH groups to the phosphoryl group of the phosphodiester, [13] which obviously contradicts the experimental results.

On basis of determined binding constant for BNPP one may conclude that with β-CD concentrations below 2.5 mM employed in kinetic studies (see below) the degree of the substrate complexation by CD is less than 50% and for HPNP it should be even smaller.

In all kinetic experiments hydrolysis of BNPP proceeded though intermediate formation of mono-4-nitrophenyl phosphate, which further was hydrolyzed to the second 4-nitrophenolate anion and inorganic phosphate but with a different smaller rate constant. The details of kinetic analysis are given in the Section 4. The observed first-order rate constants (k_{obs}) for BNPP hydrolysis discussed below correspond to the first step of the reaction. In the case of HPNP usually overlooked problem is that the compound prepared by traditionally employed procedure [14] contains ca. 5% of 1-hydroxy-2-propyl isomer, which is ca. 10 times more reactive than the main 2-hydroxypropyl isomer [15] (see also [16]). The contribution from the hydrolysis of the more reactive isomer practically disappears after ca. 20% of hydrolysis and this initial part of the reaction was excluded from the calculation of the rate constant.