Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **17:** 393–397, 1994.

Solubilization of Lanthanide Ions by Cyclodextrins in Basic Aqueous Solutions

MORIO YASHIRO, SACHIKO MIYAMA, TOHRU TAKARADA and MAKOTO KOMIYAMA*
Department of Chemistry and Biotechnology, Faculty of Engineering, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

(Received: 26 April 1994)

Abstract. Cyclodextrins form complexes with lanthanide ions in basic aqueous solutions. This complex formation in basic solution dramatically enhances the solubility of lanthanide ions, which are otherwise insoluble due to the formation of hydroxide gels. Solutions of the γ-cyclodextrin–Ce^{3+} complex effectively hydrolyze 2′-deoxyadenosine-5′-monophosphate to 2′-deoxyadenosine.

Key words: Lanthanide ion, Pr^{3+} ion, cyclodextrin, DNA hydrolysis.

1. Introduction

The surprisingly high activities of lanthanide ions in the hydrolysis of biologically important compounds have recently attracted a great deal of attention [1–4]. The first nonenzymatic 'hydrolytic' cleavage of linear DNAs was achieved by cerium ion [1, 2]. Lanthanide ions having such remarkable properties are expected to become tools in biotechnology and gene therapy. However, lanthanide ions readily precipitate under basic conditions due to the formation of insoluble hydroxide gels, and such behavior has been a significant obstacle to their use in aqueous solutions and *in vivo*. Thus, it is important to design a suitable complex which is stable enough to prevent the formation of hydroxide gels, while maintaining the remarkable activities of the lanthanide ions.

We here report that cyclodextrins (CD) can dramatically enhance the solubility of lanthanide ions in basic aqueous solutions by formation of non-inclusion outer sphere complexes.

2. Experimental

To 1.5 mL of an aqueous solution of CD was added 0.5 mL of an aqueous solution of $PrCl_3 \cdot 7H_2O$, and the pH of the solution was then adjusted to 11.5 with NaOH. The final concentration of Pr^{3+} is 20 mM and that of CD is 0–26 mM. The solution was ultracentrifuged (12 000 rpm) for 15 min, and the supernatant (1.25 mL) was

* Author for correspondence.

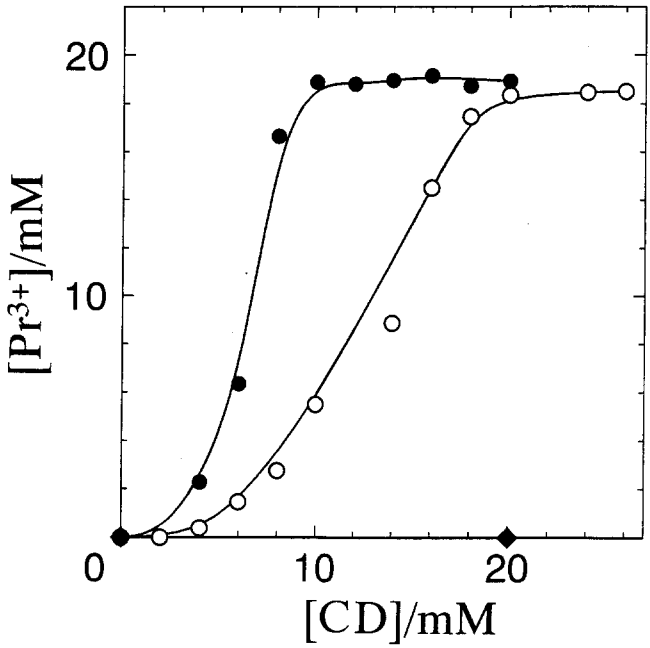


Fig. 1. Solubilization of Pr^{3+} (20 mM) by cyclodextrins at pH 11.5 o = α-CD; • = γ-CD; ♦ = Me-α-D-Glc.

diluted with 1.25 mL of 2 M aqueous HCl. The dissolved ion was quantified by absorbance at 443.5 nm. The results are shown in Figure 1.

3. Results and Discussion

In the absence of CD, the Pr^{3+} ion precipitates promptly under the conditions employed, which results in the complete removal of the ion from the aqueous phase. However, CDs increase the solubility remarkably. Totally homogeneous solutions are obtained when the molar ratio of α-CD to Pr^{3+} is 1 or greater. With γ-CD, the γ-CD/Pr^{3+} ratio should be 0.5 or greater for complete solubilization. The results indicate that a 1 : 1 complex is formed in the α-CD–Pr^{3+} system, whereas a 1 : 2 complex is formed in the γ-CD–Pr^{3+} system. In contrast, when methyl-α-D-glucoside (Me-α-D-Glc), a monomeric analog of CD, is used instead of CD, no solubilization of Pr^{3+} is observed, even in the presence of a large excess (up to 64 equivalents) of Me-α-D-Glc. The cyclic structures of CDs are definitely required for solubilization [5].

Circular dichroism spectra of the CD–Pr^{3+} (1 : 1) solutions showed Cotton effects [6] corresponding to the electronic absorption of Pr^{3+} in the visible region [7]. The Me-α-D-Glc–Pr^{3+} (6 : 1) solution, however, showed very faint Cotton effects at pH 7.5, which are considerably smaller than those of the α-CD–Pr^{3+} (1 : 1)

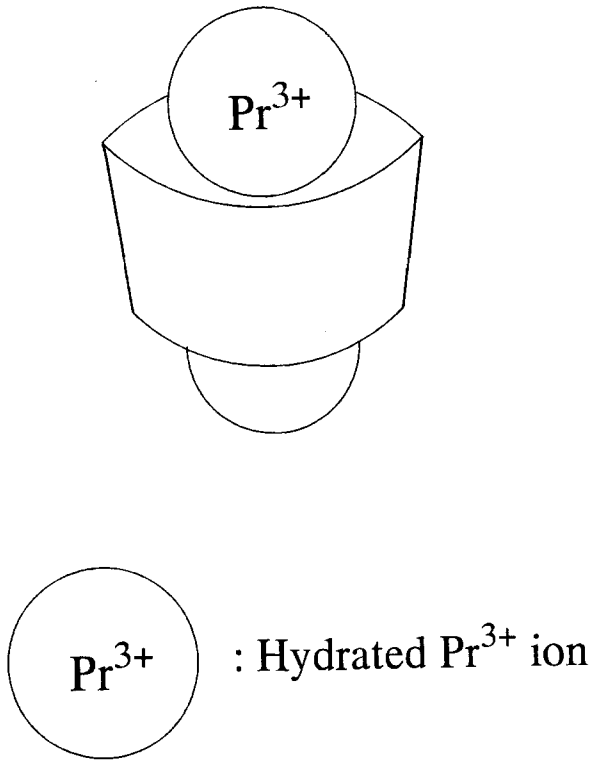


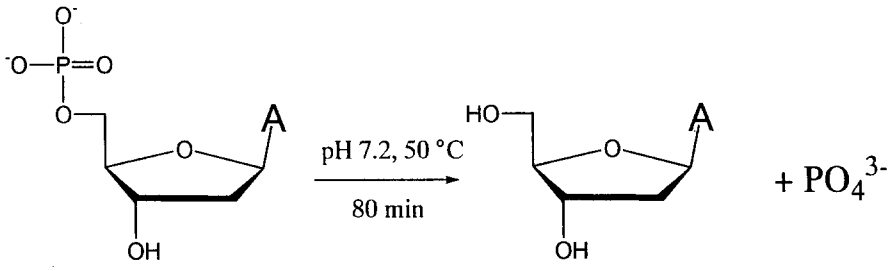
Fig. 2. Proposed structure for the γ-CD–Pr^{3+} complex.

system. Apparently, the solubilization of Pr^{3+} is due to the complex formation with CD.

The solubilization of Pr^{3+} by γ-CD is not suppressed by 1-adamantanamine (equimolar to γ-CD), which is known to form an inclusion complex with γ-CD under the conditions employed. In contrast, heptakis(2,6-di-O-methyl)-β-cyclodextrin does not solubilize the Pr^{3+} ion at all. The results indicate that the CD cavity does not participate in the solubilization of Pr^{3+}, and hydroxide groups arranged on the edges of the CD ring are important for the formation of a complex with Pr^{3+}. Since lanthanide ions are strongly hydrated in aqueous solutions, Pr^{3+} is not favorably incorporated into the hydrophobic cavity of CD [8]. The hydrated Pr^{3+} ions, whose diameter is ca. 8 Å [9], is assumed to occupy the outer sphere of CD [10]. A plausible structure for the complex is shown in Figure 2 [11].

CDs are also effective for the solubilization of other lanthanide ions, e.g. La^{3+}, Ce^{3+}, Ce^{4+}, Er^{3+}, and Lu^{3+}. It is worth noting that cerium ion, which is the most active in the hydrolysis of DNAs [2], is successfully solubilized by CDs over a wide pH range [12].

According to a preliminary study, lanthanide ions in the form of CD complexes maintain their remarkable activities in the hydrolysis of phosphoesters. For



Scheme I. Hydrolysis of 2′-deoxyadenosine-5′-monophosphate by Ce^{3+}–γ-CD.

example, 14% of 2′-deoxyadenosine-5′-monophosphate (5 mM) was hydrolyzed to 2′-deoxyadenosine with Ce^{3+}-γ–CD (10 mM, 1 : 5) at pH 7.2, 50°C, 80 min, which is comparable with the value (23% conversion) obtained in the absence of γ-CD (Scheme I) [13].

4. Conclusion

The solubilities of lanthanide ions can be remarkably enhanced by formation of non-inclusion complexes with CDs under basic conditions. This discovery has opened a route to the molecular design of highly active and selective catalysts for the cleavage of nucleic acids and other biologically important compounds. Further, CDs will be useful as a carrier of lanthanide ions into the biological systems.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan, and by the Kawakami Memorial Foundation.

Notes and References

1. (a) Y. Matsumoto and M. Komiyama: *Chem. Express* **7**, 785 (1992). (b) M. Komiyama, K. Matsumura, K. Yonezawa, and Y. Matsumoto: *Chem. Express* **8**, 85 (1993). (c) M. Komiyama, M. Yashiro, Y. Matsumoto, J. Sumaoka, and K. Matsumura: *Nippon Kagaku Kaishi*, 411 (1993). (d) M. Komiyama, Y. Matsumoto, N. Hayashi, K. Matsumura, N. Takeda, and K. Watanabe: *Polym. J.* **25**, 1211 (1993). (e) T. Shiiba, K. Yonezawa, N. Takeda, Y. Matsumoto, M. Yashiro, and M. Komiyama: *J. Mol. Catal.* **84**, L21 (1993). (f) M. Komiyama, T. Shiiba, Y. Takahashi, N. Takeda, K. Matsumura, and T. Kodama: *Supramol. Chem.* in press. (g) M. Komiyama, T. Shiiba, Y. Takahashi, N. Takeda, K. Matsumura, T. Kodama, and M. Yashiro: *Nucleosides and Nucleotides* in press. (h) B.K. Takasaki and J. Chin: *J. Am. Chem. Soc.* **116**, 1121 (1994).

2. (a) M. Komiyama, T. Kodama, N. Takeda, J. Sumaoka, T. Shiiba, Y. Matsumoto, and M. Yashiro: *J. Biochem.* **115**, 809 (1994). (b) M. Komiyama, T. Shiiba, Y. Takahashi, N. Takeda, H. Uchida, J. Sumaoka, T. Kodama, and M. Yashiro: submitted.
3. (a) M. Komiyama, K. Matsumura, and Y. Matsumoto: *J. Chem. Soc., Chem. Commun.*, 640 (1992). (b) N. Hayashi, N. Takeda, T. Shiiba, M. Yashiro, K. Watanabe, and M. Komiyama: *Inorg. Chem.* **32**, 5899 (1993). (c) J.R. Morrow, V.M. Shelton, L.A. Buttrey, and K.A. Berback: *J. Am. Chem. Soc.* **114**, 1903 (1992). (d) J.R. Morrow and K.A. Kolasa: *Inorg. Chem.* **32**, 3983 (1993). (e) J. Ciesiolka, T. Marciniec, and W.J. Krzyzosiak: *Eur. J. Biochem.* **182**, 445 (1989).
4. (a) K. Matsumura and M. Komiyama: *J. Inorg. Biochem.* in press. (b) J. Sumaoka, M. Yashiro, and M. Komiyama: *J. Chem. Soc., Chem. Commun.*, 1707 (1992).
5. 5. β-CD is also effective in the solubilization of the lanthanide ions, although detailed analysis is difficult due to the low solubility of β-CD.
6. Circular dichroism spectra (50 mM, pH 11.5): for the α-CD–Pr^{3+} system: λ 491 nm ($\Delta\varepsilon - 6 \times 10^{-4}$), 487 ($+8 \times 10^{-4}$), and 440 ($-6 \times 10^{-4}$), and for the γ-CD–Pr^{3+} system: λ 596 nm ($\Delta\varepsilon - 5 \times 10^{-4}$), 492 ($-6 \times 10^{-4}$), 487 ($+12 \times 10^{-4}$), and 474 ($+5 \times 10^{-4}$).
7. 7. C.V. Banks and D.W. Klingman: *Anal. Chim. Acta* **15**, 356 (1956).
8. M.L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer-Verlag: New York, 1977; F. Vögtle: *Supramolecular Chemistry*, Wiley: New York, 1991.
9. The diameter of the hydrated Pr^{3+} ion is estimated by the simple summation of the Pr^{3+}–O distances (A. Habenschuss and F.H. Spedding: *J. Chem. Phys.* **70**, 3758 (1979)) and the diameter of the water molecule.
10. Such a type of coordination of metal ions with CD has been anticipated in a recent review (J. Szejtli: *Stärke* **42**, 444 (1990)).
11. ^{1}H-NMR spectra of the α-CD–Pr^{3+} and the γ-CD–Pr^{3+} systems (20 mM in D_2O, pD 12) show very small lanthanide-induced shifts (less than 0.03 ppm) which are almost independent of the kinds of the protons in the glucose unit. These observations suggest that Pr^{3+} is not located at the specific site of the sugar [14].
12. The solubilization of lanthanide ions by cyclodextrins is commonly observed even at pH from 8 to 11. Therefore, the proton dissociation of the hydroxyl groups (pK_a ca. 12) is not necessary for the solubilization of lanthanide ions, in contrast to the solubilization of Cu^{2+} by CDs (Y. Matsui, T. Kurita, M. Yagi, T. Okayama, K. Mochida, and Y. Date: *Bull. Chem. Soc. Jpn.* **48**, 2187 (1975)).
13. In contrast, coordination with ethylenediamine-tetraacetate (EDTA), iminodiacetate, or hexaaza macrocyclic ligands [1, 2] resulted in a significant decrease of the remarkable activities of lanthanide ions.
14. H. Grasdalen, T. Anthonsen, O. Harbitz, B. Larsen, and O. Smidsrød: *Acta Chem. Scand. A* **32**, 31 (1978); S.J. Angyal and D. Greeves: *Aust. J. Chem.* **29**, 1223 (1976).