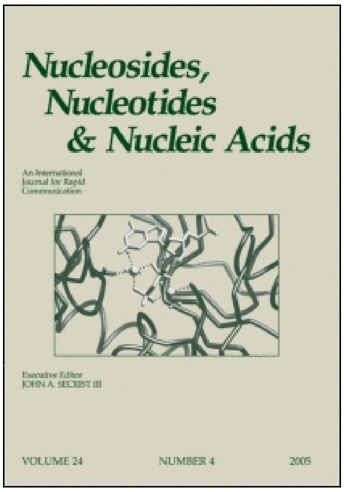

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CERIUM(IV)/LANTHANIDE(III)/DEXTRAN TERNARY SOLUTIONS FOR EFFICIENT AND HOMOGENEOUS DNA HYDROLYSIS[1]

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ABSTRACT: Homogeneous and neutral solutions are prepared by mixing $Ce(NH_4)_2(NO_3)_6$ and dextrans at pH 7. These homogeneous solutions are active for DNA hydrolysis. Still more importantly, the activities of the binary solutions are greatly promoted by the addition of various lanthanide(III) ions.

Recently, significant interests have been focusing onto non-enzymatic hydrolysis of DNA.[2] However, DNA is enormously stable and is difficult to hydrolyze without using natural enzymes (the intrinsic half-life of the phosphodiester linkage therein at pH 7 and 25 °C is estimated to be 200 million years).[3-6] A few years ago, the authors succeeded in the first non-enzymatic hydrolysis of linear DNA by use of lanthanide ions.[7] The activity of cerium ion was especially remarkable. Later, the active species was shown to be cerium(IV) ion.[8-10] Furthermore, it was reported that bimetallic Ce(IV)/Pr(III) and Ce(IV)/Nd(III) combinations were still more active than Ce(IV), because of the cooperation of two kinds of metal ions.[11,12]

However, all of these metal ions are heterogeneous due to the formation of metal hydroxide gels in the reaction mixtures. Preparation of homogeneous catalysts is un-

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This paper is dedicated to the memory of Prof. Tsujiaki Hata, who is one of the most important pioneers in nucleoside and nucleotide chemistry.

*Telephone and fax number :+81-3-5802-3340. E-mail : mkomi@chembio.t.u-tokyo.ac.jp doubtedly important to widen the scope of applications of non-enzymatic DNA hydrolysis.[13]

In this paper, homogeneous solutions are prepared by mixing Ce(IV) with dextran in water. These solutions are active for DNA hydrolysis under physiological conditions. Significantly, the DNA-hydrolyzing activities of these solutions are greatly promoted by the addition of various lanthanide(III) ions. Homogeneous and highly active solutions for DNA hydrolysis are for the first time reported.

EXPERIMENTAL SECTION

Materials. Dextrans [degrees of polymerization (DP) = 25-27, 50-74, and 310-430] were purchased from Nacalai or Funakoshi. Poly(vinylalcohol) (DP = 500), $Ce(NH_4)_2(NO_3)_6$, and $LaCl_3$ were obtained from Nacalai. Other lanthanide(III) salts were from Soekawa. Amyloses (DP = 3, 6, 18, 30-40, and 90) were cordial gifts from Ensuiko Sugar Refining Co, Hayashibara Biochemical Laboratories, or Nihon Shokuhin Kako Co. Poly(acrylic acid) (DP = 6000) was from Aldrich. Thymidylyl(3'→5') thymidine (TpT) was purchased from Sigma.

Water was purified by a Millipore purification system (Milli-XQ) so that its specific resistance was larger than 18.3 MΩ·cm. Furthermore, the water and the reaction vessels were sufficiently sterilized in an autoclave immediately before use. The greatest care was paid to avoid contamination by natural enzymes and other metal ions.

Preparation of Homogeneous Solutions of Ce(IV)/Lanthanide(III)/Dextran Ternary Systems. Required amounts of $Ce(NH_4)_2(NO_3)_6$, lanthanide(III) chloride, and dextran were mixed, and were added to a Hepes buffer solution (50 *mM*). Then the pH of the solution was adjusted to 7.0 by using a small amount of NaOH.

Kinetic Study on the Hydrolysis of TpT. The hydrolysis of TpT was achieved at pH 7.0 (50 *mM* Hepes buffer) and 50 °C. The initial concentration of the substrate was 0.1 *mM*. The reactions were followed by a reversed-phase HPLC (a Merck LiChrosphere RP-18(e) ODS column; water/acetonitrile = 92/8 (v/v)). The HPLC peaks were assigned by coinjection with authentic samples.

All the reactions satisfactorily obeyed pseudo-first-order kinetics. The change in pH during the reactions was less than 0.2 unit. All the rate constants presented here were the averages of the results of duplicate or more runs, which coincided with each other within 10%.

Determination of the Amount of Pr(III) Ion Which is Incorporated into the Ce(IV)/Pr(III)/Dextran Ternary Complex. The solutions of Ce(IV)/Pr(III)/dextran ternary system at pH 7.0 were ultrafiltrated under a static pressure 2 kg cm^{-2} of nitrogen. The membrane used (a model UP-20 ultrafilter from ADVANTEC) had an average cut-off molecular weight 20000. According to careful control experiments, free Pr(III) ions completely passed through the membrane, but the dextran (DP = 310-430) could not. Furthermore, free Pr(III) ions were passable through the membrane even in the presence of the dextran, as long as there existed no Ce(IV) ions in the solutions. Only when all the three components (Ce(IV), Pr(III), and dextran) coexisted in the solutions, some portion of the Pr(III) was cut off by the membrane. Thus, the amount of Pr(III) ions which were incorporated into the Ce(IV)/Pr(III)/dextran ternary complex, was evaluated from the difference between the concentration of Pr(III) in the solution before the ultrafiltration and the value after that. The Pr(III) concentrations were measured by UV/VIS absorption spectroscopy (at 442.5 nm).

Light-Scattering Photometry on the Ce(IV)/Dextran Binary and the Ce(IV)/Pr(III)/Dextran Ternary Systems. Homogeneous solutions of the Ce(IV)/dextran and the Ce(IV)/Pr(III)/dextran systems were subjected to an Otsuka Electronics ELS-800 dynamic photo-scattering photometer. The analyzing system can detect the particles which are greater than 3 nm in diameter, and provide the number-averaged size distribution of the particles.

RESULTS AND DISCUSSION

Preparation of Homogeneous Solutions of the Ce(IV)/Dextran Binary System. When $Ce(NH_4)_2(NO_3)_6$ was added to a pH 7 Hepes buffer in the absence of dextran, white precipitates of Ce(IV) hydroxide were rapidly formed. In the presence of sufficient amounts of dextran, however, homogeneous and pale yellow solutions of Ce(IV) were prepared.[14] All the dextrans available (DP = 25-27, 50-74, and 310-430) were effective to prepare homogeneous solutions. The charged ratio of the monomeric residue of dextran to the Ce(IV) salt should be greater than 1, in order to efficiently solubilize the Ce(IV) ions at pH 7. Apparently, polymeric aggregation of the Ce(IV) hydroxide to gels was notably suppressed by the dextrans.

These binary homogeneous solutions were active for the hydrolysis of DNA. According to the reversed-phase HPLC, a dinucleotide TpT was gradually converted to thymidine. The hydrolysis intermediates, thymidine 3'- and 5'-monophosphates, were not accumulated in the mixtures, since these intermediates were hydrolyzed faster than TpT was hydrolyzed to them[15]. Non-enzymatic hydrolysis of DNA in homogeneous solution has been successful. However, the DNA hydrolysis by this binary system was not very fast. The pseudo-first-order rate constant for the hydrolysis of TpT at pH 7.0 and 50 °C was 0.0003 h^{-1}, when $[Ce(IV)]_0 = 10$ and $[\text{monomeric residue of dextran (DP = 310-430)}]_0 = 20 \text{ mmol dm}^{-3}$ (see TABLE 1: note that the hydrolysis by the binary solutions is remarkably accelerated by the cooperation with various lanthanide(III) ions, as described below).

When amylose (DP = 18, 30-40, or 90) was used in place of the dextrans, white precipitates of the metal hydroxide were soon formed, and heterogeneous mixtures were obtained. In order to provide homogeneous binary solutions, the glycoside linkage between the glucose residues in the sugar additives must be an α-1,6 type (as in dextran), rather than α-1,4 type (as in amylose). Both poly(vinylalcohol) and poly(acrylic acid) showed no measurable Ce(IV)-solubilizing activities (either in the presence of lanthanide(III) ions or in their absence).

Promotion of the DNA-Hydrolyzing Activity of the Cerium(IV)/Dextran System by Lanthanide(III) Ions. The ternary solutions of Ce(IV)/lanthanide(III)/dextran system were prepared by adding a mixture of $Ce(NH_4)_2(NO_3)_6$, lanthanide(III) chloride, and dextran to Hepes buffer solutions. The mixtures were homogeneous, when the ratio of the monomeric residue of dextran to Ce(IV) was 1 or greater (otherwise, the mixtures gradually turned turbid and finally white precipitates were formed).

Quite interestingly and significantly, the DNA-hydrolyzing activities of the ternary solutions were far greater than that of the Ce(IV)/dextran binary solution. The rate constant for TpT hydrolysis by the Ce(IV)/Pr(III)/dextran system was 0.10 h^{-1}, under the conditions that $[Ce(IV)]_0 = 10$, $[Pr(III)]_0 = 5$, and $[\text{monomeric residue of dextran (DP = 310-430)}]_0 = 20 \text{ mmol dm}^{-3}$ at pH 7.0 and 50 °C (TABLE 1).[16] The TpT hydrolysis (the half-

TABLE 1. Pseudo-first-order rate constants for the hydrolysis of TpT at pH 7.0 (50 mmol dm^{-3} Hepes buffer) and 50 °C [a]

Metal ion	Pseudo-first-order rate constant (in h^{-1})	
	with dextran	without dextran
Ce(IV)/Pr(III)	0.10	1.7
Ce(IV)	0.0003	0.16
Pr(III)	0.00	0.00

a. $[Ce(IV)]_0 = 10$, $[Pr(III)]_0 = 5$, and [monomeric residue of dextran (DP = 310-430)$]_0 = 20$ mmol dm^{-3}.

life 6.9 h) was more than 300 fold faster than that by the Ce(IV)/dextran system. In contrast, the Pr(III)/dextran binary solution was totally inactive for DNA hydrolysis, although it was homogeneous. Thus, a synergetic cooperation of Ce(IV) and Pr(III) in the ternary solutions was conclusive.

Other lanthanide(III) ions also promoted the DNA-hydrolyzing activity of the Ce(IV)/dextran system (see FIG. 1). The activities of these Ce(IV)/lanthanide(III)/ dextran systems were in the following order: Pr > Nd > Eu > La > Lu. Even the Ce(IV)/Lu(III)/dextran system, which was the least active of them, was 9 times as active as the Ce(IV)/dextran binary system. It was noteworthy that all the lanthanide ions showed a synergetic cooperation with Ce(IV) in these ternary systems. This result is in contrast with the fact that, in the absence of dextran, only Pr(III) and Nd(III) are effective for the cooperation with Ce(IV).[11] There, the other lanthanide(III) ions suppress the hydrolytic activity of Ce(IV) (the activity of the Ce(IV)/La(III) system for DNA hydrolysis is about 1/2 that of Ce(IV), whereas the Ce(IV)/Lu(III) system is almost inactive).

All the ternary mixtures for the DNA hydrolysis were homogeneous throughout the reactions. As is the case in the TpT hydrolysis by the Ce(IV)/dextran system, the hydrolysis intermediates (thymidine 3'- and 5'-monophosphates) were promptly hydrolyzed to thymidine and thus only thymidine was perceived as the product by HPLC.[17] No byproducts assignable to oxidative cleavage of the deoxyribose were formed. The present DNA scission proceeds via the hydrolysis of phosphodiester linkage. Homogeneous and sufficiently active solution for DNA hydrolysis are for the first time prepared.[18]

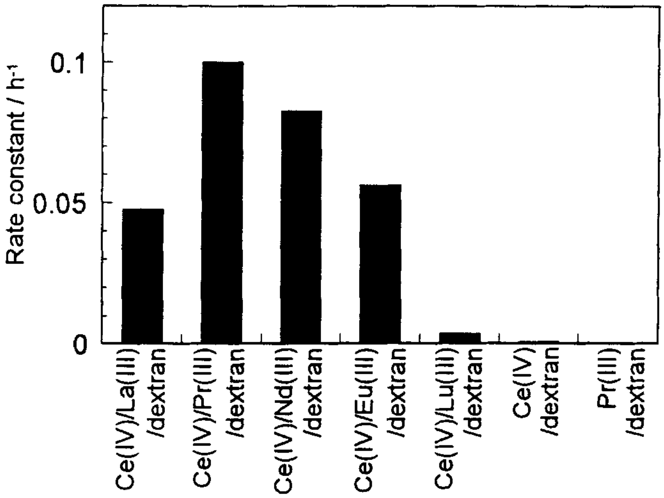

FIG. 1. Activities of the ternary solutions composed of Ce(IV) ion, various lanthanide(III) ions, and dextran (DP = 310-430) for TpT hydrolysis at pH 7.0 and 50 °C. The activities of the Ce(IV)/dextran system and the Pr(III)/dextran systems are also depicted for the purpose of comparison. $[Ce(IV)]_0 = 10$, $[lanthanide(III)]_0 = 5$, and [monomeric residue of dextran (DP = 310-430)]$_0$ = 20 mmol dm^{-3}.

Effect of $[Pr(III)]_0/[Ce(IV)]_0$ Ratio on the DNA-Hydrolyzing Activity of the Ce(IV)/Pr(III)/Dextran System. As depicted by the open circles in FIG. 2, the activity of the Ce(IV)/Pr(III)/dextran system for TpT hydrolysis significantly increased with increase in the $[Pr(III)]_0/[Ce(IV)]_0$ ratio up to the ratio of 0.5, and then decreased with increasing ratio. Here, $[Ce(IV)]_0$ was kept constant at 10 mmol dm^{-3}. The maximum activity was achieved around the molar ratio 0.5. This result is consistent with the previous result for the reactions by the binary Ce(IV)/Pr(III) system (in the absence of dextran).[11] It is indicated that the DNA hydrolysis is ascribed to a species which involves Ce(IV) and Pr(III) in 2:1 ratio. The gradual decrease of the hydrolysis rate at the $[Pr(III)]_0/[Ce(IV)]_0$ ratio > 0.5 is probably associated with the competitive inhibition by free Pr(III) ions in the solutions (*vide infra*). These metal ions form complexes with the substrate TpT and suppress the formation of the substrate-active spices complexes.

Interactions in the Ce(IV)/Pr(III)/Dextran Ternary System. In order to obtain further information on the cooperative DNA hydrolysis by the Ce(IV)/Pr(III)/dextran

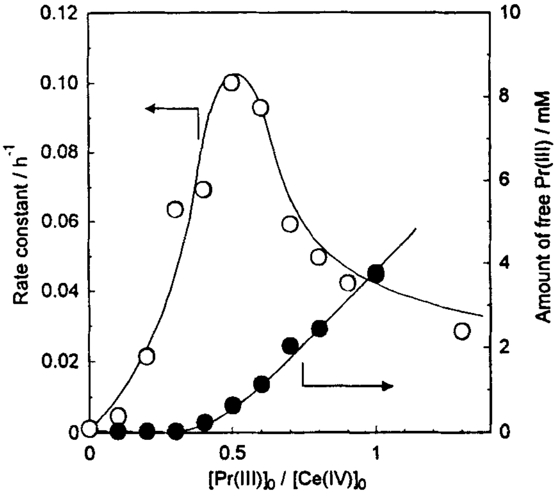

FIG. 2. Dependence of the hydrolysis rate (the open circles) on $[Pr(III)]_0/[Ce(IV)]_0$ ratio for the TpT hydrolysis by Ce(IV)/Pr(III)/dextran ternary system at pH 7.0 and 50 °C. The closed circles show the amounts of Pr(III) ions which are not incorporated into the ternary complex and thus can pass through the ultrafiltration membrane (the details are described in the EXPERIMENTAL SECTION). The $[Ce(IV)]_0$ and [monomeric residue of dextran (DP = 310-430)]$_0$ were kept constant at 10 and 20 mmol dm^{-3}, respectively. The reaction conditions were chosen so that the homogeneity of the reaction mixtures throughout the DNA hydrolysis could be substantiated (see ref. 16).

ternary system, these ternary solutions were filtrated with an ultrafiltration membrane (the details on the ultrafiltration study are described in the EXPERIMENTAL SECTION). By measuring the concentrations of Pr(III) before and after the ultrafiltration, the amounts of the Pr(III) ions, which were incorporated into Ce(IV)/Pr(III)/dextran ternary complex in the mixtures, were determined. When the $[Pr(III)]_0/Ce(IV)]_0$ ratio was smaller than 0.5, virtually all of the Pr(III) ions in the mixtures were incorporated into the Ce(IV)/Pr(III)/dextran ternary complex and thus could not pass through the membrane (see the closed circles in FIG. 2). Only when the $[Pr(III)]_0/[Ce(IV)]_0$ ratio was greater than 0.5, there existed considerable amounts of free Pr(III) ions in the mixtures (only free Pr(III) ions could pass through the membrane). These results are fairly in accord with those observed previously on the Ce(IV)/Pr(III) systems in the absence of dextran.[11]

The UV-VIS absorption spectra on the Ce(IV)/Pr(III)/dextran ternary system were identical with those for the corresponding Ce(IV)/dextran binary systems. In both cases, the absorption maxima were located around 280 nm, which were assignable to the absorption by the Ce(IV).

Evaluation of the Size of the Particles in the Binary and the Ternary Systems. According to the light-scattering measurements, the Ce(IV)/dextran binary solutions involved small colloidal particles which had the average diameter of about 15 nm (FIG. 3 (a)). The distribution in particle size was rather narrow (most of the particles had the diameters of 12-20 nm). The diameter of the dextran in aqueous solutions (without Ce(IV)) could not be measured by the present analyzing system for lack of the scattering of light by the solutions. Thus the particles in Ce(IV)/dextran binary solutions should be composed of both Ce(IV) and dextran.

Small particles of similar average diameter and size distribution were formed in the Ce(IV)/Pr(III)/dextran ternary system (see FIG. 3 (b)). These particles were responsible for the DNA hydrolysis, since all the DNA-hydrolyzing activities disappeared when they were removed by ultrafiltration.

Proposed Mechanism of DNA Hydrolysis. In the Ce(IV)/lanthanide(III)/dextran ternary systems, Ce(IV) and lanthanide(III) ions form bimetallic mixed hydroxide clusters.[11] Since the dextran functions as a protecting polymer and inhibits the formation of polymeric aggregates, these bimetallic clusters exist as small colloidal particles of diameters around 15 nm. The arguments are concretely supported by both the light-scattering measurements (FIG. 3) and the ultrafiltration study (*vide ante*).

Assumedly, the DNA hydrolysis by the ternary systems proceeds as nucleophilic attack by a metal-bound hydroxide ion towards the phosphate, and another metal ion in the mixed hydroxide clusters functions simultaneously as an acid. This greater DNA-hydrolyzing activity of ternary systems is presumably due to the cooperation of two kinds of metal ions, Ce(IV) and Pr(III).[19] The cooperation can work effectively in the mixed clusters, where both the Ce(IV) and Pr(III) are located in a close proximity to each other. The proposed mechanism is consistent with those for the DNA hydrolysis in the absence of dextran.[8,10]

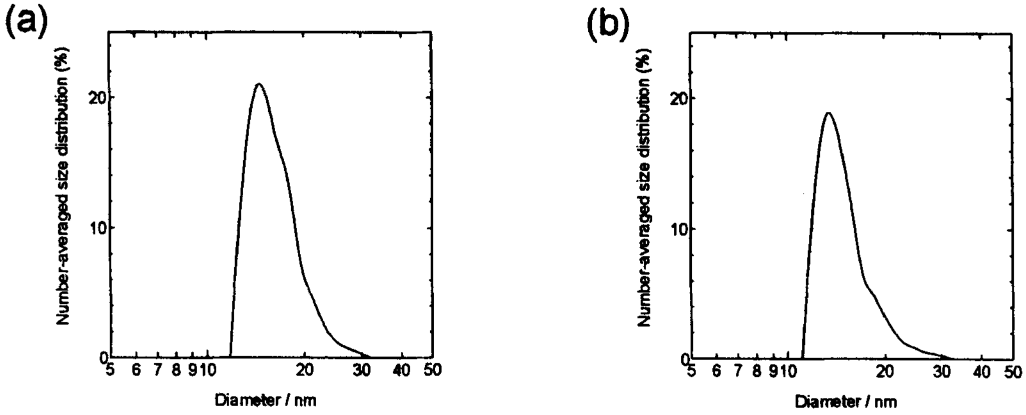

FIG. 3. Number-averaged size distributions of the particles in the homogeneous solutions of (a) Ce(IV)/dextran binary system and (b) Ce(IV)/Pr(III)/dextran ternary system: $[Ce(IV)]_0 = 10$, $[Pr(III)]_0 = 5$, and [monomeric residue of dextran (DP = 310-430)$]_0 = 20$ mmol dm^{-3}.

CONCLUSION

Homogeneous solutions, which efficiently hydrolyze linear DNA under physiological conditions, have been for the first time prepared by mixing $Ce(NH_4)_2(NO_3)_6$, lanthanide(III) salts, and dextran. The activities of these ternary solutions are in the following order: Pr > Nd > Eu > La > Lu. Dextran and its derivatives have been widely used for medical and industrial purposes, and thus the present homogeneous DNA-hydrolyzing agents would be promising for versatile applications. These attempts, as well as detailed studies on the structures of the ternary complexes and the mechanism of their DNA hydrolysis, are currently under way in our laboratory.

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16. When the ratio [monomeric residue of dextran]$_0$/[Ce(IV))]$_0$ is 1.0, homogeneous solutions are obtained for both the Ce(IV)/dextran and the Ce(IV)/Pr(III)/dextran systems. However, these solutions are rather unstable, and gradually become turbid and heterogeneous as the TpT hydrolysis proceeds. The ratio must be 2 or greater, in order to keep the mixtures homogeneous throughout the reactions.
17. The HPLC profile for TpT hydrolysis by Ce(IV)/Pr(III)/dextran was presented in ref. 1. The pseudo-first-order rate constants for the hydrolyses of authentic samples of 3'- and 5'-monophosphate were 0.79 and 0.88 h^{-1}, respectively.
18. None of amylose, poly(vinylalcohol), and poly(acrylic acid) provided homogeneous ternary solutions, which is consistent with the results for the binary systems.
19. In Ce(IV)/dextran binary system, both the acid and the base must be provided by the Ce(IV) ion(s). The cooperation can not be very effective here, since the Ce(IV) ion is intrinsically eminent as an acid but not very active as a base. Consistently, the Pr(III)-bound hydroxide is much stronger a base than the Ce(IV)-bound one (the pK$_a$ of Pr(III)-bound water (7.1) is greater than that of Ce(IV)-bound water (~ 0)) (the pK$_a$ of the water bound to lanthanide(III) ions, other than Pr(III), is also between 7 and 9, and thus the corresponding metal-bound hydroxide is sufficiently basic for the catalysis: Burgess, J. *Metal Ions in Solution*, Horwood, **1978**, Chichester).