Homogeneous DNA Hydrolysis by Cerium(IV)/Lanthanide(III)/Dextran Ternary Complexes

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Homogeneous solutions for DNA hydrolysis are prepared from cerium(IV) ion, lanthanide(III) ion, and dextran. When [Ce(IV)] = 10, [Pr(III)] = 5, and [dextran (monomeric residue)] = 20 mmol dm^{-3} at pH 7.0 and 50 °C, the pseudo-first-order rate constant for the hydrolysis of thymidylyl(3'→5')thymidine (TpT) is 0.10 h^{-1}.

Non-enzymatic hydrolysis of DNA has been attracting interests of chemists, biochemists, and others.[1,2] However, DNA is enormously resistant to hydrolysis.[3] It was only a few years ago that the first non-enzymatic hydrolysis of linear DNA was achieved by the present authors by using lanthanide ions.[4] Cerium(IV) ion is especially eminent.[5] Furthermore, it was shown that Ce(IV)/Pr(III) and Ce(IV)/Nd(III) combinations are still more active than Ce(IV).[6]

However, all of these reaction mixtures are heterogeneous because of the formation of metal hydroxide gels, making the systems complicated. Homogeneous and effective catalysts for DNA hydrolysis have never been prepared.[7,8] Here we show that dextran satisfactorily solubilizes all of Ce(IV) ion, lanthanide(III) ions (Ln(III)), and Ce(IV)/Ln(III) combinations at pH 7. The resultant homogeneous solutions of Ce(IV)/Ln(III)/dextran ternary complexes efficiently hydrolyzed TpT under the physiological conditions.

The typical procedure for the preparation of homogeneous solutions for DNA hydrolysis is as follows. To 0.1 mmol of $Ce(NH_4)_2(NO_3)_6$ in Hepes buffer (5 cm^3), were added $LnCl_3$ (0.05 mmol in 2.5 cm^3 of Hepes buffer) and dextran (0.2 mmol residue in 2.5 cm^3) in this order. The pH of the mixture was adjusted to 7.0 by small amount of NaOH. The dextran (from Nacalai) has an averaged molecular weight of 50000-70000.

At pH 7.0 and 50 °C, TpT was promptly hydrolyzed to thymidine (Thd) by the ternary solution composed of Ce(IV), Pr(III), and dextran, as shown in Figure 1 (a). The solution was homogeneous throughout the DNA hydrolysis. The pseudo-first-order rate constant for TpT hydrolysis is 0.10 h^{-1}. The conversion of TpT to Thd is stoichiometric, and no by-products assignable to oxidative cleavage of the ribose were formed. Thus, the scission is totally hydrolytic. The hydrolysis intermediates, thymidine 3'- and 5'-monophosphates, were rapidly converted to Thd and were not accumulated. When the concentration of dextran was decreased to 10 mmol dm^{-3} (in residue), the rate constant of TpT hydrolysis was 0.28 h^{-1} (the half-life of TpT is 2.5 h) . Thus, the solution is 1.4 times as active as the Ce(IV) hydroxide gel formed in the absence of dextran.[4,5,9] The activities of homogeneous solutions of other Ce(IV)/Ln(III)/dextran systems are as follows: Pr > Nd > Eu > La > Lu (even the Ce(IV)/Lu(III)/dextran system is nearly 10 fold more active than Ce(IV)/dextran).

Homogeneous solutions were also prepared by mixing either $Ce(NH_4)_2(NO_3)_6$ or $LnCl_3$ with dextran at pH 7. However, these binary solutions are quite poor in the catalytic activities (see (b) and (c) in Figure 1; the rate constants are less than 3 x 10^{-4} h^{-1}). When both the Ce(IV) salt and the Ln(III) salt were

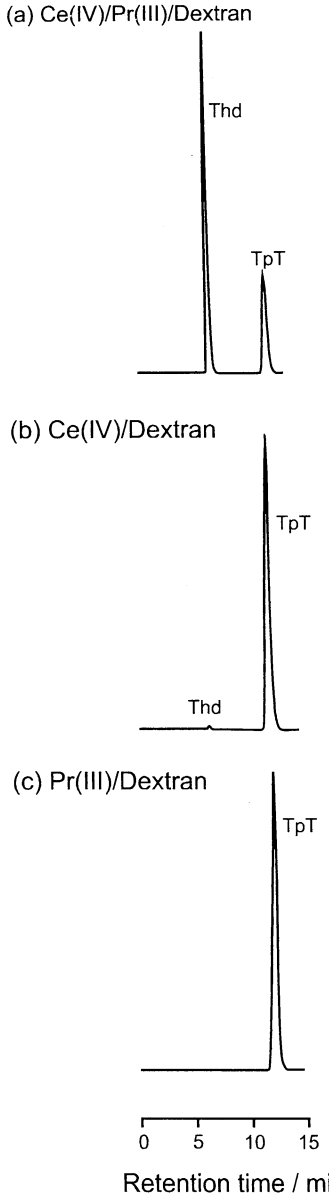

Figure 1. Reversed-phase HPLC profiles for the hydrolysis of TpT at pH 7.0 (50 mmol dm^{-3} Hepes buffer) and 50 °C for 10 h by (a) Ce(IV)/Pr(III)/dextran, (b) Ce(IV)/dextran, and (c) Pr(III)/dextran systems: [Ce(IV)] = 10, [Pr(III)] = 5, and [monomeric residue in dextran] = 20 mmol dm^{-3}. $[TpT]_0$ = 0.1 mmol dm^{-3}.

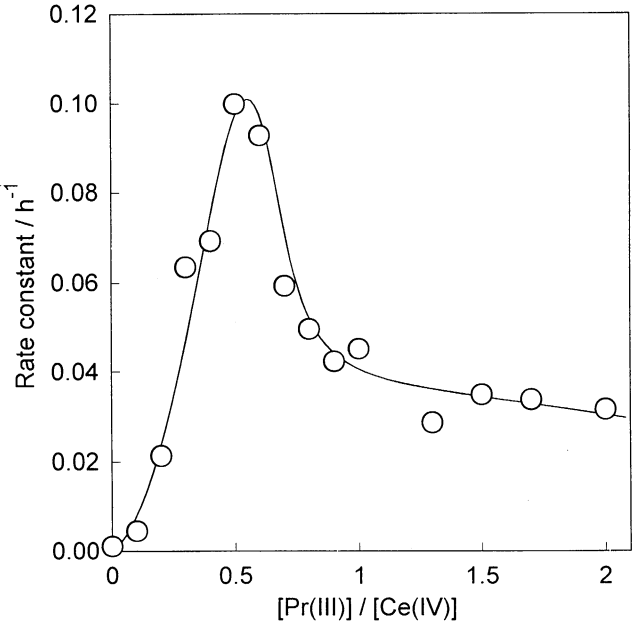

Figure 2. Dependence of the catalytic activity of Ce(IV)/Pr(III) /dextran ternary system on [Pr(III)]/[Ce(IV)] ratio for TpT hydrolysis at pH 7.0 and 50 °C: [Ce(IV)] and [monomeric residue in dextran] were kept constant at 10 and 20 mmol dm^{-3}, respectively.

added to pH 7 buffer in the absence of dextran, white precipitates were immediately formed. All the three components (Ce(IV), Ln(III), and dextran) are essential for homogeneous DNA hydrolysis.[10]

Figure 2 depicts the plot of rate of TpT hydrolysis as a function of the amount of $PrCl_3$; the concentrations of $Ce(NH_4)_2(NO_3)_6$ and dextran (in monomeric residue) are kept constant at 10 and 20 mmol dm^{-3}, respectively. All the solutions are homogeneous throughout the [Pr(III)]/[Ce(IV)] ratio investigated. The catalytic activity dramatically increases with the increase in [$PrCl_3$], and takes the maximum around [Pr(III)]/[Ce(IV)] ratio = 0.5.[11] This result is consistent with the previous conclusion[6] (obtained in the absence of dextran) that 2:1 Ce(IV)/Pr(III) mixed hydroxide cluster is responsible for the remarkable DNA hydrolysis. Formation of a ternary complex from Ce(IV), Pr(III), and dextran (as well as acid/base cooperation of these metal ions for the catalysis) is strongly indicated.

In conclusion, homogeneous polymer complex solutions, which efficiently hydrolyze linear DNA, have been prepared for the first time. The finding should open the way to design of still more active and useful catalysts for DNA hydrolysis.

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9 In this case, however, the homogeneous solution gradually became turbid as the reaction proceeded.
10 No homogeneous solutions were obtained when either amylose or poly(vinylalcohol) was used in place of dextran. The metal complexes with poly(acrylic acid) were inactive for DNA hydrolysis.
11 The gradual decrease of the rate at the [Pr]/[Ce] ratio > 0.5 is probably associated with the competitive binding of the substrate by free Pr(III) ion in the solutions.