# Catalysis of DNA cleavage by lanthanide complexes with nucleophilic or intercalating ligands and their kinetic characterization

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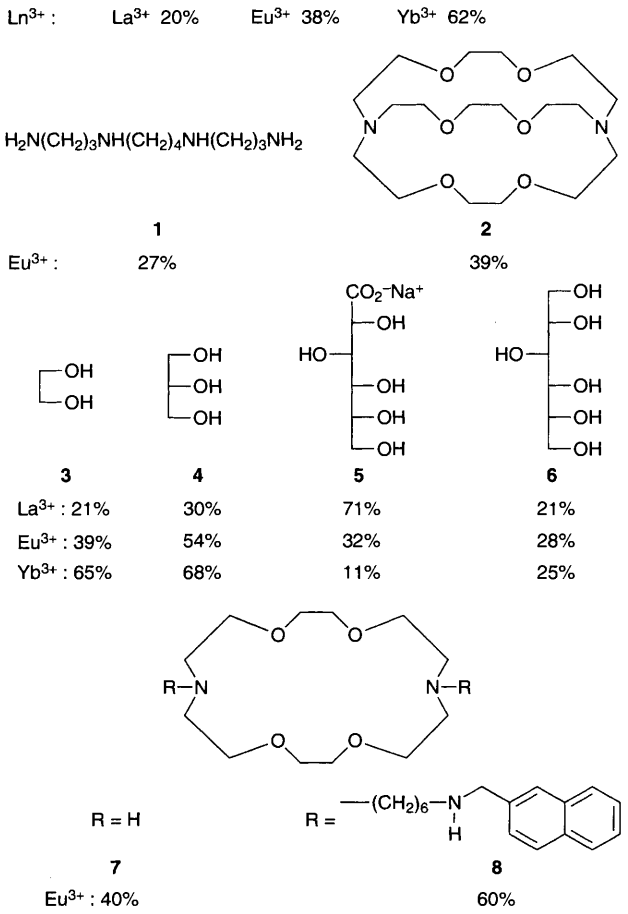
**The catalysis of plasmid ds-DNA cleavage by lanthanoid cations and their complexes is characterized by saturation kinetics; addition of polyamines or cryptand does not hinder catalysis, which can be enhanced to a total factor of $10^7$ by alcoholic co-substrates such as glycerol, or by intercalating ligands.**

The hydrolytic cleavage of nucleic acids by lanthanides[1] is of considerable interest, in particular for possible biotechnological applications. The extremely long half-life time of DNA approaching more than 100 million years for total hydrolysis[2] makes the development of supramolecular catalysts a particular challenge. With few exceptions,[3] the up to date available chemical nucleases cleave DNA by radical processes,[4] with the disadvantage of less discriminate reactions of *e.g.* highly reactive hydroxy radicals, and of concomitant partial destruction of nucleosides. The rate enhancements found until now with lanthanides[1] for such synthetic catalysts are unusually large, but still smaller by orders of magnitudes in comparison to natural nucleases. Furthermore, enzyme-like kinetic behaviour of the artificial nucleases has until now not been characterized. We report for the first time on the saturation kinetics with native DNA, and on preliminary attempts to increase the activity by ligands containing additional nucleophiles. A further increase was expected by implementation of intercalating groups in the ligands. In addition we investigated the effect of different lanthanide ions including $Ce^{4+}$, and the hitherto unknown effect of polyamines on the lanthanide-induced cleavage of DNA.

Cleavage of one of the two strands of the native supercoiled form I of plasmidic DNA is known to lead to the open circular form II, and, by further reaction at the second strand, to the linear form III. The cleavage products can be separated by gel electrophoresis and quantified by densitometry after staining with ethidiumbromide (data corrected for the decreased stainability of form I by a factor of 1.22[5]). The presence of polyamines such as spermine **1**, or nitrogen-containing macrocyclic ligands, however, inhibit or slow down the migration of DNA in the electric field by neutralizing the anionic charge. We therefore developed a method, which using ion-exchange resins, allows microscale removal of the polyamines after incubation. It was then established that even the presence of a 2500-fold excess (calculated per DNA base pair) of the highly charged spermine barely interferes with the action of $Eu^{3+}$ ions (Scheme 1).

That the DNA cleavage occurs hydrolytically and not by radical redox-type mechanisms was supported by the finding




**Scheme 1** Structures of co-substrates and metal complex ligands. The proportion of form II of plasmid DNA is given for each structure, measured after incubation for 2 h with $5 \times 10^{-4}$ mol $dm^{-3}$ metal and $5 \times 10^{-3}$ mol $dm^{-3}$ co-substrate/ligand at 37 °C, pH 7.0 in $1 \times 10^{-2}$ mol $dm^{-3}$ EPPS.

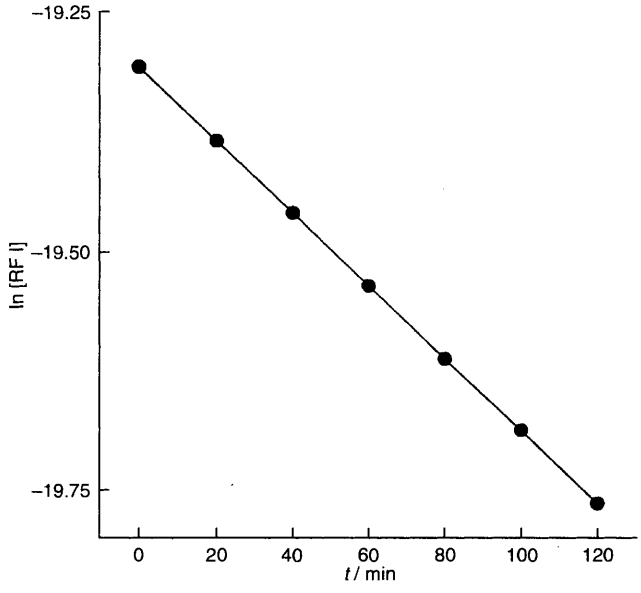



**Fig. 1** Example for pseudo-first order linearization for ds (plasmidic) DNA cleavage with $5 \times 10^{-4}$ mol $dm^{-3}$ $Eu^{3+}$, 37 °C, pH 7.0, incubation 20–120 min

that addition of hydrogen peroxide did not enhance the observed rates. Thus, even $10^{-3}$ mol dm$^{-3}$ $H_2O_2$ after 2 h incubation in the presence of $5 \times 10^{-3}$ mol dm$^{-3}$ $Eu^{3+}$ showed the same rate (68 ± 2% form II) as the metal ion alone. By contrast, $[Cu^{2+}] = 10^{-4}$ mol dm$^{-3}$ with $[H_2O_2] = 10^{-3}$ mol dm$^{-3}$ was reported[4e] after 30 min to leave only about 10% of form I and to produce substantial amounts of form III. A hydrolytic pathway is furthermore in line with the absence of other cleavage products as indicated by electrophoresis, as well as with the results of the recent analysis by Komiyama *et al.*,[1b] who observed only hydrolytic products even with the redox-active $Ce^{4+}$-ion reaction with dinucleotides.

The kinetic analysis of plasmid DNA cleavage exhibited an excellent linear behaviour based on a pseudo-first order equation (see Fig. 1.). The plot of the thus accessible rate constants against catalyst concentration allowed for the first time a Michaelis–Menten analysis[6] (Fig. 2) with native DNA. This yields a value of $k_{cat} = 4.2 \times 10^{-3}$ min$^{-1}$, which could be realized also experimentally with $[Eu^{3+}] = 2.5$ mmol dm$^{-3}$. Compared to the only approximatly known uncatalysed hydrolysis rate[7] of ds-DNA, the acceleration reaches a factor of $7 \times 10^6$. The $K_M$ value obtained from the non-linear curve fit (Fig. 2) is $3.9 \times 10^{-5}$ mol dm$^{-3}$. This indicates binding to two phosphate residues in view of the observed $K_M = 2.9 \times 10^{-3}$ mol dm$^{-3}$ (ref. 9) for the hydrolysis of singly charged diphenylphosphates with $Eu^{3+}$, in accord with the average value of $K_D \approx 10^{-1}$ mol dm$^{-3}$ per single salt bridge. The latter value has been found with many ion pairs,[9] including those with ds-DNA.[10a] The high binding constant obtained from the saturation kinetics explains the negligible influence of spermine on the catalysis, as the latter has (with $K = 2 \times 10^5$ mol dm$^{-3}$)[10b] a similar affinity to DNA.

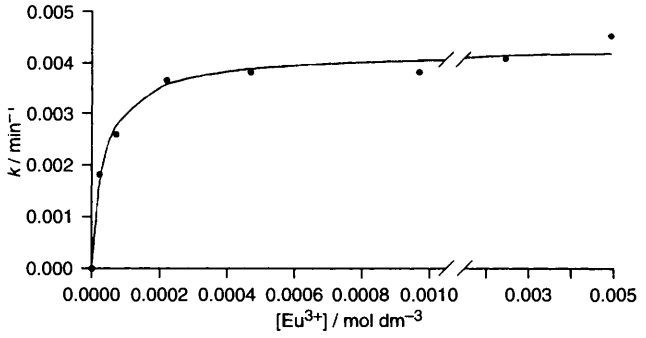
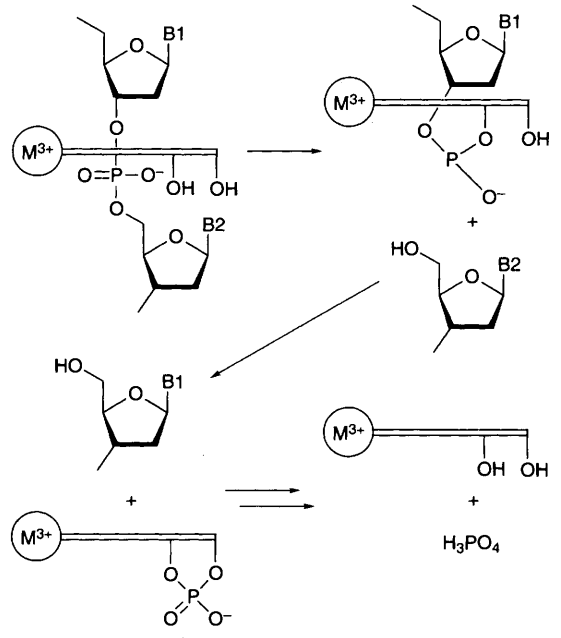



**Fig. 2** Saturation kinetics for ds (plasmidic) DNA with $2.5 \times 10^{-5}$–$5 \times 10^{-3}$ mol dm$^{-3}$ $Eu^{3+}$, 37 °C, pH 7.0; non-linear least-squares fit[6] and experimental points; $k_{cat} = 4.2 \times 10^{-3}$ min$^{-1}$, $K_M = 3.9 \times 10^{-5}$ mol dm$^{-3}$




**Scheme 2** Proposed mechanism of polynucleotide hydrolysis by phosphoryl transfer on several nucleophiles (such as OH groups) contained in the metal ligand

Measurements with diarylphosphates as model esters have already shown[8] negligible inhibiting effect on the $Eu^{3+}$ catalysis even with ligands such as [2.2.2]cryptand which encapsulates the metal ion to a large degree. The same result is now obtained with measurements with DNA (Scheme 1); this is important in view of the high thermodynamic and kinetic stability of such complexes[11] required for applications and modifications.

With respect to the different metal ions we found the activity of $Yb^{3+}$ to be larger than that of $Eu^{3+}$, which again is above that of $La^{3+}$, in agreement with results of Komiyama *et al.*[1a] Thus, after 2 h incubation, form II is formed in 20, 38 and 62% abundance with $La^{3+}$, $Eu^{3+}$ and $Y^{3+}$, respectively ($[Ln^{3+}] = 5 \times 10^{-4}$ mol dm$^{-3}$). In contrast to the hydrolytic activity of $Ce^{4+}$ reported independently by Matsumoto and Komiyama[1a] and Takasaki and Chin[12] for reactions with deoxydinucleotides we found the activity of $Ce^{4+}$ towards (plasmid) DNA similar, or even less active than *e.g.* $Eu^{3+}$. At concentrations $> 10^{-3}$ mol dm$^{-3}$ one also observes cloudiness or precipitation with $Ce^{4+}$ at pH 7.

In order to achieve possibly a complete and hydrolytic release of the phosphates from intact nucleoside ends, and to enhance the catalytic activity, we explored the strategy outlined in Scheme 2. The presence of several nucleophilic groups, such as OH substituents in the metal ligand sphere, should lead to a consecutive transfer of the phosphoryl group to several nucleophiles. This may lead to similar rate enhancements as seen in RNA, in which the presence of the 2′-OH group *via* the cyclic phosphate leads to a reactivity which is several magnitudes higher than that of DNA. After transfer of the phosphoryl group to the first OH group a second neighbouring nucleophile could take over, and thus enable the total and fast release of the phosphate.

The ligands and/or co-substrates shown in Scheme 1 indeed lead partially to a substantial effect beyond the activity of the metal ion alone. The largest accelerating effects of co-substrates are observed with glycerol **4** and with gluconate **5**, which,

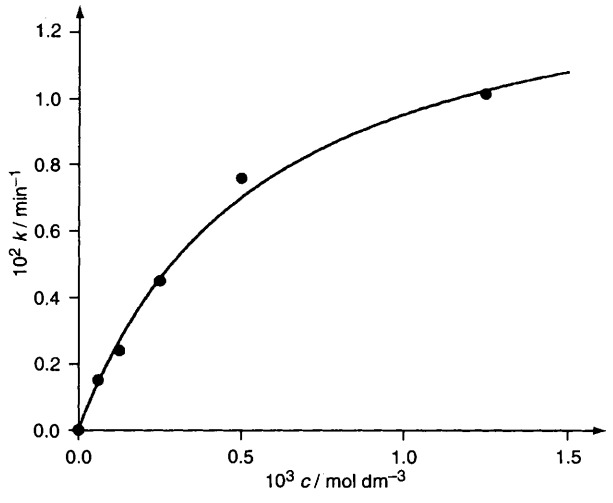



**Fig. 3** Saturation kinetics measured with $Eu^{3+}$ complex of ligand **8** (conditions: see Fig. 2); $k_{cat} = 3.5 \times 10^{-2}$ min$^{-1}$; $K_M = 5.7 \times 10^{-4}$ mol dm$^{-3}$

however, in water show only weak complexation with the metal ions studied. In contrast, ligands **7** and **8** bind more strongly to the cations, and show enhanced activity. As NMR measurements with naphthylalkylamines have unequivocally established intercalation into ds-DNA,[13] we have prepared the corresponding ionophore **8**, which also contains additional nitrogen atoms as nucleophiles. The percentage increase of form II obtained with differing catalysts in Scheme 1 appears undramatic, but it should be borne in mind that the maximum increase even with 100% form II cannot exceed an improvement factor of 2–3. With ligand **8** we additionally carried out a Michaelis–Menten analysis (Fig. 3), which indicated an approximately ten-fold increase of $k_{cat}$ in comparison to $Eu^{3+}$ alone.

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