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Journal of Chromatography B, 800 (2004) 169–173
JOURNAL OF CHROMATOGRAPHY B
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Cleavage of double stranded plasmid DNA by lanthanide complexes

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Abstract

The use of free lanthanide ions and their complexes for plasmid DNA pBR322 and chromosomal DNA cleavage was studied. Plasmid pBR322 DNA was treated by lanthanide chlorides (Eu^{3+}, La^{3+}, Nd^{3+}, Pr^{3+}, Gd^{3+}) in HEPES buffer (pH 7.0, 7.5 and 8.0) at 24, 37, 50, 63, and 76 °C. The formation of linear and nicked plasmid forms was investigated depending on the reaction conditions. Heterogeneous lanthanide complexes of ethylenediamine tetraacetic acid (EDTA) immobilized on insoluble methacrylate support and iminodiacetic acid (IDA) immobilized on styrene support were used as catalysts plasmid for DNA pBR322 cleavage, too. The temperature of reaction mixture had substantial influence on cleavage rate. The precipitation of DNA occurred during the measurement of interactions between chromosomal DNA and La^{3+} ions.

Keywords: Cleavage; Lanthanide complexes; Plasmid DNA pBR322

1. Introduction

Molecular biotechnology applications are based on isolation and transfer of genes (DNA) from one organism into another [1,2]. In vitro, DNA molecules of bacteria, viruses and higher species are manipulated using naturally occurring enzymes—including restriction nucleases. The development of synthetic, sequence-selective cleavage agents and structure probes for DNA itself and for DNA-bound drugs are essential for further expected applications in molecular biology, medicine and related fields.

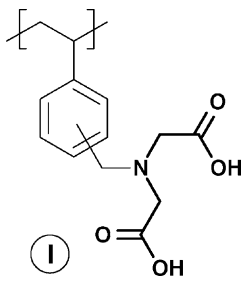
Most of the known synthetic catalysts for DNA hydrolysis are metal ions and their soluble or even insoluble complexes. In particular, lanthanides are known as excellent catalysts of DNA cleavage [3–5]. They hydrolyze phosphodiester linkages in simple phosphate esters, oligonucleotides, RNA and single stranded DNA at reasonable rates under physiological conditions [3,6–8]. There are some papers dealing with the cleavage of plasmid DNA by non-lanthanide ions [9,10] and lanthanide ions [11,12]. The use of small-molecule complexes for lanthanide-mediated oligonucleotide hydrolysis are reviewed in paper [13]. Insoluble complexes are advantageous for their simple removal from reaction mixture [10,14].

The aim of this work was to prepare insoluble polymeric lanthanide complexes and to compare the effect of both free lanthanide ions and their complexes for chromosomal and plasmid DNA pBR322 cleavage.

2. Materials and methods

2.1. Chemicals

Agarose was purchased from Lachema (Brno, Czech Republic), ethidium bromide, restrictase *Eco*RI, DNA marker (used in gel electrophoresis) lambda DNA/*Hin*dIII, lanthanide chlorides from Sigma (St. Louis, USA). Plasmid DNA pBR322 was obtained from New Englands BioLabs (Hertfordshire, Great Britain), chromosomal DNA from Reanal (Budapest, Hungary). Linearized DNA pBR322/*Eco*RI (4322 bp) was prepared according to the published protocol [15]. Chelex 100 (I), sorbent with immobilized iminodiacetic acid functional groups on 1% cross-linked polystyrene (concentration: 0.6 mmol/g) was from Sigma (St. Louis, USA).



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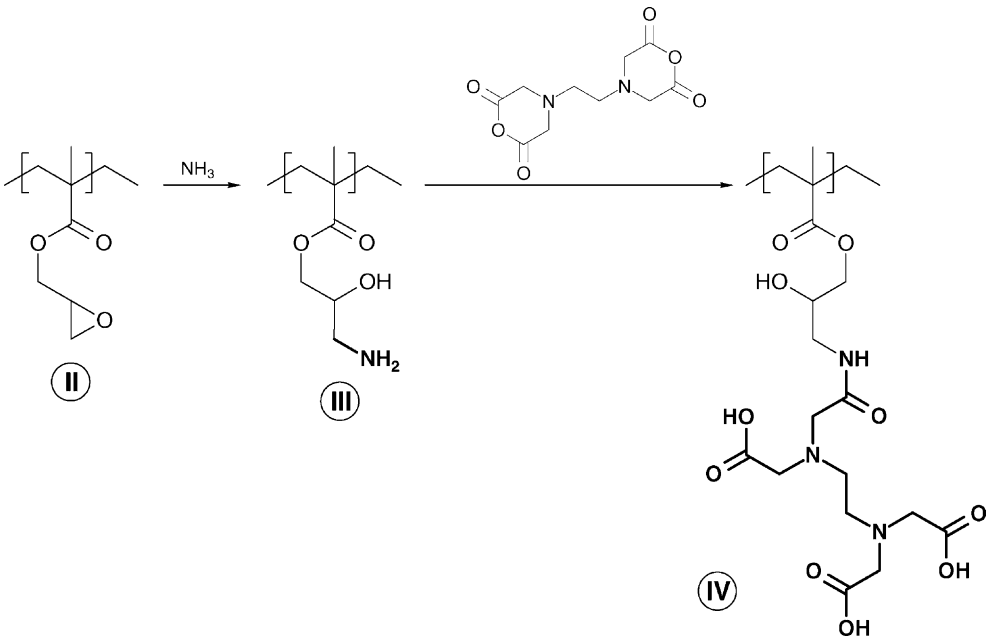
doi:10.1016/j.jchromb.2003.09.011

The other common chemicals and solvents were of analytical grade.

2.2. Equipment

Agarose gel electrophoresis was carried out using a 3000 Xi power supply (Bio-Rad Lab., Richmond, USA). Cleaved reaction products of pBR322 were visualized on UV transilluminator EB-20E (UltraLum, Paramount, USA), and photographed with a CD 34 Polaroid Camera (Polaroid, Cambridge, USA). Spectrophotometric measurements were carried out on a UV spectrophotometer DMS 100 (Varian, Mulgrave, Australia).



2.3. Methods

2.3.1. Preparation of carrier

The chelating macroporous methacrylate copolymer with ethylenediamine tetraacetic acid (EDTA) ligand was prepared from poly(glycidyl methacrylate-*co*-ethylene dimethacrylate-60/40) P(GMA-*co*-EDMA) II [16], particle size 4–8 μm, by two-step reaction. First step was ammonolysis [15] of epoxide groups II with aqueuos ammonia (28 wt.%, 14 ml) [16], for 2 days at room temperature [17,18]. The amino sorbent III was washed with water. It contained 1.67 mmol NH_2/g dry substance (according to nitrogen elemental analysis: N: 2.34%).

Amino derivative III (1 g) was suspended in 10 ml of anhydrous pyridine, refluxed under stirring with EDTA dianhydride (2.05 g) [19] for 2 h and then left standing over night at room temperature. The product was washed with water and remaining anhydride groups were hydrolyzed under mild conditions. Carrier IV contains 3.37% N. It corresponds 0.48 mmol EDTA groups/g dry substance (calculated from difference in nitrogen in III and IV).

2.3.2. Preparation of lanthanide complexes

Sorbent IV (0.06 g) was added to 3 ml of 0.05 M acetate buffer pH 5.5 and 0.05 M lanthanide chloride ($LnCl_3$) and the mixture was gently shaken 3 h under laboratory temperature. Then the mixture was washed three times with 10 ml of acetate buffer and three times with 10 ml of sterile distilled water. The sorbent was resuspended in 1 ml of water and diluted six times before use (0.01 g/ml).

2.3.3. Measurement of lanthanides activity

Plasmid pBR322 DNA was treated by lanthanide chlorides (Eu^{3+}, La^{3+}, Nd^{3+}, Pr^{3+}, Gd^{3+}) in HEPES buffer (pH 7.0, 7.5 and 8.0) at 24, 37, 50, 63, and 76 °C. The reaction time was upto to 3 h (for free lanthanides) or upto 10 h (for immobilized lanthanides). Using free lanthanide ions, the cleavage mixture contained: 2 μl of 100 mM solution of appropriate ion, 4 μl of 50 mM HEPES buffer, 0.2 μl of plasmid DNA (1 μg/μl). The volume of reaction mixture was adjusted to 20 μl with water. The reaction was stopped with 5 μl of 0.5 M EDTA (pH 8.0).

The cleavage activity of immobilized system was assayed after incubation of 10 μl of a water suspension containing 0.1 mg of IV–Ln^{3+} (or 0.08 mg of I–Ln^{3+}), 0.2 μl of plasmid DNA (1 μg/μl) and 4 μl 50 mM HEPES buffer. The amount of carrier was choosen so that the same amount of active groups was in the reaction mixture. The volume of reaction mixture was adjusted to 20 μl with water. In the case of immobilized systems the reaction was stopped by cooling down and gel electrophoresis analysis was carried immediately.

Chromosomal DNA was assayed after incubation of 30 μl 100 mM La^{3+} ions, 0.1 ml of chromosomal DNA (1.6 mg/ml), 0.376 ml of acetate buffer (0.2 M acetic acid + 0.2 M sodium acetate, pH 5.0) and the total volume

of reaction mixture was adjusted to 3 ml. UV absorbance measurement [20] at 220–320 nm followed.

2.4. Gel electrophoresis

Gel electrophoresis was carried out in 1% agarose, at 2.5 V/cm for 3 h using TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0). DNA was stained with 0.5 μg/ml ethidium bromide and photographed under 305 nm UV light on a TT667 film with CD34 Camera. The presence of different plasmid forms (circular covalently closed (ccc); linear (lin); open circle (oc)) was monitored on the gel according to the shift of their mobilities. Linearized pBR322/*Eco*RI was used as the control of the presence of linear form.

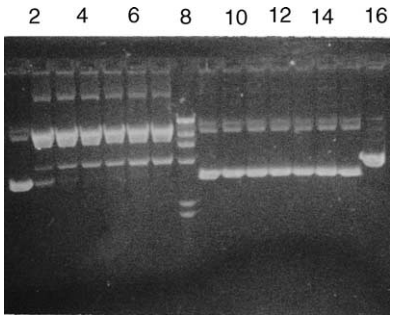


Fig. 1. Agarose gel electrophoresis of plasmid DNA pBR322 digested for upto 3 h at 63 °C by Nd^{3+} ions, HEPES buffer (pH 8.0). Lanes 1–7: plasmid DNA pBR322 treated with 10 mM Nd^{3+} ions for 0, 30, 60, 90, 120, 150, and 180 min, respectively; lane 8: λ DNA/*Hin*dIII marker; lanes 9–15: control plasmid DNA pBR322 (without Nd^{3+} ions) treated for 0, 30, 60, 90, 120, 150, and 180 min, respectively; lane 16: linearized DNA pBR322/*Eco*RI.

3. Results and discussion

DNA is highly negatively-charged polyelectrolyte, due to negative charges associated with their phosphate groups. In solutions, the charge neutralization of nucleic acids is presumably achieved through the interactions with positively-charged metal ions. From this point of view the DNA counter-ion interactions have critical importance in the study of nucleic acids properties. The phosphate groups of DNA are strongly affected among many metal ions by lanthanide ions [21] too.

3.1. Cleavage of plasmid DNA

A model system using plasmid pBR322 was used for the evaluation of optimal experimental cleavage conditions. This model was successfully used in the characterization of the biological activity of DNase I immobilized on magnetic particles and activated by Mn^{2+} and Co^{2+} ions [22].

The cleavage of plasmid DNA pBR322 was tested in the presence of free lanthanide cations—Eu^{3+}, La^{3+}, Nd^{3+}, Pr^{3+} and Gd^{3+}. Hydrolysis of DNA was carried out in 10 mM HEPES buffer (at pH 7.0, 7.5, 8.0) and at temperatures 24, 37, 50, 63, and 76 °C for upto 3 h. The cleavage of one of the two strands of the supercoiled form of plasmid DNA was followed by its conversion to the open circular form and, after cleavage of the second strand, to the linear form. This cleaved plasmid products can be easily separated and characterized by agarose gel electrophoresis. The time of incubation in which linear plasmid form appeared and ccc plasmid form disappeared was chosen as evaluation criteria for lanthanide ion activity. Weak cleavage of plasmid DNA was detected at 50 °C (the results are not shown), pronounced at 63 and 76 °C. The results are given in Table 1. All the tested lanthanide ions introduced single-stranded and double-stranded DNA nicks into plasmid DNA creating oc and lin forms of different intensities. The catalytic activity of tested lanthanide ions was slightly different due to their various physico-chemical characteristics. The pH influence was not important in tested range (pH 7.0 and 8.0) in used experimental arrangement. For this reason, the cleavage was carried out at pH 8.0 in further experiments. The results of pBR322 DNA hydrolysis at pH 8.0 in the presence of Nd^{3+} ions at temperatures of 63 °C are given in Fig. 1.

The lanthanides are toxic to biological systems due to their similarity to Ca^{2+} ions [23]. The cleavage of plasmid DNA using lanthanide EDTA and IDA complexes in solution (non-immobilized) and immobilized form (I–Ln^{3+}, IV–Ln^{3+}) was studied due to their stability and resistence to metal exchange. No cleavage of plasmid DNA was de-

Table 1
Plasmid DNA pBR 322 cleavage at different temperatures using lanthanide ions (Ln^{3+}) and their chelates

Lanthanide	Temperature (°C)	Ln^{3+}/time (min)									
		Eu^{3+}		Gd^{3+}		La^{3+}		Nd^{3+}		Pr^{3+}	
		ccc	lin	ccc	lin	ccc	lin	ccc	lin	ccc	lin
Ln^{3+} (free)	63	30	30	30	30	60	60	120	30	120	30
	76	30	30	30	30	30	30	30	30	30	30
IV–Ln^{3+}	63	+++	−	+++	−	+++	−	+++	−	+++	−
	76	+	60	+	60	180	90	180	60	180	60
I–Ln^{3+}	63	60	30	90	30	180	30	60	30	60	30
	76	30	30	30	30	30	30	30	30	30	30

IV–EDTA complex, I–IDA complex; decrease of band intensity in reaction time 3 h: (+++); (+) band of strong and weak intensity; (−) no band.

tected at laboratory temperature (24 °C) after 3 days treatment if EDTA-lanthanides complexes in solution were used. No cleavage of plasmid DNA was detected within 3 h at temperatures of 76 °C, too. This fact was apparently caused by abundance of EDTA excess in the reaction mixture as immobilized lanthanide EDTA complexes has been active (see further). These results agree with literature [24,25] as the complexation of lanthanide ions with EDTA is used for the stopping of reaction. Plasmid DNA cleavage was detected in time shorter than 3 h at elevated temperature 76 °C only with the immobilized lanthanide complexes of EDTA (Table 1). No linear plasmid form but smear occurred using the carrier amount which corresponded to the 10 mM lanthanide concentration in solution due to the accident double stranded breaks in plasmid molecules. The linear form appeared using 10× lower amount of carrier. The reason of the change of catalytically active lanthanide concentrations is not clear. The cleavage of plasmid DNA pBR322 using immobilized Eu^{3+}, La^{3+}, Nd^{3+}, Gd^{3+}, and Pr^{3+} EDTA complexes at 76 °C is shown in Fig. 2. There are also other EDTA ion chelates than lanthanides which cleaved DNA. The EDTA complex of Fe^{2+} cleaved DNA by free radical mechanism in the presence of hydrogen peroxide. The fragments could not be re-ligated by ligase [26] as they were different from those produced by nucleases. The use of this system in biotechnology applications is, therefore, limited. Preferential hydrolysis of gap and bulge sites in DNA by Ce^{4+}/EDTA complexes at pH 7.0 and 37 °C was described [25].

The cleavage of plasmid DNA pBR322 using I–Ln^{3+} was carried out in next experiments. The results are given in Table 1. A comparable cleavage of plasmid DNA pBR322 using IDA complexes appeared at lower temperature (50 °C; results are not shown) than IV–Ln^{3+}. The time of incubation in which linear plasmid form appeared corresponded to the decrease of the stability constants [27,28] of related complexes (EDTA, IDA) or to the decrease of effective ionic radii [27]. The time of ccc plasmid form disappearance also corresponded to the decrease of the stability constants and effective ionic radii of tested lanthanides (except La^{3+}).

Sequence-selective and hydrolytic artificial nucleic (DNA and RNA) acid cleavage agents were prepared by the attachment of lanthanide ion-iminodiacetate complexes to DNA oligomer (sequence-recognizing sites) [29]. Supports with immobilized IDA as chelating reagents were also used for chromatographic separation of lanthanides [28,30]. The silica gel and hydrophilic methacrylate supports seem to give better separation compared with those on polystyrene [30]. Different bonding and steric environments affect the basicity of the IDA groups and hence produce different stability constants.

3.2. Interactions of free La^{3+} ions with chromosomal DNA

The cleavage of chromosomal DNA (for example using DNase I) is manifested by the increase of ultraviolet light absorption at 260 nm. In the case of DNA condensation the

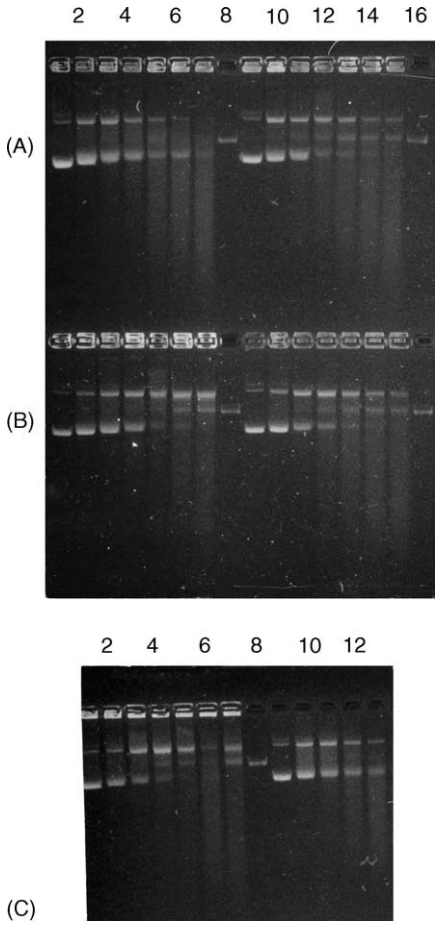


Fig. 2. Agarose gel electrophoresis of plasmid DNA pBR322 digested for upto 180 min at 76 °C by EDTA (IV) complexes with Eu^{3+}, Gd^{3+}, La^{3+}, Nd^{3+}, and Pr^{3+}. HEPES buffer (pH 8.0). (A) Eu^{3+} and Gd^{3+} complexes. (B) La^{3+} and Nd^{3+} complexes. (C) Gd^{3+} complex. Lanes 1–7 and 9–15: plasmid DNA pBR322 treated for 0, 30, 60, 90, 120, 150, and 180 min; lanes 8, 16: DNA pBR322/*Eco*RI. Lanes 1–7: plasmid DNA pBR322 treated for 0, 30, 60, 90, 120, 150, and 180 min; lane 8: DNA pBR322/*Eco*RI, lanes 9–13: control without particles for 0, 60, 120, 150, and 180 min.

absorbance increases, too [31]. However, no cleavage of double stranded DNA can be detected in this case using neutral agarose gel electrophoresis.

The precipitation of DNA occured during the spectrophotometrical measurement of interactions between chromosomal DNA and La^{3+} ions (the results are not shown). Precipitated DNA, whose absorbance at 260 nm decreased, was visible as a suspension of “cotton wool”. The precipitation of DNA occured in the presence of La^{3+} ions at 1 and 10 mM concentrations. Using agarose gel electrophoresis no cleavage of tested DNA was observed (after re-desolving of precipitated DNA). The La^{3+} salt of chromosomal DNA was more resistant to the action of DNase I activated by Mg^{2+} ions. DNA was not visualized after 3 min of cleavage with DNase I activated by Mg^{2+} ions using gel agarose electrophoresis. The same degree of cleavage was observed after 45 min DNase I treatment if the mixture of Mg^{2+} and

La^{3+} ions (10 mM) was used for their activation. However, no precipitation and the decrease of absorbance occured if EDTA was added to the reaction mixture.

4. Conclusion

The results presented in this report show that lanthanides ions (Eu^{3+}, La^{3+}, Nd^{3+}, Pr^{3+}, Gd^{3+}) are suitable nonenzymatic cleavage agents for the cleavage of plasmid DNA pBR322. In the pH range 6–8, their activity was not markedly changed. However, temperature had substantial influence on cleavage rate. Catalytically active are also heterogeneous IDA and EDTA lanthanide complexes on polymethacrylate or polystyrene type of supports. Higher cleavage activity was shown for carrier with IDA ligands. The carriers prepared in this way will be applied in the next applications.

Acknowledgements

Financial support by grants No. 202/02/1361 of the Grant Agency of the Czech Republic is gratefully acknowledged.

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