# Lanthanide Cleavage of Phosphodiester Liposomes

**Robert A. Moss,*[a] Byeong D. Park,[a] Paolo Scrimin*[b,c] and Giovanna Ghirlanda[b]**

[a] *Department of Chemistry, Rutgers University, New Brunswick, NJ, 08903, USA*
[b] *Department of Organic Chemistry, University of Padova, via Marzolo 1, 35131 Padova, Italy*
[c] *Department of Chemical Sciences, University of Trieste, Via Giorgieri, Trieste, Italy*

Cleavages of a liposomal phosphodiester by $Eu^{3+}$ or $La^{3+}/H_2O_2$ are attended by 50 to 70-fold rate accelerations relative to a non-aggregated model phosphodiester, whilst trans-bilayer $Eu^{3+}$ transport can be mediated by an hydrophobic ethylene diamine derivative.

The attempted construction of model artificial nucleases has focused attention on the catalytic hydrolysis of the polymeric phosphodiester, RNA. Many of the enzymes that catalyse nucleophilic displacement at phosphorus(v) require metal ions,[1] and lanthanide ions are now particular objects of investigation. They are good catalysts for the cleavage of phosphodiesters (*e.g.* **1**) which are normally resistant to nucleophilic reagents, presumably because the lanthanides supply both electrophilic acceptor sites for the phosphodiester's P–O$^-$, and M–OH nucleophiles for simultaneous attack at P=O.

Thus, even uncomplexed $Eu^{3+}$ ions enhanced the hydrolysis of **1** by *ca.* $10^8$ at pH 7, with saturation kinetics and turnover consistent with an 'enzyme-like' mechanism.[2] A $La^{3+}/H_2O_2$ complex (perhaps tetradeprotonated **2**) provided a 4.4 × $10^8$-fold enhancement in the cleavage of **1** at pH 7, whereas $La^{3+}$ cations alone afforded an enhancement of 1.3 × $10^4$.[3] Similarly, a quaternary complex of a bipyridyl-linked duplex β-cyclodextrin, $La^{3+}$, $H_2O_2$, and substrate **1** underwent cleavage with *ca.* $10^8$-fold rate enhancement.[4]

More recent reports describe a bis-8-hydroxyquinoline phosphodiester substrate that self-destructs in the presence of $La^{3+}$,[5] a mixed hydroxide cluster of $La^{III}$ and $Fe^{III}$ that exhibits cooperative catalysis in the cleavage of **1**,[6] and *ca.* $10^4$-fold catalysis in the cleavage of *p*-nitrophenyl(mono)phosphate by a $La^{III}$ (2.2.1) cryptate complex.[7] These observations are parallelled by applications to the cleavage of biological targets (*e.g.* dinucleotides), where $Tm^{III}$,[8] hexadentate aza complexes of $Eu^{III}$, $La^{III}$, $Gd^{III}$, and $Tb^{III}$,[9] as well as $Ce^{IV}$[10] proved particularly effective.

Some time ago, we prepared the pyridinium salt of the anionic phosphodiester lipid substrate **3** (PNP = *p*-nitrophenyl), liposomes of which could be *exo*/*endo* surface differentiated by exoliposomal *p*-nitrophenylate (PNPO) cleavage by external hydroxide ions at pH 11.8.[11] It occurred to us that (anionic) liposomes of **3** should strongly bind lanthanide cations which would then cleave the activated *p*-nitrophenyl phosphate moieties of the substrate lipids. Moreover, concentration of the lanthanide cations into the small volume of reaction space represented by the liposomes would be expressed as additional catalysis, over and above that afforded by the lanthanide ions themselves. In the event, these suppositions proved correct, and we describe here the initial examples of aggregate-amplified lanthanide cleavage of phosphodiesters.

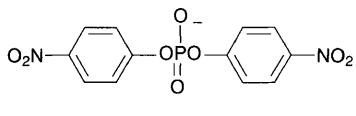
The sodium salt of 1,2-dipalmitoyl-3-glyceryl *p*-nitrophenyl phosphate was prepared in 72% yield by reaction of *p*-nitrophenyl phosphodichloridate with 1,2-dipalmitoyl-*rac*-glycerol in $CH_2Cl_2/Et_3N$ (0–25 °C, 2 h), followed by hydrolysis (5% aq. $NaHSO_3$ acidified to pH 1–2 with HCl, 10 min), isolation from the (dried) $CH_2Cl_2$ phase, and recrystallization from EtOAc (mp 50–52 °C).†

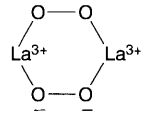
A suspension of 6 mg of **3** in 15 ml of pH 5.6, 0.01 mol dm$^{-3}$ aq. HEPES buffer was repetitively extruded (10x) through two stacked 200 nm and 100 nm polycarbonate filters (< 200 psig of nitrogen, 50–55 °C), affording a clear solution of liposomal **3** with an average diameter of 1370 Å (dynamic light scattering) and a critical temperature ($T_c$) of 41.8 °C (differential thermal analysis).

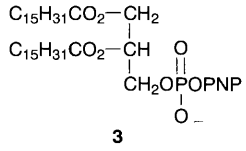
Liposomal **3** (6.5–9.9 × $10^{-5}$ mol dm$^{-3}$), prepared at pH 5.6 to minimize prior cleavage, was reacted with excess (1.2–6.0 × $10^{-3}$ mol dm$^{-3}$) $EuCl_3$ in 0.01 mol dm$^{-3}$ pH 7.2–7.3 HEPES buffer (μ = 0.01 KCl) at 25–27 °C. Pseudo-first-order kinetics were observed for the cleavage of PNPO from **3**, followed by UV spectrometry at 400 nm. Typical kinetic runs with 1.2 × $10^{-3}$ mol dm$^{-3}$ $Eu^{3+}$ at 25 °C gave $k_{obs}$ = 2.1 ± 0.3 × $10^{-4}$ s$^{-1}$. There was little increase in $k_{obs}$ with increasing concentration of $Eu^{3+}$ up to 5.3 × $10^{-3}$ mol dm$^{-3}$ ($k_{obs}$ = 2.4 × $10^{-4}$ s$^{-1}$), suggesting that at these excess $Eu^{3+}$ concentrations the liposomes were fully saturated with bound $Eu^{3+}$ ions. Importantly, the $Eu^{3+}$-mediated cleavage of liposomal **3** ceased after 50–55% of theoretical PNPO release, consistent with $Eu^{3+}$ cleavage proceeding only on the exoliposomal surface of the liposomes.‡ This point is elaborated below.

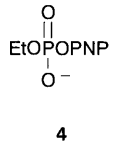
In order to estimate the catalysis due to liposomal aggregation, the kinetics of the $Eu^{3+}$ cleavage of PNPO from the non-aggregated model substrate (lithium) ethyl *p*-nitrophenyl phosphate **4**§ were determined under conditions and concentrations comparable with those described above. This afforded $k$ = 3.77 ± 0.01 × $10^{-6}$ s$^{-1}$, so that we estimate a kinetic advantage of *ca.* 56 for the $Eu^{3+}$-bound liposomal reactions of **3**, relative to the analogous reaction of **4**. Substrate **4** is *ca.* 40 times less reactive toward $Eu^{3+}$ than **1**,[2] for which the catalysis of PNPO cleavage due to $Eu^{3+}$ generates a kinetic enhancement of *ca.* $10^8$. However, aggregate amplification of the $Eu^{3+}$ cleavage of liposomal **3** more than compensates for the intrinsically lower reactivity of the alkyl *p*-nitrophenyl phosphate **3**, relative to bis-*p*-nitrophenyl phosphate **1**.

Liposomal **3** was also subjected to cleavage by the lanthanum-$H_2O_2$ reagent:[3] 7.4 × $10^{-5}$ mol dm$^{-3}$ liposomal **3** was treated with 1.4 × $10^{-3}$ $La(ClO_4)_3$/1.4 × $10^{-2}$ $H_2O_2$ in 0.01 mol dm$^{-3}$ HEPES buffer (μ = 0.01, KCl) at pH 7.0–7.18, 25 °C. The pseudo-first order rate constant for PNPO release was 1.4 × $10^{-3}$ s$^{-1}$, about seven times greater than that observed for the $Eu^{3+}$ cleavage, in keeping with the known greater reactivity of the $La^{3+}/H_2O_2$ reagent.[3] Cleavage of substrate **4** under analogous conditions afforded $k$ = 2.12 ± 0.05 × $10^{-5}$ s$^{-1}$, so that a kinetic advantage of *ca.* 66 is estimated for



the $La^{3+}/H_2O_2$ cleavage of liposomal phosphodiester **3**, relative to nonaggregated model **4**.

An important difference between the $Eu^{3+}$ and $La^{3+}/H_2O_2$ cleavages of liposomal **3** is that the La reaction does not stop after 50–55% (exoliposomal) PNPO cleavage, as is the case with $Eu^{3+}$. Rather, $La^{3+}$ cleavage continues, affording 70–80% of the theoretical PNPO after 7 h. Both $La^{3+}/H_2O_2$ and $Eu^{3+}$ mediate the cleavage of *p*-nitrophenyl(mono)phosphate,[2,3] but the more reactive La reagent might also be able to cleave the exoliposomal 1,2-dipalmitoyl-3-glyceryl phosphate molecules remaining after initial PNPO cleavage from liposomal **3**, thus creating leaky dipalmitolyglycerol 'patches' on the exoliposomal surface, or otherwise damaging the liposome so that reagent permeation and endoliposomal PNPO cleavage can proceed.

The surface-specific nature of the $Eu^{3+}$ cleavage of liposomal **3** is dramatically illustrated by following experiment, carried out with 580 Å sonicated liposomes. These were prepared in 0.025 mol $dm^{-3}$ HEPES buffer at pH 7, using 55% power output of a Braunsonic immersion probe sonicator for 10–15 min at 35 °C, followed by filtration of the liposomes through a 0.44 μ Millipore filter. When $5.4 \times 10^{-5}$ mol $dm^{-3}$ liposomal **3** was subjected to cleavage with $1.7 \times 10^{-3}$ mol $dm^{-3}$ $EuCl_3$ at 25 °C in 0.025 mol $dm^{-3}$ HEPES buffer at pH 7.0, liberation of PNPO occurred with $k = 4.1 \times 10^{-5}$ $s^{-1}$ and ceased after *ca.* 71% of the theoretical quantity had been released. At this point, addition of $2.7 \times 10^{-5}$ mol $dm^{-3}$ *N*-hexadecyl-*N,N,N*-trimethylethylene diamine[12,13] reinitiated PNPO cleavage, which continued at $2.7 \times 10^{-5}$ $s^{-1}$ until completion.¶

Two explanations can be offered for these observations. The hydrophobic diamine might chelate $Eu^{3+}$ ions, transporting them across the liposomal bilayer where they initiate endoliposomal PNPO cleavage. Alternatively, the single-chain diamine molecules could insert into the liposomes, affording leaky 'patches' that facilitate $Eu^{3+}$ permeation. The permeation of $Eu^{3+}$ ions, whatever its precise mechanism, appears to be rate limiting for the endoliposomal cleavage process: variation of the concentration of added diamine between $0.48–2.7 \times 10^{-5}$ mol $dm^{-3}$ (eight points) gave a linear correlation with the apparent rate constants $0.98–2.7 \times 10^{-5}$ $s^{-1}$ for the endoliposomal cleavage of **3**.

We are continuing our studies of lanthanide-aggregate phosphodiester cleavage reactions, including the use of other lanthanide ions, other kinds of aggregates, and other ionophores.

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## Footnotes

† Appropriate $^1H$ and $^{31}P$ NMR spectra and a satisfactory C, H, N analysis were obtained. A previous preparation of **3** afforded the pyridinium salt with a lower mp and liposomal critical temperature.[11]

‡ PNPO was determined after 13 h from its absorbance at 400 nm, read after adjustment of the pH to 8. Little change in PNPO absorbance was observed after the initial 6 h of reaction. 'Total' PNPO absorbance was determined after complete PNPO cleavage, subsequently induced by reaction with 0.4 mol $dm^{-3}$ KOH at 90 °C for 10 min.

§ Substrate **4** was prepared by LiBr deethylation of diethyl *p*-nitrophenyl phosphate in refluxing acetone (26 h). After trituration in pentane/diethyl ether, **4** (mp 260 °C, decomp.) contained 0.2 equiv. of LiBr and was a monohydrate. The $^1H$ and $^{31}P$ NMR spectra and the elemental analysis (C, H, N) were appropriate to this composition.

¶ The 580 Å sonicated liposomes of **3** have a higher curvature than the 1370 Å extruded liposomes, and exhibit a higher *exo/endo* liposomal functional group ratio. The sonicated liposomes also appear to react with $Eu^{3+}$ more slowly than the extruded liposomes. The smaller, sonicated liposomes are preferred for the permeation experiments because extensive precipitation occurs when the extruded liposomes are treated with the diamine.

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