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Vesicles and Micelles from Amphiphilic Zinc(II)−Cyclen Complexes as Highly Potent Promoters of Hydrolytic DNA Cleavage

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Supporting Information

ABSTRACT: Phosphate esters are essential to any living organism and their specific hydrolysis plays an important role in many metabolic processes. As phosphodiester bonds can be extraordinary stable, as in DNA, great effort has been put into mimicking the active sites of hydrolytic enzymes which can easily cleave these linkages and were often found to contain one or more coordinated metal ions. With this in mind, we report micellar and vesicular Zn(II)−cyclen complexes which considerably promote the hydrolytic cleavage of native DNA and the activated model substrate bis(4-nitrophenyl)phosphate (BNPP). They are formed by self-assembly from amphiphilic derivatives of previously employed complexes in aqueous solution and therefore allow a simple and rapid connection of multiple active metal sites without great synthetic effort. Considering the hydrolytic cleavage of BNPP at 25 °C and pH 8, the micellar and vesicular metal catalysts show an increase of second-order rate constants (k_2) by 4−7 orders of magnitude compared to the unimolecular complexes under identical conditions. At neutral pH, they produce the highest k_2 values reported so far. For pBR322 plasmid DNA, both a conversion of the supercoiled to the relaxed and linear form, and also a further degradation into smaller fragments by double strand cleavages could be observed after incubation with the vesicular Zn(II)−complexes. Finally, even the cleavage of nonactivated single-stranded oligonucleotides could be considerably promoted compared to background reaction.

Phosphate esters are ubiquitously found in nature and essential to any living being as DNA with its phosphodiester backbone contains the genetic code of an organism. Therefore, these biomolecules need to possess a sufficient stability under physiological conditions to prevent premature degradation or accidental altering. DNA has an estimated half-life of 30 million years concerning the spontaneous hydrolysis of a single phosphodiester bond.[1]

Nevertheless, the specific hydrolysis of such very stable phosphodiester bonds plays an important role in metabolic and regulatory processes[2] and is easily performed by specific enzymes, which often contain metal ions in their active sites.[3] Understanding and mimicking these enzymes still represents a challenge for chemists. A variety of different synthetic catalysts for hydrolysis of DNA and corresponding model substrates have been reported, usually based on lanthanide[4] or transition metal ions like Cu(II), Ni(II), Co(II), and Zn(II).[5] Although Zn(II) ions were occasionally reported to be less efficient, they are the choice of hydrolytic enzymes. Therefore, the design of zinc-based catalysts for phosphodiester hydrolysis is a favorable biomimetic strategy.[6] Covalent connection of two or more Zn(II)-catalytic centers by a defined spacer has allowed to achieve higher catalytic rates for the cleavage of nonactivated phosphate ester bonds than those observed only for a single Zn(II)-center.[6a] Combination of catalytic centers through noncovalent interactions in a metallomicelle[7] as well as immobilization on polymer supports[8] or on nanoparticles[9] are other successful strategies to increase hydrolytic activity.[10]

We report here self-assembled catalytic systems which are able to promote the hydrolysis of both native DNA and activated model substrates. The catalysts are based on amphiphilic derivatives of the Zn(II)-complex of 1,4,7,10-tetraazacyclododecane, which was previously found to promote the hydrolytic cleavage of phosphodiester substrates.[11] In buffered aqueous solution the amphiphilic catalysts Zn**1** and Zn_2**2** spontaneously assemble either into homomicelles or into vesicular membranes if mixed with a suitable lipid component like 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC; Scheme 1).

Syntheses and preparation procedures have been previously described by us[12] or are given in the Supporting Information (SI). A series of mono- and bis-cyclen-based zinc complexes (Zn**1**−Zn_2**5**) have been tested for the hydrolysis of the simple DNA model substrate bis-(4-nitrophenyl) phosphate (BNPP) in the pH range from 7 to 9 by following the absorption (Abs) increase of released nitrophenolate (NP) at 408 nm.[11] We have employed our amphiphilic catalyst derivatives as micellar and vesicular solutions by adding them to 1−10 mM solutions of BNPP and recorded kinetics using an initial slopes method in TRIS buffer (50 mM) at a slightly basic pH of 8.0, as these are common conditions for DNA hydrolysis experiments and allow a comparison of rate constants with reported values, and in HEPES buffer (25 mM) at neutral pH (7.4), which renders the hydrolysis conditions more challenging.

While at pH 8 Zn_2**4** represents one of the most active Zn(II) complexes for this reaction,[7d] we observed that hydrolytic rates are exceeded by more than 4 orders of magnitude using Zn_2**2** micelles (at 2.3 mM BNPP and 0.045 mM Zn_2**2**; Table 1).[13] The rate constant for Zn**1**-micelles under these conditions is 1 order of magnitude lower. However, compared to Zn**3** as reference, the micellar system outperforms the unimolecular catalyst by 7 orders of magnitude at identical experimental

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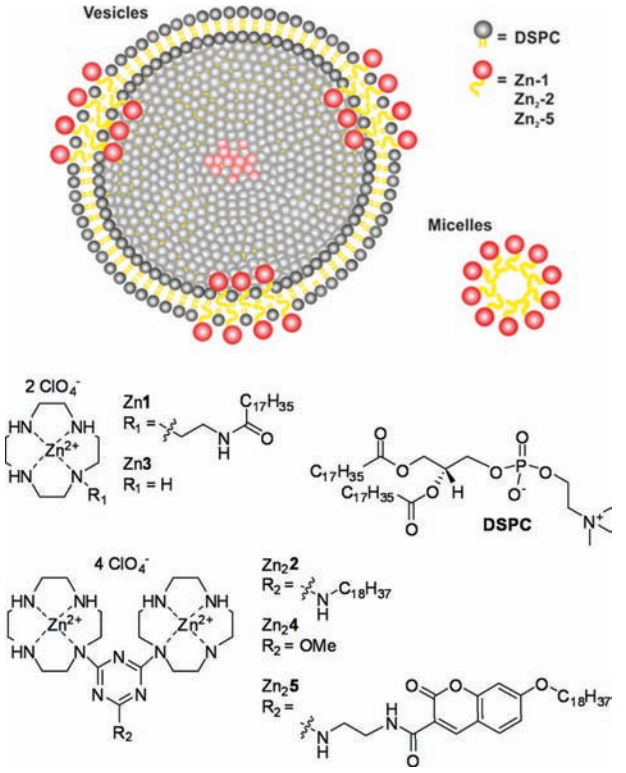
Scheme 1. (Amphiphilic) Zn(II)-Complexes and Vesicular/Micellar Catalyst Systems



conditions. At pH 7.4, Zn**1**-micelles give about the same rate constants as at pH 8, whereas the rate constant of Zn_2**2**-micelles drops by about 1 order of magnitude. As a result, both micellar catalysts give similar k_2-values under these conditions and still show a remarkable promotion of BNPP hydrolysis, whereas the unimolecular complexes Zn**3** and Zn_2**4** are not able to produce significant amounts of NP here.

After observing the remarkable rate increases for the micellar catalysts, we used the amphiphilic zinc complexes in vesicular catalysts optimizing a number of parameters: We prepared vesicles in sizes ranging from about 60−150 nm and compared their rate constants which were all in the same order of magnitude; however, the maximum activity was obtained at about 100 nm (see SI, Table S2 and Figure S9). As a result, we used these for all further experiments. Next we varied particle composition in terms of Zn(II)-content of the DSPC vesicle membrane. The peak activity here was found at loading levels of 10 mol % Zn_2**2** as shown by pseudo-first-order rate constants k_{obs} in Table S1. Higher amounts of metal complexes led to increasing particle destabilization (indicated by DLS, size exclusion experiments and shifted emission wavelengths, cf. Figure S4) and reduced catalytic efficiency. Generally best results were achieved at BNPP concentrations of 2.3 mM and 0.02 equiv of Zn(II) (see also Figures S7 and S8). At these conditions, the vesicular catalysts derived from Zn**1** and Zn_2**2** yielded second-order rate constants in the same order of magnitude as the Zn_2**2**-micelles (Table 1).

All kinetic data showed excellent reproducibility (cf. Figure S14) and control experiments with pure DSPC vesicles containing no metal complexes and buffer solution were performed to determine background hydrolysis. No significant amounts of the hydrolysis product nitrophenolate could be detected here (Figure S13).

Table 1. Second-Order Rate Constants (k_2) for BNPP Hydrolysis at Neutral pH and 25 °C unless Otherwise Stated and Second-Order Rate Constants Relative to the Hydroxide Promoted Reaction (k_{rel})

entry	catalyst	T (° C)	pH	k_2 (M^{-1} s^{-1})	k_{rel}	ref
1	OH^-	35	7	2.4×10^{-5}	1	20a
2	Zn**1**-vesicles (DSPC/Zn**1** 9:1)	25	8	1.5	n.d.	this work
3	Zn**1**-vesicles (DSPC/Zn**1** 9:1)	25	7.4	1.8	75000	this work
4	Zn_2**2**-vesicles (DSPC/Zn_2**2** 9:1)	25	8	24	n.d.	this work
5	Zn_2**2**-vesicles (DSPC/Zn_2**2** 9:1)	25	7.4	9.4	391667	this work
6	Zn**1**-micelles	25	8	7.9	n.d.	this work
7	Zn**1**-micelles	25	7.4	7.4	308333	this work
8	Zn_2**2**-micelles	25	8	68	n.d.	this work
9	Zn_2**2**-micelles	25	7.4	7.9	329167	this work
10	Zn**3**	25	8	6.3×10^{-7}	n.d.	11
11	Zn**3**	25	7.4	-[a]	-[a]	this work
12	Zn_2**4**	25	8	1.3×10^{-3}	n.d.	11
13	Zn_2**4**	25	7.4	-[a]	-[a]	this work
14	Zn(II)-micelles	35	10	4.3×10^{-4}	n.d.	7a
15	Zn(II)-MPGNs	40	7	1.5	62500	9
16	Ce(IV)-micelles	37	7	0.6	25000	20b
17	Ce(IV)-MPGNs	40	7	0.93	38750	20b

[a]Below detection limit.

We explain the dramatically increased reaction rates of our self-assembled catalyst systems by the high local concentration of coordinated Zn(II) ions either in the metallomicelles or in tightly packed domains of metal complexes embedded in the bilayer membranes[14] and the decreased polarity[15] at the vesicle bilayer−water interface, which can be compared to MeOH/$CHCl_3$-mixtures (Figure S5) and therefore facilitates a nucleophilic attack of the phosphodiester substrate compared to bulk water. Despite the high reaction rates, however, no complete conversion of the BNPP substrate could be achieved by micelles or vesicles as indicated by the saturation Abs values and the produced NP equivalents (see also SI). Although this was not observed in the previous studies with Zn**3** and Zn_2**4**, product inhibition might explain limited turnovers as much lower amounts of Zn^{2+} (0.02 equiv) were used for micellar and vesicular catalysis compared to the unimolecular complexes which were usually employed stoichiometrically.[11] A large excess of BNPP and NP(P) might replace counterions (like reactive OH^-) in the surrounding layer of the densely packed Zn^{2+} aggregates and therefore reduce their catalytic activity or even lead to particle destabilization due to charge neutralization (cf. Figure S8).

The high hydrolysis rates observed with the activated phosphate diester BNBP encouraged us to expand the study to the hydrolysis of less activated oligonucleotides. pBR322 plasmid DNA is a widely used test substrate for artificial hydrolases. The dsDNA is transformed by strand cleavage from

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its initial supercoiled strained form (I) into its relaxed circular form (II) and further on to its open linear form (III). All forms are distinguishable by agarose gel electrophoresis.[16] Supercoiled pBR322 (100 ng) with a size of 4.3 kb was incubated with Zn_2**2**-vesicles (1×10^{-4} Zn(II)) at pH 7.4 and 40 °C for different periods of time before the reactions were stopped with an excess of SDS and EDTA. The obtained samples were then separated on agarose mini gels (0.7% w/w) and stained with ethidium bromide. As shown in Figure 1, the supercoiled DNA

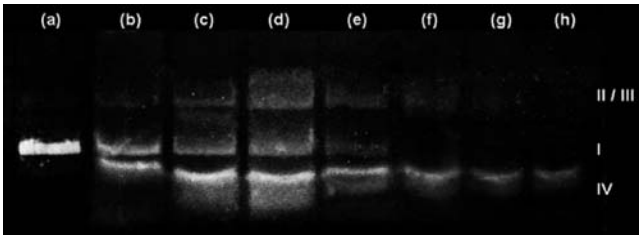

Figure 1. Agarose gel (0.7%) electrophoresis of pBR322 (100 ng per lane) vs Zn_2**2**-vesicles (1×10^{-4} mM) incubated at 40 °C at pH 7.4 for (a) 0, (b) 4, (c) 11, (d) 24, (e) 33, (f) 38, (g) 42, (h) 48 h.

form (I) is completely degraded with increasing incubation times of up to 38 h. However, it is not only transformed to forms II/III, but also to smaller DNA fragments (IV) with a size of approximately 2.0 kb[17] corresponding to about half the size of the plasmid. The band of these fragments appears on the gel even before the bands of form II/III indicating that double-strand cleavages take place. The cleavage is not selective and all bands broaden with progressing incubation times; after 24 h, all four forms are present. The bands become subsequently weaker with time and convert into a smear around band IV. Such degradation is remarkable under these conditions and usually only reported for oxidative degradation conditions.[18] Control experiments using vesicles without Zn_2**2** and only buffer solution showed that the supercoiled DNA remained stable under the otherwise unchanged experimental conditions. Additionally, separate samples of the vesicular catalysts were applied to the gels without DNA to exclude the occurrence of fluorescent bands by vesicle−ethidium bromide interactions (Figure S17).

After observing high hydrolysis rates for BNPP and on strained plasmid dsDNA, we tested our catalytic systems on a single-stranded oligonucleotide as a more challenging substrate. We chose the commercially available TaqMan probe consisting of a 24mer oligonucleotide with the random sequence CAAGTTTGACCAAGTCACAACGGC and a 5′ fluorescence label (FAM; λ_{exc} = 495 nm, λ_{em} = 520 nm) and 3′ quencher (BHQ-1 λ_{max} = 534 nm). The intact probe shows only very weak fluorescence intensity, but upon its cleavage at an arbitrary site, the effect of the quencher on the fluorophore diminishes and an increasing emission intensity can be monitored. For our hydrolysis assay, this probe (10^{-8} M) was incubated with Zn_2**2**-micelles and -vesicles at 40 °C for increasing periods of time. The reactions were stopped by addition of SDS and EDTA and fluorescence emission intensities at 520 nm were recorded and plotted against incubation time as shown in Figure 2 (inset). In contrast to the results for BNPP, the vesicular catalysts of Zn_2**2** clearly outperformed the micellar catalyst, which gave only a small enhancement over background hydrolysis similar to the monomeric water-soluble complex Zn_2**3** illustrated by the

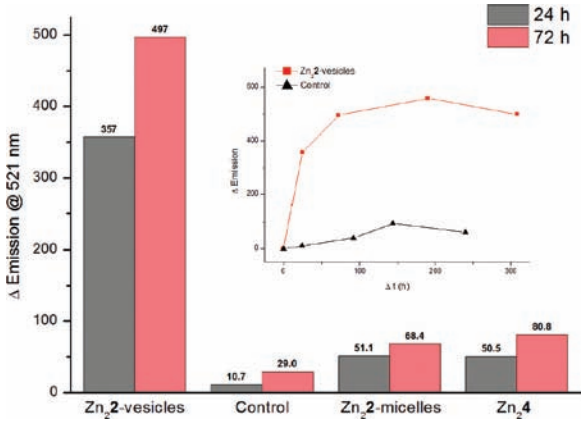

Figure 2. Emission increase of TaqMan probe (1×10^{-8} M) incubated with Zn_2**4**, Zn_2**2**-vesicles and -micelles (1×10^{-5} M Zn(II)) and buffer only at 40 °C and pH 8.0 for 24 and 72 h. Inset: Kinetics for Zn_2**2**-vesicles and background hydrolysis for comparison.

comparison of a FAM emission increase after 24 and 72 h. According to the saturation fluorescence of the vesicular kinetics, it was assumed that about 75%[19] of the probe molecules have been cleaved at least at a single site which is remarkable for a stable nonactivated phosphodiester substrate. As control, the kinetics were again repeated with pure DSPC vesicles and buffer solution which resulted in no fluorescence increase compared to background reaction.

The better performance of vesicles compared to micelles in the catalysis of polyphosphates may be explained by multivalence effects resulting in a higher substrate affinity of the vesicular catalyst. This hypothesis is supported by the much higher affinity of fructose-1,6-bisphosphate, used as a non-hydrolyzable model substrate, to coumarin-labeled amphiphilic Zn(II)-complex Zn_2**5** (10^{-5} M in HEPES) if embedded in DSPC vesicles (10 mol % Zn_2**5**) compared to homomicelles. Its attached fluorescence label previously allowed the direct observation of phosphate ion binding to the zinc complex in a vesicular environment by emission quenching[12a] and is assumed to respond in a similar way if assembled in micelles (see Figure S19, left). Emission titrations with fructose-1,6-bisphosphate revealed a binding constant toward the vesicles in the micromolar range,[12a] while no significant zinc-phosphate coordination could be detected in micelles under these conditions (Figure S19, right).

Micellar and vesicular catalysts that promote the hydrolysis of phosphate esters were self-assembled from amphiphilic Zn(II)−cyclen complexes and commercial lipids. The approach avoids laborious synthesis to covalently connect multiple catalytic centers or attaching them to polymers or nanoparticles. Vesicles with embedded domains of dinuclear Zn(II) complexes showed a remarkable promotion of phosphodiester cleavage reactions. Although substrate conversion is limited here due to considerable product inhibition, the highest so far reported activities for Zn(II) based catalysts in terms of second-order rate constants for the hydrolysis of BNPP at neutral pH and 25 °C were observed, exceeding even those of lanthanide complexes.[20] pBR322 plasmid DNA is readily degraded by double strand cleavage, which is difficult to achieve with nonenzymatic catalysts.[6a] The catalytic vesicles also considerably enhance the cleavage of a nonactivated single-strand oligonucleotide compared to background reaction control.

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In conclusion, vesicles with embedded metal complexes as catalytic active sites show a remarkable catalytic performance in phosphodiester hydrolysis. Turnover numbers need to be improved to enable an effective applicability of these systems. Their preparation, however, is easy and very versatile, which greatly facilitates further improvement of properties. In particular, the hydrolysis of nonactivated oligophosphates is promoted, which might be due to the presence of dense clusters of bis-Zn(II) cyclen complexes as catalytic centers on the vesicle surface. Mechanistic details will be investigated further. Combining such catalytically active sites with binding sites for sequence recognition by co-embedding might pave the way to functionalized vesicles, which are able to discriminate in their hydrolysis between different substrates or target specific DNA sequences mimicking the properties of hydrolytic enzymes more closely.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of Zn**1**, detailed data and experimental conditions for BNPP, oligonucleotide and plasmid DNA hydrolysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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