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Metallodendrimers as Transphosphorylation Catalysts

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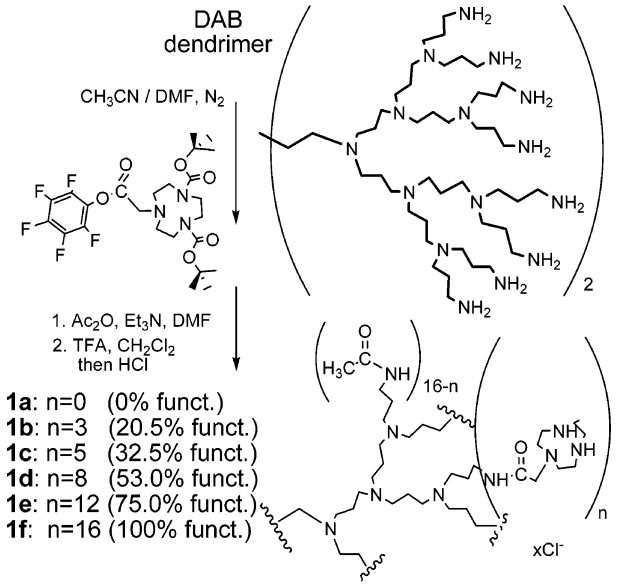
A common trait found in enzymes is the cooperativity between several functional units in order to perform, in a more efficient way, their catalytic tasks. Scientists have devised several synthetic, polyfunctional systems potentially able to work on a cooperative basis between catalytic units with alternating success. Among polyvalent systems, successful examples are those constituted by functional polymers (synzymes)[1] and monolayer-passivated nanoparticles (nanozymes).[2,3] For dendrimers,[4] contrasting results in support or against cooperativity have been reported.[5] We report here a straightforward example of a cooperative catalyst based on a functional dendrimer. Our system was designed as a potential catalyst for the cleavage of a phosphate diester model of RNA. Phosphate esters are rather sluggish substrates to hydrolyze, particularly under neutral or basic conditions. The process is, however, strongly catalyzed by transition metal ions that typically operate in a concerted manner.[6] Accordingly, the metal-catalyzed cleavage of a phosphate ester provides an excellent way to test if, in a given system, several metal ions work cooperatively.

For this purpose, we have functionalized third generation DAB (poly(propylene imine)) dendrimers[7] with a triazacyclononane-bearing acetate via amide bond formation. The triazacyclononane macrocycle is an excellent ligand for ions such as Zn^{II} and Cu^{II}, and its complexes with these metal ions are known to be good catalysts for the cleavage of phosphate esters.[8] In order to specifically address the issue of cooperativity between different metal ions present on the periphery of the dendrimer, we have prepared derivatives with different degrees of functionalization. In this way, the relative concentration of the complexes on the surface of each dendrimer could be changed, thus allowing us to test how this affects the activity of the different catalysts. Usually, the activity of increasing generation of functional dendrimers is compared, but this is not a reliable way to assess cooperativity. In fact, by increasing dendrimer generation, both the size and the number of functional groups present on the periphery are increased. It is thus difficult to correlate the activity with the concentration of the active functions at the reaction loci (i.e., the dendrimer interfacial region).

Dendrimers **1a**−**f** were synthesized following the procedure described in Scheme 1. Thus Boc-diprotected triazacyclononane was reacted with 2-Br-methylacetate, and the ester was converted in the activated pentafluorophenol derivative that was eventually reacted with the dendrimer. By working with different molar ratios of activated ester and DAB dendrimer, derivatives **1b**−**f** were obtained with different degrees of functionalization after acetylation of the remaining free amines. Each of the compounds **1b**−**e** is constituted by a mixture of dendrimers with a different degree of functionalization. The percentage of ligand introduced is the average estimated from the ^{1}H NMR spectra (see Supporting Information).

Scheme 1. Synthesis of Dendrimers **1a**−**f**



On the contrary, both **1a** and **1f** are single compounds obtained by treating the DAB dendrimer with an excess of acetic anhydride (**1a**) or activated ester (**1f**). The concentrations of the ligand units in stock solutions of **1b**−**f** were obtained by spectrophotometric titration with a standard Cu^{II} solution.

Nuclease-like activity was assayed in the presence of Zn^{II} ions using 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP), a substrate used as a standard model of an RNA phosphodiester. Zn^{II} is one of the metal ions most commonly present in the catalytic site of nucleases. Figure 1 reports the dependence of the rate constant for the cleavage of HPNPP by functionalized dendrimers **1b**−**f** as a function of the number of equivalents of Zn^{II} added. All plots of Figure 1 have been obtained by using the same nominal concentration of ligand (not dendrimer!). In all cases, the most active system is that in which all triazacyclononane units form a 1:1 complex with Zn^{II}. It is also immediately apparent that as the functionalization of the dendrimer increases so does the maximum rate constant observed. Thus the 1:1 Zn^{II} complex of dendrimer **1f** is the most active catalyst ($k_{obs} = 1.9 \times 10^{-3}$ s^{-1}) with a 270-fold rate acceleration compared to that observed with the Zn^{II}−1,4,7-triazacyclonane complex (7.0×10^{-6} s^{-1}) and a 9900-fold acceleration over the uncatalyzed process (2.0×10^{-7} s^{-1}).

This behavior can be understood if one considers that the reaction occurs in the functionalized region of the dendrimer and that the local concentration of the catalytically relevant Zn^{II} complex increases with the functionalization of the dendrimer.[9] This situation is very similar to that found with metallomicelles.[10]

A plot (Figure 2) of the rate constant for each Zn^{II} dendrimer as a function of the mole fraction of the complex present on its

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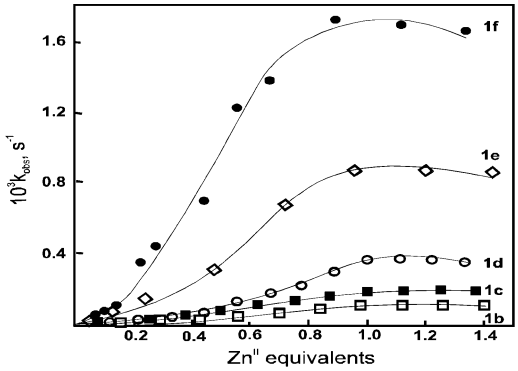

Figure 1. Observed rate constant for the cleavage of HPNPP by dendrimers **1b**−**f** as a function of the equivalents of Zn^{II} added. The rate constant in the presence of [**1a**] = 2.0×10^{-4} M is 2.0×10^{-7} s^{-1}, identical to that of the uncatalyzed process. Conditions: [triazacyclononane] = 6.6×10^{-4} M, [HPNPP] = 3.3×10^{-5} M, pH = 7.5, 40 °C.

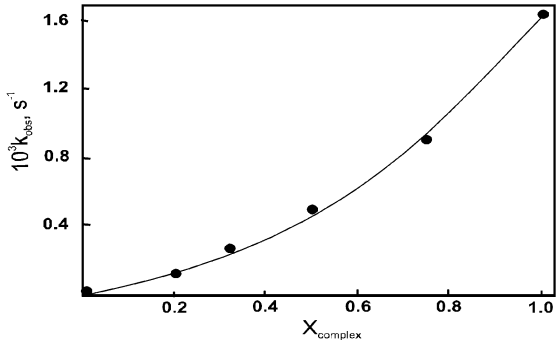

Figure 2. Observed rate constant for the cleavage of HPNPP as a function of the molar ratio of complex on the periphery of the different dendrimers. The curve represents the best fit obtained by using the equation discussed in the text.

periphery (keeping constant the complex analytical concentration at 6.6×10^{-4} M) does not give a linear dependence.[11] By fitting these data with the equation $k_{obs} = k_o + k_n X^n$, where k_o is the rate observed for the fully acetylated dendrimer **1a**, k_n is the rate constant for the fully functionalized dendrimer, X is the mole fraction of the complex present on the periphery of the dendrimer, and n is the number of Zn^{II} complexes in the catalytically active species, we obtain $n = 2.10 \pm 0.17$.[12] This indicates that two complexed Zn^{II} ions are involved in the cleavage of HPNPP or, said in other words, that two metal ions cooperate in the process.

The analysis of the dependence of the reactivity on pH reveals that, depending on the dendrimer, the rate levels out in the pH 6.5−7.5 region, suggesting that the role of the metal ions is that of stabilizing the complexed substrate toward the transition state where a further negative charge develops and in facilitating deprotonation of the nucleophilic species. By using dendrimers **1f**−Zn^{II} and **1e**−Zn^{II},[13] the Michaelis−Menten plots with an excess of substrate give $k_{cat} = 1.55 \times 10^{-2}$ and 8.72×10^{-3} s^{-1}.[14] By comparing the activities of **1f**−Zn^{II} and **1e**−Zn^{II} with that of gold nanoparticles functionalized with an analogous Zn^{II} triazacyclononane complex,[2] we observe that these dendrimers are, respectively, 3.7- and 2-fold better, and consequently, they set a new limit for these multivalent catalysts. This higher activity could be due, inter alia, to a tighter packing of the functional groups on the dendrimer surface than that attained in the larger nanoparticles, which allows a better exploitation of the cooperation between the metal centers.

In conclusion, the Zn^{II} complexes of triazacyclononane-functionalized DAB dendrimers are among the most active catalysts (based on this biologically relevant metal ion) reported for the cleavage of the RNA model substrate HPNPP. Furthermore, they show clear evidence of cooperativity between two metal ions in the catalytic site. This finding adds further evidence in favor of the analogy of behavior between functional dendrimers and enzymes.[15]

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Supporting Information Available: Details for the synthesis of the dendrimers and for their characterization and kinetic analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) By weighting for the statistical distribution of the functionalization in the dendrimers (see Supporting Information).
(13) The notation **1x**−Zn^{II} indicates a dendrimer to which a stoichiometric amount of $Zn(NO_3)_2$ has been added to saturate all triazacyclononane units present.
(14) By operating at constant [HPNPP] and increasing [**1f**−Zn^{II}], the k_{cat} obtained is about 1 order of magnitude lower. In analogy with that found with synzymes (Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Org. Chem.* **2001**, *66*, 5866−5874), this may be taken as the k_{cat} per catalytic site.
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