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Phosphate Triesters Cleavage by Gold Nanozymes †

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† Presented at the 2nd International Online-Conference on Nanomaterials, 15–30 November 2020; Available online: https://iocn2020.sciforum.net/.

Published: 15 November 2020

Abstract: Phosphate triesters are cleaved by gold nanoparticles functionalized with metal complexes (Zn(II), Cu(II), Co(II), Co(III), Eu(III), Yt(III), Zr(IV)) of -triazacyclononnonane and cyclen ligands with a mononuclear mechanism with impressive rate accelerations with respect to the uncatalyzed processes, constituting remarkable example of nerve agents-hydrolyzing nanoazymes.

Keywords: phosphate triesters; gold nanoparticles; nanozymes; nerve agents

1. Introduction

Phosphate triesters share with phosphate diesters one of the most important bonds (P-OR) present in nature for its relevance in the chemistry of life. To the class of phosphate triesters belong nerve agents that are among the most noxious compounds known to man, with lethal effects even at very low doses.[1] Some of these chemicals were developed for use in agriculture for pest control.[2] They operate by irreversibly reacting with the enzyme acetylcholinesterase which is involved in controlling neuronal signalling.[3,4] Although they are banned by the Chemical Weapons Convention (1997) enforced by the Organisation for the Prohibition of Chemical Weapons,[5] they are still available and have been used in regional wars, terroristic attacks or for other criminal purposes. Defined protocols exist for the destruction of large stockpiles of nerve agents, mostly relying on their hydrolysis under strongly basic conditions. Mild methods for the hydrolysis of these compounds are still required and actively sought particularly for use when civilians or military personnel are exposed to them [6–8].

We have reported in the past that gold nanoparticles (AuNPs) functionalized with metal ion complexes are powerful catalysts of the cleavage of phosphate diester including DNA.[9–11] We thought they were excellent candidates as catalysts for the cleavage of phosphate triesters and nerve agents, in particular. Typically, catalysts developed for this purpose are not tested with the real nerve agents for their toxicity and simulants, far less toxic, are used. We report here are results for the development of AuNPs-based catalysts for the cleavage of nerve agents simulants *p*-nitrophenyl diphenyl phosphate (PNPDPP) and dimethyl *p*-nitrophenyl phosphate (DMNP, methyl paraoxon) shown in Figure 1.

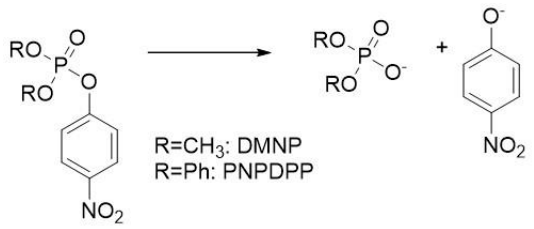

Figure 1. Cleavage of nerve agents simulants PNPDPP and DMNP (methyl paraoxon) by AuNPs.

2. Experimental Details

The details of the synthesis and characterization of the ligands reported in Figure 2 and the gold nanoparticles obtained by passivation of ca. 2 nm gold clusters will be reported elsewhere.

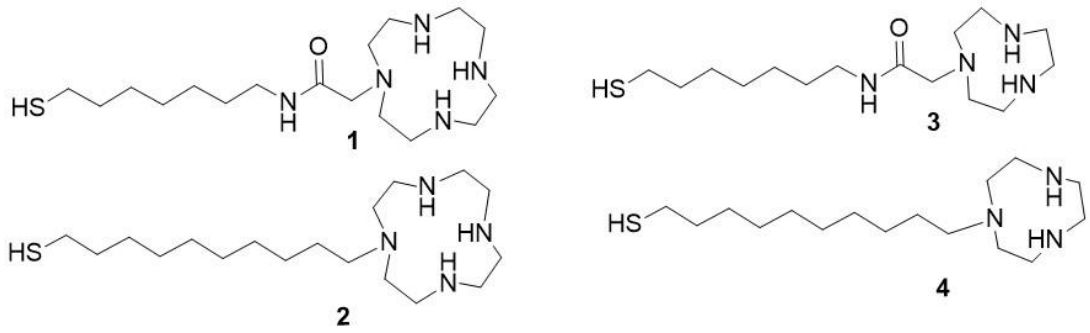

Figure 2. Ligands used for the passivation of the AuNPs.

3. Cleavage of *p*-Nitrophenyl Diphenyl Phosphate (PNPDPP) by AuNP1–4 and Metal Ions

Because of its faster reactivity, PNPDPP was used for a quick screening of a small 28-member library constituted by AuNP**1–4** in the presence of metal ions Zn(II), Cu(II), Co(II), Co(III), Eu(III), Yb(III) and Zr(IV). The ease of passivation of AuNPs renders the preparation of such a library a relatively simple task and highlights the versatility of AuNPs for rapid nanocatalysts screening. The size of the nanoparticles studied was slightly lower than 2 nm (diameter). This means that each of them is passivated with ca. slightly less than 70 ligands. In analyzing the rate of cleavage of the substrates we always used the concentrations of the ligands and not that of the nanoparticles. The results of this quick screening are reported in Table 1. The structure of the four ligands studied is reported in Figure 2.

Table 1. Observed rate constantn (kobs) obtained for the 28-member library of AuNPs for the cleavage of PNPDPP (25 °C, pH = 8) [a].

AuNP	Metal Ion	$10^4 k_{obs}$, s^{-1}	AuNP	Metal Ion	$10^4 k_{obs}$, s^{-1}
AuNP1	Zn(II)	6.03	**AuNP3**	Zn(II)	6.49
AuNP1	Cu(II)	0.55	**AuNP3**	Cu(II)	6.37
AuNP1	Co(II)	1.01	**AuNP3**	Co(II)	0.51
AuNP1	Co(III)	0.61	**AuNP3**	Co(III)	0.33
AuNP1	Eu(III)	2.44	**AuNP3**	Eu(III)	0.96
AuNP1	Yb(III)	2.42	**AuNP3**	Yb(III)	1.00
AuNP1	Zr(IV)	1.47	**AuNP3**	Zr(IV)	0.74
AuNP2	Zn(II)	20.00	**AuNP4**	Zn(II)	8.28
AuNP2	Cu(II)	4.94	**AuNP4**	Cu(II)	8.53
AuNP2	Co(II)	6.17	**AuNP4**	Co(II)	5.09
AuNP2	Co(III)	4.92	**AuNP4**	Co(III)	2.76
AuNP2	Eu(III)	10.30	**AuNP4**	Eu(III)	10.30
AuNP2	Y(III)	7.79	**AuNP4**	Yb(III)	1.00
AuNP2	Zr(IV)	5.49	**AuNP4**	Zr(IV)	4.72

[a] [Catalyst] = 2×10^{-5} M.

The results indicate that AuNP**2**-Zn(II) and Eu(III); AuNP**4**-Zn(II), Cu(II) and Eu(III) are the best performing catalysts.

4. Cleavage of Dimethyl p-Nitrophenyl Phosphate (DMNP) by the Best Performing Catalysts

The above best performing catalysts were tested in the hydrolysis of DMNP. Apart from being much less reactive than PNPDPP (more than 2 orders of magnitude) and, hence, more similar to the real nerve agents, DMNP is significantly more hydrophilic. The interaction with the monolayer of the

nanoparticles is expected to be driven by a hydrophobic interaction and not by the very weak binding constants of a neutral phosphate with the metal ions. This means that DMNP, at the very low catalyst concentration we have used (2×10^{-5} M), is mostly not bound to the nanoparticles. However by performing kinetics under Michealis-Menten conditions, behaving hence was nanozymes [12,13], (Figure 3) we were able to obtain relevant activity parameters for these catalysts. The analysis allowed us to determine the half-lives for the best performing catalysts (AuNP**2**-Zn(II) and AuNP**4**-Cu(II)) that were 27 and 38 min, respectively for the substrate fully bound to the nanoparticles, at pH = 8 and 25 °C.

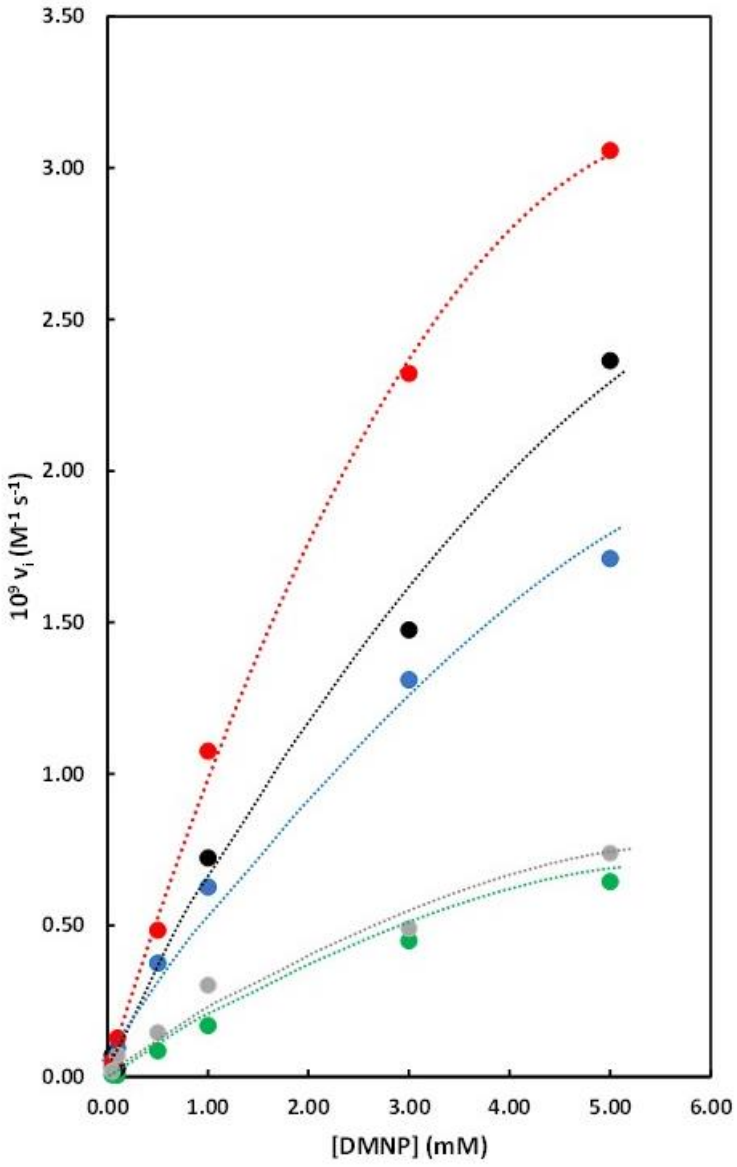

Figure 3. Michaelis-Menten kinetics for the cleavage of DMNP by the five catalysts AuNP**2**-Zn(II) (black), AuNP**2**-Eu(III) (green); AuNP**4**-Zn(II) (blue), AuNP**4**-Cu(II) (red) and AuNP**4**-Eu(III) (gray) at 25 °C and pH = 8.

5. Conclusions

The experimental evidence gathered for the cleavage of DMNP let us conclude that the picture is quite different from that observed with phosphate diesters hydrolysis. With phosphate diesters two metal ions cooperate in the cleavage process (Figure 4, left). One coordinates the anionic phosphate oxygen the other one delivers the nucleophilic OH-. In the case of phosphate triesters only a single metal ion appears to be involved in the catalytic site (Figure 4, right). This metal ion delivers

both the nucleophilic species and coordinates the phosphate as the negative charge is forming going towards the transition state. In comparison to other reported catalysts able to cleave nerve agents, the gold nanoparticles perform only slightly worse than the best performing ones placing them among the most attractive systems working close to physiological pH.

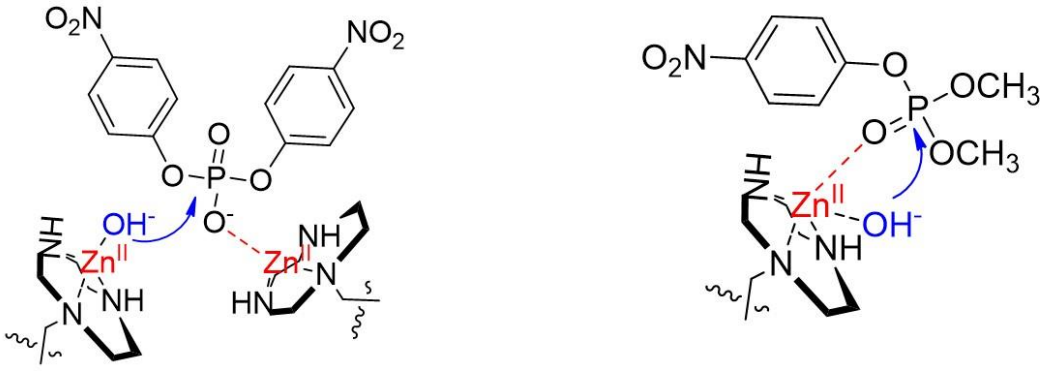

Figure 4. Proposed mechanism of cleavage of the phosphate diester BNP (left) and phosphate triester DMNP by gold nanozymes.

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