Kinetic Analysis of the Hydrolysis of Methyl Parathion using Citrate-stabilized 10 nm Gold Nanoparticles

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Abstract "Ligand-free" citrate-stabilized 10 nm gold nanoparticles (AuNPs) promote the hydrolysis of the thiophosphate ester methyl parathion (MeP) on the surface of gold as a function of pH and two temperature values. At 50 °C, the active surface gold atoms show catalytic turnover ~ 4 times after 8 hours and little turnover of gold surface atoms at 25 °C with only 40 % of the total atoms being active. From Michaelis-Menten analysis, k_{cat} increases between pH 8 and 9 and decreases above pH 9. A global analysis of the spectral changes confirmed the stoichiometric reaction at 25 °C and the catalytic reaction at 50 °C and mass spectrometry confirmed the identity of *p*-nitrophenolate (PNP) product. Additional decomposition pathways involving oxidation and hydrolysis independent of the formation of PNP were also seen at 50 °C for both catalyzed and un-catalyzed reactions. This work represents the first kinetic analysis of ligand-free AuNP catalyzed hydrolysis of a thiophosphate ester.

1. Introduction

Methyl parathion (MeP, *O,O*-dimethyl-*O-p*-nitrophenyl phosphorothioate) is a broad spectrum organothiophosphate insecticide and hazardous pollutant. Both MeP and the analogous ethyl parathion continue to be used extensively (and often illegally in some countries) for the treatment of large-scale agricultural crops such as cotton, soybean, corn and wheat as well as other cultivated fruits and vegetables. (Tabassum et al., 2014) The accumulation of MeP and its degradation products in the environment is considered to be a significant widespread threat to regional ecologies. MeP also serves as a structural mimic for organophosphate nerve agents, and is often used as a model substrate for studies on the catalytic degradation of chemical warfare agents, which also act as acetylcholinesterase inhibitors (e.g. VX, soman, tabun). (Khanna, 2008) Thus, new methods for MeP removal from the environment, and studies on the mechanistic pathway(s) for MeP breakdown are of great interest to environmental scientists. The degradation of MeP can proceed through both ox-

idative and hydrolytic pathways under basic conditions, and can occur both photochemically and in the dark. At least 20 breakdown/rearrangement products have been identified using NMR or MS. (Seger and Maciel, 2005) Non-photochemical reaction products are reproduced in Figure 1. (Seger and Maciel, 2005) Hydrolysis can proceed through either loss of the methoxy or *p*-nitrophenoxy groups to give thiophosphate ions **1** and **2** (shown in the protonated form). *O,S*-isomerization of MeP gives *S*-Me and *S*-Ar species **3** and **4**, and oxidation results in the oxon **5**.

Fig. 1. Initial non-photochemical breakdown products of methyl parathion. Tautomeric forms of **1** and **2** are not shown.

Ligand stabilized gold nanoparticles (AuNPs) are known to catalyze organic transformations under homogeneous conditions and commonly require either thiol, amine or phosphine ligands in which the ligand donor atom is covalently bound to the AuNP surface. (Zhang et al., 2011) CTAB stabilized AuNPs have been used for the detection of MeP at the ppm level by following its degradation products. (Barman et al., 2013a) Applications of "ligand-free" or anion-stabilized gold colloids in catalysis are rare. Barman et al reported the use of bio-fabricated AuNPs as a colorimetric sensor for

MeP by following the amount of nitrophenolate product, although no other reaction pathways or kinetic analyses were reported. (Barman et al. 2013b) In this communication we report the kinetic analysis of MeP degradation as a function of temperature and pH by commercially available ligand-free citrate stabilized AuNPs.^a We selected 10 nm diameter citrate-stabilized AuNP for our analysis due to the simplicity of the system for kinetic studies related to the participation of AuNP surface on catalysis and to limit potential interference due to covalently bound ligands.

2. Results and discussion

The reactions for the non-photochemical degradation of MeP using commercially available 10 nm AuNP under several conditions were monitored using the absorbance of the product *p*-nitrophenolate (*PNP*) anion at 405 nm (Figure 2). Mass spectral analysis of the reaction confirmed the hydrolysis products *PNP* and [(MeO)₂P(S)(OH)] in addition to products due to oxidation and alternative hydrolytic pathways (Supporting Information). ³¹P NMR analysis provided confirmation of product identities (Supporting Information). The reactions were performed in 20 mM MOPS or CHES buffer with 5% methanol to aid solubilization of MeP which has a low solubility in water. At 25 °C, control reactions performed in the absence of AuNPs showed no formation of *PNP*, however at 50 °C, a small degree of background hydrolysis was observed. The kinetic experiments confirm that AuNPs accelerate the degradation of MeP to *PNP*. At 25 °C the data fits to a single exponential rise to a maximum value of [*PNP*] = 4.9 M corresponding to ~ 41% of the total number of surface gold atoms (12 M). At 50 °C the exponential rise levels off to a linear region and after 200 minutes, [*PNP*] = 2.1 M suggesting that the “active gold” surface atoms have undergone turnover several times. From the linear region of [*PNP*] vs. time, we used a Michaelis-Menten formalism normalized to the active molar amount of surface gold atoms adjusting the MeP concentration for attachment to AuNP. The 10 nm AuNP core diameter was confirmed by TEM (Supporting Information) and a Spherical Cluster Approximation (SCA) (Johnston, 2002) was used, from which we calculated a value of 3,936 Au atoms. We make the assumption that the spherical nanoparticles remain intact throughout the course

of the kinetic analysis. Table 1 lists the resultant K_M and k_{cat} at 50 °C for several pH values.

Fig. 2. The formation of nitrophenolate (*PNP*, 405 nm) from MeP ([MeP] = 0.2 mM) under several conditions for the first 200 minutes. **A.** pH 8.3 at 25 °C: green = MeP, red = MeP + citrate-stabilized 10 nm AuNPs, [AuNP_{surface atoms}] = 12 M. The dashed line is a fit to a single exponential. **B.** pH 8.5 at 50 °C: orange = MeP, blue = MeP + citrate-stabilized 10 nm AuNPs. The dotted line is a fit to a single exponential plus a linear function. [AuNP_{surface atoms}] = 16 M. Table 1.

Table 1. Kinetic analysis of the hydrolysis of MeP by AuNPs. ^apH values corrected for temperature at 50 °C. ^b

Of note is the pH dependence of k_{cat} which increases between pH 8 and 9, followed by a slow decrease (Figure 3). This suggests that OH⁻ is playing an active role, however, at a pH value higher than 9, the AuNP surface exhibits decreased activity which we do not currently understand.

To obtain a better understanding of the nature of the spectral changes during the course of the reaction, we examined the changes between 260 to 800 nm

showing both the formation of

pH	k_{cat} , s ⁻¹	K_M , M	k_{cat}/K_M , M ⁻¹ s
7.	$2 \pm 1 \times 10^{-5}$	-	-
8.	$1.8 \pm 0.2 \times 10^{-4}$	$2 \pm 1 \times 10^{-4}$	-
8.	$4.1 \pm 0.3 \times 10^{-4}$	$1.0 \pm 0.2 \times 10^{-4}$	4.0
9.	$3.9 \pm 0.3 \times 10^{-4}$	$5.6 \pm 3.1 \times 10^{-5}$	6.9
9.	$2.6 \pm 0.3 \times 10^{-4}$	$4.2 \pm 1.6 \times 10^{-5}$	6.9

PNP, and the changes in the MeP and AuNP spectra as the reaction progressed. We used the software ReactLab™ KINETICS which provides global analysis for fitting chemical reaction schemes and their parameters to spectroscopic data. In these experiments, we chose reaction conditions which allowed the recording of a measureable absorbance in the UV range and gave tractable concentrations of MeP. In addition, a pH 9 value was selected which exhibited considerable stability in regard to AuNP aggregation compared to lower pH values.

^a Ligand-free citrate-stabilized 10 nm Au nanoparticles are referred to as AuNP throughout.

^b <http://www.liv.ac.uk/buffers/buffercalc.html>

Fig. 3. A. The rate of *PNP* formation vs. MeP concentration fit to a Michaelis-Menten formalism at several pH values with $[AuNP_{active\ surface\ atoms}] = 4.9\ M$ at $50\ ^\circ C$. B. k_{cat} vs. pH. Buffer = $20\ mM$ MOPS (pH 7.7 to 8.1) and CHES (pH 8.5 to 9.6). Assays were performed in triplicate.

At pH 8 in $20\ mM$ MOPS buffer, the addition MeP to $10\ nm$ Au colloid results in an immediate $6\ nm$ red shift in the plasmon band which indicates an interaction of the thiophosphate substrate with the Au surface. After 8 hours, large ($> 1\ \mu m$) irregular aggregates were isolated following centrifuga-

Figure 4A shows the spectral characteristics of the MeP and AuNP reaction at pH 9 and $50\ ^\circ C$.

Fig. 4. A. A representative experiment showing the UV-Vis spectral changes for the reaction of MeP with AuNP over a 2.86 hour period ($1 \times 10^{-4}\ M$ MeP, $4.9 \times 10^{-6}\ M$ AuNP active surface atoms at pH 9 ($0.02\ M$ CHES buffer) and 5% MeOH at $50\ ^\circ C$). B. The fitted plots of absorbance vs. time for several selected wavelengths. C. The UV-Vis spectra of the reactants, and products used in the analysis. AuNP and PNP were entered as known spectra. B was entered as non-colored and C was entered as known spectra from the analysis of Figure S2. D. The concentration profiles calculated from fitted the data. In the modeling, $k_1 = 13.9 \pm 0.07\ M^{-1}s^{-1}$ and $k_2 = 0.85 \pm 0.001\ M^{-1}s^{-1}$ for equations 1 and 2 listed in the text. Background rates for equation 3 ($k_3 = 3.2 \pm 0.02 \times 10^{-6}\ s^{-1}$) and equation 4 ($k_4 = 2.4 \pm 0.01 \times 10^{-5}\ s^{-1}$) were used in the analysis from the control experiment without AuNPs (Supporting Information Figure S2).

The UV-Vis spectra change as a function of time reflecting the formation of the *PNP* product at $405\ nm$ concomitant with changes in the MeP and AuNP spectra. Using the software ReactLabTM KINETICS, we analysed the spectral changes using the model shown in eq. 1 – 4. We propose this model based on the observations from wavelength

data at $405\ nm$ which suggests a catalytic step at $50\ ^\circ C$. In the global model, we did not include more detailed elementary steps like on/off rates or equilibrium, hence the observed 2nd order rate constants from the analysis are more complex.

Equation 1 represents a 2nd order reaction in which MeP reacts with a surface site on the AuNP to form the product *PNP* and a new surface site AuNP-B. The reaction in eq. 2 includes the catalytic step in which the surface product on the gold, AuNP-B, is displaced by an incoming MeP molecule and then regenerated after hydrolysis. We used the number of active surface atoms as the molar concentration of the AuNP. Equations 3 and 4 correspond to the background decomposition pathways measured without AuNP (see Supporting Information). Figure 5B, shows the quality of the fit of the model to the data at several selected wavelengths by comparing the experimental data to the calculated results. Figure 5C shows the UV-Vis spectra of the reactants and products used in the analysis while Figure 5D shows the concentration profile of the reactants and products as the reaction progresses. In the supporting information, the global analysis at $25\ ^\circ C$ with AuNP shows the fit of the data with and without a catalytic step (eq 1) and a slow background hydrolysis rate (eq 3). The spectral analysis of MeP hydrolysis without AuNP at $25\ ^\circ C$ and $50\ ^\circ C$ is shown in Supporting Information. At $50\ ^\circ C$, we see an additional product, (C) in the UV spectra with a λ_{max} at $285\ nm$ in addition to the decrease of MeP with growth of the *PNP* peak for both un-catalyzed and catalyzed reactions at $50\ ^\circ C$. The product C is not seen at $25\ ^\circ C$ for either the uncatalyzed or catalyzed reactions (Supporting Information). Likely candidates include the hydrolysis product **1** or the oxidation product **5** shown in Figure 1. Qualitative mass spectral analysis of dichloromethane extractions of acidified reaction mixtures ($20\ mM$ CHES buffer, 5% methanol) confirmed the presence of the hydrolysis product *PNP*, the starting material MeP, and putatively the hydrolysis product **1**, the oxidation product **5**, and less confidently the *O,S*-isomerization products **3** or **4** (Supporting Information). Mass spectral analysis of direct reaction mixtures ($20\ mM$ CHES buffer, 5% methanol) also showed the reduction of starting material MeP and the accumulation of the hydrolysis product *PNP* over time. Overall concentrations

for both were lower than expected and we attribute this to post-processing methods prior to mass spectral analysis (Supporting Information). Nonetheless, the qualitative analysis from the MS supports the utilization of the reactant (MeP), formation of the main product (*PNP*), and presence of further less abundant products. A full qualitative analysis of products formed in situ will be reported in due course.

The use of ligand-free AuNPs for the degradation of other phosphate esters was also investigated to determine the scope of the reaction. Neither the diarylphosphate ester bis(4-nitrophenyl) phosphate (*p*-BNPP), which is commonly used as a substrate for testing metal-based hydrolysis catalysts, nor the organophosphate paraoxon, showed any activity for hydrolysis as determined by lack of formation of *PNP*. Similarly, citrate stabilized ligand-free 10 nm silver nanoparticles showed no activity for the hydrolysis of MeP under the conditions described above. No activity for hydrolysis of MeP was observed when using either HAuCl_4 or sodium citrate. However, the thiophosphate ester malathion underwent degradation in the presence of AuNP as evidenced by ^{31}P NMR spectroscopy. These sets of experiments suggest that the interaction of AuNP surface with the aurophilic $\text{P}=\text{S}$ sulfur atom of MeP is a requirement for reactivity. One possible candidate is that the formation of a Au-S bond on the surface activates the thiophosphate ester towards nucleophilic attack by hydroxide ion i.e. the surface Au atoms are acting as Lewis acid sites (Figure 5). This is compelling, as similar explanations were provided for the degradation of organophosphate esters using a heterogeneous MgAl_2O_4 spinel catalyst (Zimmermann et al., 2013). While the formation of other hydrolytic breakdown products is expected, and have also been observed for non-catalytic systems, the origin of the oxidation products (oxon 5) is less clear. We do not expect the catalytic formation of reactive oxygen species e.g. singlet oxygen under the present conditions.

Fig. 5. One possible mechanism for activation of methyl parathion by 10 nm Au towards nucleophilic attack by the hydroxide ion releasing *p*-nitrophenolate (here shown as ArO^-).

3. Conclusions

We have described a kinetic analysis of the catalytic degradation of the organothiophosphate pesticide methyl parathion in aqueous solution mediated by commercially available ligand-free citrate stabilized 10 nm gold nanoparticles. Temperature and pH dependencies are also described. Mass spectral data and ^{31}P NMR analysis indicate that the catalytic reaction does not follow a simple reaction but a more complex pattern in which alternative hydrolytic and oxidative pathways are followed. To the best of our knowledge this work represents the first kinetic analysis of ligand-free AuNP catalyzed hydrolysis of a thiophosphate ester. Experiments are underway to determine the details of the mechanism for the catalytic hydrolysis/oxidation.

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Appendix A. Supplementary Material

Supplementary data associated with this article can be found, in the online version, at:

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