Mechanistic insight on the catalytic detoxification of Paraoxon mediated by imidazole: Furnishing optimum scaffolds for scavenging organophosphorus agents

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ABSTRACT

Any agricultural growth concomitantly increases the demand for pesticides and there is a major concern in monitoring these agents, in order to identify their indiscriminate use, avoid contamination and also to detect prohibited species. Organophosphorus compounds (OPC) comprise many pesticides and chemical warfare, hence, there is an increasing interest for developing efficient dephosphorlylation/detoxification methods. A promising approach is mimicking enzymatic active sites that promote dephosphorylating biological processes, with emphasis for the imidazole (IMZ) group. Notwithstanding, we present a concise and detailed mechanistic study regarding the degradation of the toxic pesticide Paraoxon with IMZ, not yet reported. Results consistently enlighten the powerful nucleophilic detoxifying activity of IMZ by a clear-cut catalysis with the regeneration of the catalyst and formation of less toxic products. Given the impressive high rate enhancements (10^6-fold), IMZ is among the most promising OPC scavenger reported. 1D and 2D nuclear magnetic resonance (^{1}H, ^{13}C and ^{31}P) and mass spectroscopy analysis support that reaction follows exclusively on the phosphorus center, forming a phosphorylated intermediate. Overall, IMZ can be consecutively reused and comprises an optimum scaffold for scavenging OPC, since the catalytic activity and mechanism with IMZ can be extended to other phoshoryl centers (*e.g.*, OPC). The present study sheds light to the strategic area of developing prominent IMZ-derived catalysts targeting detoxifying processes and design of sensors for contaminants in the environment or in food distribution centers.

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1. Introduction

The agricultural growth of a country indubitably dictates its prominent participation in the world economy, whereby is less volatile in economic crises, as projected by organizations, such as "Food and Agriculture Organization of the United Nations" and "Organization for Economic Co-Operation and Development" [1]. Concomitantly, the demand for pesticides increases accordingly, some of which are highly toxic. There is no global consensus regarding prohibition policies for using these agents, hence there is a major concern in monitoring pesticides, in order to identify their indiscriminate use, avoid contamination and also to detect illegal use of some species. In this context, organophosphorus compounds (OPC) stands out, which constitute many pesticides, insecticides

and chemical warfare, and its misuse or application can be fatal [2]. Further, despite the worldwide banning of many of these toxic agents, their uncontrolled production in the past led to large stocks of difficult handling and disposal. Thus, there is great interest in developing efficient detoxification methods, promising to combat chemical weapon attacks and develop monitoring sensors for contaminants in the environment or in food distribution centers. Accordingly, the Nobel Peace Prize 2013, received by the "Organisation for the Prohibition of Chemical Weapons" reinforces the global concern to eradicate toxic chemicals [3], many derived from OPC [2].

Phosphate esters (*i.e.*, organophosphates) are a class of OPC that has attracted increasing interest and their detoxification can be achieved by cleaving the highly stable P—O bond, leading to less toxic products [4]. Moreover, this dephosphorylation process is similarly furnished in biological systems by prominent enzymes [5]. Whereas, mimicking enzymatic active sites is promising for designing novel efficient catalytic systems for detoxification purposes
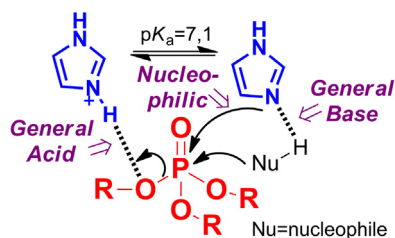
Scheme 1. IMZ multi-catalyses.

aiming the catalyst's recycling. This approach is also a benchmark for developing artificial enzymes [6]. Many interesting catalytic scaffolds have been proposed for degrading toxic OPC agents, *e.g.*, polymers [7] and carbon nanotubes [8], dendrimers [9], cyclodextrins [10], ionic liquids [11] and functionalized fatty acids [12]. Furthermore, the imidazole (IMZ) moiety, recurrent in innumerous enzymes, is capable of multi-catalyses (acid, basic and nucleophilic, Scheme 1), hence, has outlined many dephosphorylation studies with impressive catalytic enhancements, mainly with highly reactive phosphate esters [13,14]. Notwithstanding, a concise and detailed mechanistic study regarding the degradation of the toxic pesticide diethyl 4-nitrophenyl phosphate (Paraoxon) with IMZ has not been reported. Herein, we report kinetic results and elucidate the Paraoxon detoxification mechanism by nuclear magnetic resonance (^{1}H, ^{13}C, ^{31}P 1D and 2D NMR) and mass spectroscopy (MS) analyses. Results consistently enlighten the powerful IMZ activity in detoxifying Paraoxon by a clear-cut catalysis with the regeneration of the catalyst. We demonstrate that even a simple and small catalyst, such as IMZ, can be widely explored, consecutively reused and sheds light to the strategic area of developing complex IMZ-derived catalysts.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade and were obtained commercially and were used without further purification.

2.2. Kinetics

An aliquot of a stock solution of Paraoxon (0.01 M in acetonitrile) was added to 3 mL of a buffered aqueous solution containing IMZ, under pseudo-first order conditions. The reaction solutions were followed spectrophotometrically by monitoring the onset of the phenolate **8** at 400 nm, with controlled temperature (20 °C). The pseudo-first order rate constants (k_{obs}) were determined using an iterative least-squares program (with correlation coefficients > 0.99), from the absorbance *vs* time profiles. The pH was kept at 0.01 M buffers of KHCO$_3$ (pH 8–10) and in the pH range of 6–8, solutions were self-buffered with imidazole.

2.3. NMR analysis

Kinetic experiments by ^{31}P and ^{1}H NMR spectroscopy were performed in H$_2$O containing some D$_2$O at room temperature (~298 K), on a 200 NMR spectrometer operating at 4.7 Tesla, observing ^{31}P and ^{1}H nucleus at 81.01 and 200.13 MHz, respectively. The spectrometer was equipped with a 5-mm direct detection quadrinuclear probe. The ^{1}H, ^{31}P spectra as well as one-bond and long-range ^{1}H–^{13}C and ^{1}H–^{31}P correlation experiments were acquired in D$_2$O, at 303 K, on a 600 NMR spectrometer operating at 14.1 Tesla, observing ^{1}H, ^{13}C and ^{31}P at 600.13, 150.90 and 242.94 MHz, respectively. The spectrometer was equipped with

a 5-mm inverse detection quadrinuclear probe with *z*-gradient. ^{1}H NMR chemical shifts are given in ppm related to TMSP (3-(trimethylsilyl) propionic-2,2,3,3-*d*$_4$ acid, sodium salt) signal at 0.00 ppm as internal reference, while those of ^{31}P NMR are related to H$_3$PO$_4$ (85% in D$_2$O) signal from a capillary reference.

Kinetic experiments followed by NMR were performed by adding an aliquot of Paraoxon to the IMZ solution directly on NMR tubes. Then, ^{31}P NMR and ^{1}H NMR spectra were daily recorded, until there were no alterations on the signal intensities. Concentration *vs* time profiles for the species detected (Fig. 3) were obtained by determining the area of the signal of each species in relation to the ^{31}P NMR reference.

2.4. MS analysis

Mass spectrometry analyses were performed on a LTQ XL linear ion trap mass spectrometer, equipped with an ESI ion source. Typically, reactions were analyzed with IMZ (1 M) and Paraoxon (3×10^{-4} M) in aqueous medium at pH 8.5, in the negative and positive-ion mode. Main conditions: curtain gas nitrogen flow of 20 mL min-1; ion spray voltage of −4500 eV; declustering potential of −21 eV; entrance potential of −10 eV; and collision cell exit potential of −12 eV. Main species were subjected to Tandem MS by using collision-induced dissociation (CID) with helium and collision energies ranging from 5 to 45 eV.

3. Results and discussion

3.1. Kinetics for Paraoxon degradation

Paraoxon degradation mediated by IMZ was followed at different pHs and the kinetic profile obtained is given in Fig. 1, along with IMZ species distribution (pK_a = 7.10 [13]). Consistently, k_{obs} increases with pH until reaching a subtle unvarying region (~pH 9), where the reactive neutral IMZ species is at its maximum molar fraction. Upon higher pH (>9.5), k_{obs} increases due to the Paraoxon alkaline hydrolysis contribution. The kinetic data were fitted with Eq. (1) (solid red line in Fig. 1), deduced from Scheme 2, that considers the (i) spontaneous reaction with water (k_0); (ii) alkaline hydrolysis (k_{OH}) and (iii) nucleophilic reaction of IMZ (k_{IMZ}), considering the the neutral species (χ_{IMZ}). The unreactive protonated IMZH$^+$ species was not considered.

$$k_{obs} = k_0 + k_{OH}[OH] + k_{IMZ}[IMZ]_T \chi IMZ \qquad (1)$$
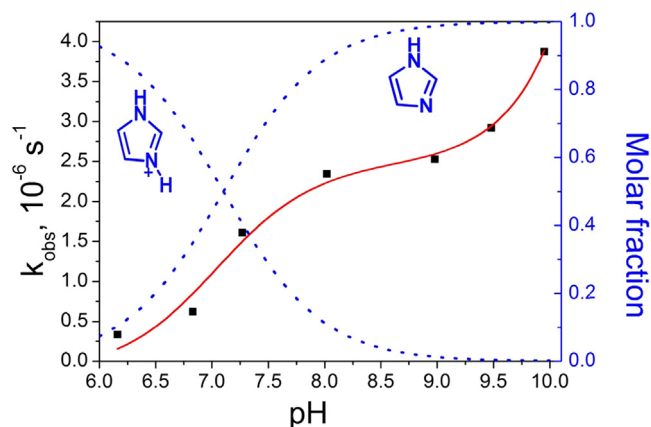

Fig. 1. Kinetic pH profile for the reaction of Paraoxon with IMZ, 1.0 M at 25 °C (left axis), along with IMZ relevant species distribution (right axis). Solid red line corresponds to the fit using Eq. (1). (For interpretation of the references to color in this figure legend as well as in the text, the reader is referred to the web version of this article.)