ORGANIC LETTERS

2006
Vol. 8, No. 15
3215−3217

A Magnetic Biomimetic Nanocatalyst for Cleaving Phosphoester and Carboxylic Ester Bonds under Mild Conditions

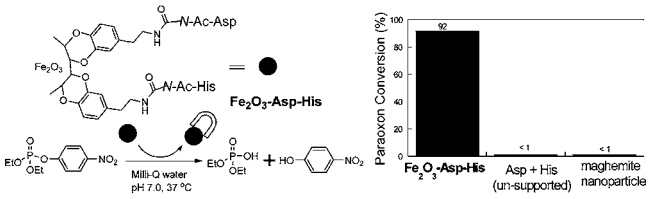
Yan Zheng, Chuansong Duanmu, and Yong Gao*

Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901-4409

ygao@chem.siu.edu

Received May 4, 2006

ABSTRACT



As a result of the unique surface structure of nanospheres, Asp and His residues supported on a 12 nm maghemite nanoparticle worked collaboratively as a biomimetic nanocatalyst for hydrolyzing paraoxon (phosphoester) and 4-nitrophenyl acetate (carboxylic ester) in Milli-Q water (pH 7.0) at 37 °C, without employing extremes of pH or heavy metals. Our nanocatalyst could be facilely recovered via magnetic concentration. The isolated catalyst exhibited long-term stability, showing no significant loss of its catalytic activity for repeated uses after 3 months.

Fission of the phosphoester and carboxylic ester bonds is involved in numerous chemical and biochemical reactions. Novel catalysts that hydrolyze phosphoesters and carboxylic esters under mild conditions could have very broad applications across many fields such as organic synthesis, industrial process, environmental treatment, and national defense. In particular, agents that promote the hydrolysis of phosphoesters could be utilized for decontamination of environmental organophosphate pollutants such as paraoxon.[1] Paraoxon is a biocide that has been widely used for crop protection. The number of worldwide human intoxications with organophosphate pesticides and insecticides is estimated at 3,000,000/year.[1] Paraoxon is also an analogue of chemical warfare agents such as sarin, soman, and VX,[2] and novel catalysts hydrolyzing paraoxon can potentially be employed for neutralizing military nerve agents.[3] In addition, catalysts cleaving phosphodiester bonds might be developed into nucleic acid scission agents[4] for use as DNA/RNA structural probes and antisense therapeutics.

Numerous strategies have been examined for cleaving phosphoester and carboxylic ester bonds, and strong acids/bases or heavy metals are usually the reagents of choice for hydrolysis reactions. However, extreme pHs could be problematic to sensitive functional groups in organic synthesis. Metal complexes are potentially complicated by toxicity for therapeutic antisense treatments. An alternative strategy is to use hydrolytic enzymes for cleaving ester, amide, and phosphoester bonds. Enzymatic hydrolysis reactions can be carried out at 37 °C in a neutral aqueous medium. A common feature of these biocatalytic reaction

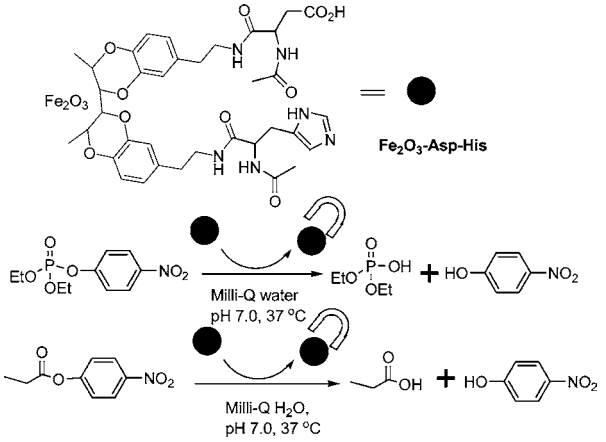
(1) Eyer, P. *Toxicol. Rev.* **2003**, *22*, 165.
(2) Yang, Y.-C. *Acc. Chem. Res.* **1999**, *32*, 109.
(3) For example, see: (a) Moss, R. A.; Gong, P. K.; Morales-Rojas, H. *Org. Lett.* **2002**, *4*, 1835. (b) Tsang, J. S.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 7602. (c) Bromberg, L.; Hatton, T. A. *Ind. Eng. Chem. Res.* **2005**, *44*, 7991. (d) Wagner, G. W.; Procell, R. L.; O'Connor, R. J.; Munavalli, S.; Carnes, C. L.; Kapoor, P. N.; Klabunde, K. J. *J. Am. Chem. Soc.* **2001**, *123*, 1636.
(4) For some reviews, see: (a) Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777. (b) Trawick, B. N.; Daniher, A. T.; Bashkin, J. T. *Chem. Rev.* **1998**, *98*, 939. (c) Claussen, C. A.; Long, E. C. *Chem. Rev.* **1999**, *99*, 2797. (d) Chow, C. S.; Bogdan, F. M. *Chem. Rev.* **1997**, *97*, 1489. (e) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089. (f) Armitage, B. *Chem. Rev.* **1998**, *98*, 1171. (g) Blaskó, A.; Bruce, T. C. *Acc. Chem. Res.* **1999**, *32*, 475. (h) Suh, J. *Acc. Chem. Res.* **2003**, *36*, 562. (i) Kool, E. *Acc. Chem. Res.* **1998**, *31*, 502.

10.1021/ol061098a CCC: $33.50
Published on Web 06/27/2006

mechanisms involves the cooperative catalysis by an acid and base pair on the side chains of two adjacent amino acids with the proper folding of the protein polymers. For example, the His-Asp catalytic dyad contributes to the activities in many RNase A proteins.[5] However, lack of availability of the hydrolytic enzymes in sufficient quantities, low enzyme stability, and substrate selectivity have limited the potential of using hydrolases for broad applications. As a consequence, novel catalysts that can cleave phosphoester and carboxylic ester bonds under mild conditions are still urgently needed.

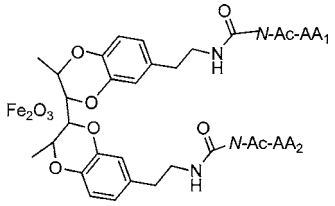
In this paper, we report a novel biomimetic nanocatalyst, **Fe_2O_3-Asp-His**, for the hydrolysis of paraoxon and 4-nitrophenyl acetate with high catalytic efficacies in Milli-Q water (pH 7.0) at 37 °C, —without employing extremes of pH or heavy metals (Scheme 1). **Fe_2O_3-Asp-His** is a lead selected

Scheme 1. Repeated Uses of **Fe_2O_3-Asp-His** for the Hydrolysis of Paraoxon and 4-Nitrophenyl Acetate



from a small library of nanocomposites (Table 1).[6] Each nanocomplex in the library comprised a 12 nm maghemite (Fe_2O_3) nanocore wrapped with a shell of either a monad ($AA_1 = AA_2$) or a dyad of amino acids (AA_1 and AA_2 molar ratio 1:1). Only those amino acids with a carboxylate, a basic or a nucleophilic group on the side chain such as Asp, Glu, His, and Lys were employed for constructing the library. Immobilization of these amino acid analogues on the surface of a nanoparticle allows their acidic and basic side chains to be positioned in close proximity to each other, potentially leading to cooperative catalysis from two neighboring amino acids. Dopamine was utilized as a linker for supporting amino acid residues since ethenediols such as dopamine have a strong affinity for undercoordinated surface sites of metal oxide.[7] The α-amino groups of the surface amino acids were acylated to mimic the amide bonds of the enzyme backbones.

Table 1. Cleavage of Paraoxon by 12 nm Maghemite Nanoparticle-Supported Amino Acids[a]



entry	AA_1, AA_2	conv (%)[b]	entry	AA_1, AA_2	conv (%)[b]
1	nanoparticle[c]	<1	11	Asp, Ser	28
2	Asp	5	12	Glu, His	51
3	Cys	15	13	Glu, Lys	50
4	Glu	<1	14	Glu, Cys	44
5	His	6	15	Glu, Ser	45
6	Lys	2	16	His, Cys	30
7	Ser	4	17	His, Ser	40
8	Asp, His	77/92[d]	18	Lys, Ser	17
9	Asp, Lys	27	19	Lys, Cys	39
10	Asp, Cys	25	20	Asp + His[e]	< 1

[a] Conditions: paraoxon (0.5 mM) and a magnetic nanocomplex (amino acid concentration 0.06 mM) in 2 mL of Milli-Q water, 37 °C, 48 h. [b] Average of at least two runs; HPLC analyses. [c] 12 nm maghemite nanoparticles coated with oleate (no amino acids attached) (ref 8). [d] Reaction time: 96 h. [e] Unsupported Asp (0.14 mM) and His (0.14 mM) and paraoxon (0.5 mM) in 2 mL of Milli-Q water at 37 °C for 48 h.

The magnetic Fe_2O_3 cores allow our nanocatalysts to be facilely concentrated and recovered for repeated uses via applying a permanent magnet externally.

The nanocomplexes in Table 1 were fabricated via surface-exchanging presynthesized 12 nm maghemite nanoparticles[8] with either a single amino acid analogue or a mixture of two amino acid dopamine derivatives (molar ratio 1:1) (Supporting Information). TEM measurements were employed for examining the iron oxide cores of the nanoparticle−amino acid complexes, and elemental analyses were utilized for evaluating the amount and composition of amino acid coatings on the surface of magnetic nanoparticles. Entries 2−7 in Table 1 are nanoparticles functionalized with a monad of an amino acid analogue, whereas nanocomplexes coated with a dyad of amino acid residues are shown in entries 8−19. The catalytic activities of these nanocomplexes were investigated by utilizing the nanoparticles to catalyze the hydrolysis reaction of paraoxon. A typical experiment employed for our assay involved the introduction of a nanocomplex (amino acid concentration 0.06 mM) to a solution of paraoxon (0.5 mM) in 2 mL of Milli-Q water at 37 °C. After 48 h, the nanocomplex was magnetically concentrated and removed from the solution. The remaining solution was then subjected to HPLC analyses using an internal standard for estimating the conversion yield of paraoxon. To minimize experimental errors, we repeated every experiment at least two times. The averages of our repeated assays for all nanocomplexes are listed in Table 1.

(5) (a) Mohan, C. G.; Boix, E.; Evans, H. R.; Zikolovski, Z.; Nogués, M. V.; Cuchillo, C. M.; Acharya, K. R. *Biochemistry* **2002**, *41*, 12100. (b) Schultz, L. W.; Quirk, D. J.; Raines, R. T. *Biochemistry* **1998**, *37*, 8886.

(6) A similar combinatorial approach using a polymer matrix was previously reported: Menger, F. M.; Eliseev, A. V.; Migulin, V. A. *J. Org. Chem.* **1995**, *60*, 6666.

(7) (a) Rajh, T.; Chen, L. X.; Lukas, K.; Liu, T.; Thurnauer, M. C.; Tiede, D. M. *J. Phys. Chem. B.* **2002**, *106*, 10543. (b) Kohler, N.; Fryxell, G. E.; Zhang, M. *J. Am. Chem. Soc*. **2004**, *126*, 7206.

(8) (a) Park, J.; An, K.; Hwang, Y.; Park, J.-G.; Noh, H.-J.; Kim, J.-Y.; Park, J.-H.; Hwang, N.-M.; Hyeon, T. *Nat. Mater*. **2004**, *3*, 891. (b) Lu, J.; Fan, J.; Xu, R.; Roy, S.; Ali, N.; Gao, Y. *J. Colloid Interface Sci.* **2003**, *258*, 427.

Nanoparticles coated with *N*-Ac-Asp and *N*-Ac-His analogues (**Fe_2O_3-Asp-His**) (Entry 8, Table 1) exhibited the highest catalytic activity. For example, after 48 h, 77% of paraoxon was hydrolyzed using **Fe_2O_3-Asp-His**; after 96 h, a conversion yield of 92% was achieved. In contrast, a mixture of *N*-Ac-Asp and *N*-Ac-His without a nanoparticular support (Entry 20) led to a conversion yield less than 1%. Within the margin of experimental error, the unsupported amino acid pair showed no catalytic activity in the hydrolysis of paraoxon. On the other hand, the nanoparticle support itself does not appear to be a catalyst, as entry 1 showed that after 48 h less than 1% of paraoxon was hydrolyzed by maghemite nanoparticles without a shell of amino acid coatings. Interestingly, nanoparticles protected with other dyad pairs of *N*-acetyl amino acids (Entries 9−19, Table 1) are less active catalysts than **Fe_2O_3-Asp-His**. For example, the nanocomplex with Glu and His led to a conversion yield of 51% after 48 h (Entry 12), which is lower than that of a dyad of Asp and His despite the fact that the structures of Asp and Glu are similar to each other. Kinetic studies suggested that the hydrolysis of paraoxon by **Fe_2O_3-Asp-His** fits into the Michaelis−Menten model (Supporting Information). Analysis of the Lineweaver−Burk plot gave $K_M = 1.1$ mM and $k_{cat} = 4.3 \times 10^{-5}$ s^{-1} in a pH 7.4 buffer at 40 °C for **Fe_2O_3-Asp-His**.

Although the detailed mechanisms are not clear at this stage, it is reasonable to hypothesize that the catalytic activity of **Fe_2O_3-Asp-His** was probably due to the carboxylate-imidazole cooperativity induced by the surface of the nanoparticle. Asp and His have a carboxylate and an imidazole group on their side chains, respectively. Anchorage of the carboxylate/imidazole pair on the surface of a nanoparticle will limit the mobility of two functional groups and position them in close proximity to each other, potentially leading to the collaboration between two amino acids. Such general acid/base pairs are frequently observed in many RNase A proteins.[5] The carboxylate anion could act as a general base activating a water molecule, and the protonated imidazole acts as a general acid transferring a proton to the developing intermediate. Alternatively, the Asp analogue could help orient the proper tautomer of His on **Fe_2O_3-Asp-His** for catalysis.[5b] The unique radial structure of the nanoparticle surface coatings also allows the acid/base pair to be readily exposed to the substrate in the surrounding solution. A recent investigation on Au nanoparticle-supported dipeptide hydrolysis reactions[9] vindicated our cooperative mechanism. Our hypothesis could also be supported by our findings in Table 1. For example, nanoparticles coated with a monad of either Asp (Entry 2) or His (Entry 5) led to much lower conversion yields than that of **Fe_2O_3-Asp-His** (Entry 8), which is probably due to the lack of a collaborative acid/base pair on the nanoparticle surface. Additional detailed kinetic studies might provide more insights on such carboxylate-imidazole cooperativity.

The long-term stability and recyclability of **Fe_2O_3-Asp-His** were also investigated. The isolated **Fe_2O_3-Asp-His** was sequentially washed with methanol (2 × 50 mL) and CH_2Cl_2 (2 × 50 mL) and air-dried. The nanocatalyst was then subjected to a new round of paraoxon hydrolysis reaction. Table 2 listed the conversion yields of using recovered **Fe_2O_3-Asp-His** for repeated uses. The fourth cycle of the hydrolysis reaction in Table 2 was carried out about three months after the first round of reaction; no significant loss of its activity was observed. **Fe_2O_3-Asp-His** was also found to promote the hydrolysis of 4-nitrophenyl acetate (Scheme 1). After 48 h, over 67% of 4-nitrophenyl acetate (0.5 mM) was hydrolyzed by **Fe_2O_3-Asp-His** (amino acid concentration 0.06 mM) in Milli-Q water (2 mL) at 37 °C.

Table 2. Repeated Uses of Iron Oxide-Asp-His for Hydrolyzing Paraoxon[a]

	reaction round			
	1	2	3	4
conversion (%)[b]	77	74	74	72

[a] Conditions: paraoxon (0.5 mM) and iron oxide-Asp-His (amino acid concentration 0.06 mM) in 2 mL of Milli-Q water, 37 °C, 48 h. [b] Yields by HPLC analyses.

In summary, we reported a novel biomimetic nanocatalyst that cleaves phosphoester and carboxylic ester bonds under mild conditions (e.g., neutral Milli-Q water at 37 °C). Paraoxon and 4-nirtophenyl acetate were utilized as two model compounds for our test reactions. **Fe_2O_3-Asp-His** was facilely recovered and reused for repeated reactions. We believe that such magnetic biomimetic nanocatalysts could have very broad application potentialities across many fields. Ongoing research work in our laboratory is to examine the use of biomimetic nanocatalysts as nucleic acid cleavage agents; our progress in these areas will be reported in due course.

Acknowledgment. This work was supported in part by the NSF (CAREER Award CHE-0343440) and the SIU Materials Technology Center. We would also like to acknowledge the contributions from Antonio Lozada (SIUC) and Jinda Fan (SIUC) for assistance with the synthesis of maghemite nanoparticles and optical rotation measurements.

Supporting Information Available: TEM micrograph of maghemite nanoparticles, detailed descriptions of experimental procedures for the synthesis of amino acid-dopamine analogues and maghemite-amino acid complexes, and kinetic studies of the hydrolysis of paraoxon by **Fe_2O_3-Asp-His**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061098A

(9) Pengo, P.; Polizzi, S.; Pasquato, L.; Scrimin, P. *J. Am. Chem. Soc.* **2005**, *127*, 1616.