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14. ABSTRACT
The objective of the proposal was 1) to investigate oxidative coupling of nitro-toluene compounds, catalyzed by vanadium bromoperoxidase or its functional mimics and 2) to investigate catalytic properties of silicate-encapsulated enzymes, in general, and haloperoxidase enzymes, in particular, or their functional mimics to assess their utility as catalysts in relevant synthetic schemes. It was found that V-BrPO catalyzed bromination reactions with TNT do not effect the oxidative coupling reaction required to form HNS. FeHeme chloroperoxidase, which functions optimally at low pH is also not suitable for catalyzing the oxidative coupling of TNT to HNS because it only chlorinates at low pH. It was also found that haloperoxidases immobilized in mesoporous silicate matrices retain their catalytic and functional properties. These enzymes may well have applications as anti-biofouling catalysts.

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vanadium bromoperoxidase, nitrotoluene, hexanitrostilbene, mesoporous silicate materials, energetic material

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Grant #: 00014-98-1-0836

Principal Investigator: Alison Butler

Institution: University of California, Santa Barbara

Grant Title: Reactivity of Haloperoxidases and Functional Mimics of Haloperoxidase

Award Period: August 15, 1998- August 14, 2001

Objective: The objective of the proposal was 1) to investigate oxidative coupling of nitro-toluene compounds, catalyzed by vanadium bromoperoxidase or its functional mimics and 2) to investigate catalytic properties of silicate-encapsulated enzymes, in general, and haloperoxidase enzymes, in particular, or their functional mimics to assess their utility as catalysts in relevant synthetic schemes.

Approach: The initial approach was to investigate whether vanadium bromoperoxidase catalyzes the oxidative coupling of 2,4-dinitrotoluene or 2,6-dinitrotoluene forming tetranitrostilbene products as a model system for the oxidation coupling of trinitrotoluene (TNT) forming hexanitrostilbene (HNS). The approach was based on the Shipp process in which bleach (OCI) chlorinates a deprotonated methyl group of TNT; subsequent coupling of two equivalents of Cl-TNT led to C-C bond formation followed by loss of HCl to produce HNS in a reaction that does not proceed in high yield.

Accomplishments: Objective 1): Because TNT is explosive, we started off by examining the reactivity of 2,4- or 2,6-dinitrotoluene (DNT) with vanadium bromoperoxidase (V-BrPO; *Ascophyllum nodosum*), FeHeme chloroperoxidase, and Ti-grafted SBA-18 mesoporous silicate materials in the presence of a peroxide (i.e., hydrogen peroxide, or t-butyl hydroperoxide), a halide (bromide or chloride) and under various pH conditions (pK 5-8). Neither, halogenated intermediates nor the coupled nitrotoluene products were observed using V-BrPO, Ti^(IV)-modified mesoporous silicate materials that are functional mimics of V-BrPO (e.g., Ti-SBA15; Ti-MCM), nor FeHeme ClPO, which may be a result of the pK_a differences between the dinitrotoluenes (pK_a 17 and 19) and trinitrotoluene (pK_a 14.5), which is more acidic, as well as the pH at which the catalysts function, which is below pH 8 for V-BrPO and Ti-silicate materials and below about pH 4 for FeHeme ClPO.

Reaction of TNT with vanadium chloroperoxidase indicates evidence for the bromination of TNT by the mass of the product (m/z = 305 and 307, resulting from the nearly equal isotopic distribution of Br-79 and Br-81). It is one of several products. Another product appears to be hydroxylated (m/z = 242), and from our experience with other haloperoxidase reactions, this could well arise from an initial bromination step followed by bromide displacement by water. This displacement is evidence for the reactivity of the benzyl-bromide moiety (an essential step for the coupling part of the reaction). The hydroxyl product is, therefore, another indication that bromination has occurred.

Objective 2): Porous silica materials, which have large surface areas and variable pore diameters, are attractive candidates to host large molecules, including proteins. Extensive research has been performed on encapsulation of proteins in sol-gels for possible applications for biosensors. However, a disadvantage in the use of sol-gels is their variability in pore size, which can not be tailored to isolate specific proteins. With the recent discovery of the well-ordered hexagonal (SBA-15, pore diameter = 50-150 Å) and mesocellular siliceous foam (MCF, pore diameter = 170-420 Å) mesoporous silica phases prepared with triblock co-polymers, in which the phases and narrowly defined pore diameter are tuned by trimethylbenzene (TMB), it is possible to design the selectivity of these hosts to specific protein characteristics, such as size and charge, by varying the pore diameter and by derivatizing the reactive silanol groups with specific functional organic silanes. We investigated the use of derivatized mesoporous silicate materials to sequester proteins based on size and charge.

Using 3-aminopropyl triethoxysilane ($3\text{-NH}_2\text{C}_3\text{H}_6\text{Si}(\text{OC}_2\text{H}_5)_3$) to functionalize the surface of SBA-15 (60 Å) and MCF (160 Å), we examined the size selectivity of APTS-derivatized SBA-15 (60 Å) and MCF (160 Å) towards anionic proteins of varying size but similar pI: i.e., conalbumin (MW 77,000, pI 6.0), chicken egg ovalbumin (MW 44,000, pI 4.9) and soybean trypsin inhibitor protein (MW 14,000, pI 5.2). APTS-derivatized SBA-15 (60 Å) selectively adsorbed (and released at elevated ionic strength) the smallest protein, trypsin inhibitor protein, whereas APTS-derivatized-MCF adsorbed and released all three proteins. This is the first utilization of mesoporous materials to sequester proteins where both size exclusion and ion-exchange chromatography techniques are employed simultaneously. Cationic proteins (e.g. lysozyme, pI 10) were reacted as well, with SBA-15 derivatized with anionic organosilane groups (e.g. $\text{HOOC-R-Si}(\text{CH}_2\text{CH}_3)_3$) which absorbed readily into the pores.
J. Am. Chem. Soc. 1999, 121, 9897-9898.

We also investigated the reactivity of FeHeme chloroperoxidase (*Caldariomyces fumago*) sequestered in MCF. MCF is a promising material for immobilizing enzymes, due to its large pore structure and high loading capacity compared to other mesoporous materials, such as MCM-48, SBA-16 and SBA-15. The immobilized chloroperoxidase in MCF retains its activity. The optimal pH at which the maximum amount of enzyme is immobilized was determined to be pH 3.4, slightly below the isoelectric point of the enzyme. A weak ionic interaction between the enzyme and the surface of the inorganic substrate is thought to be critical in maintaining the activity of the immobilized enzyme. The loading capacity of MCF is 122 mg protein/g MCF. We demonstrate the advantage of MCF as an inorganic substrate for immobilization of enzymes.
J. Mol. Cat. B: Enzymatic, 2002, 17, 1-8.

Conclusions: V-BrPO catalyzed bromination reactions with TNT do not effect the oxidative coupling reaction required to form HNS. FeHeme chloroperoxidase, which functions optimally at low pH is also not suitable for catalyzing the oxidative coupling of TNT to HNS because it only chlorinates at low pH. The benzyl-chloro TNT using vanadium chloroperoxidase and subsequently PCl_5 to convert the benzyl hydroxylated

product formed in the V-CIPO reaction to the benzyl chloride intermediate has now been published by Jonathon Dordick.

Significance: Haloperoxidases immobilized in mesoporous silicate matrices retain their catalytic and functional properties. These enzymes may well have applications as anti-biofouling catalysts.

Patent Information: NA

Award Information:

H. Burr Steinbach Fellow, Woods Hole Oceanographic Institution 1999-2000
Chaired 2002 Marine Natural Products Gordon Research Conference, February 2002, Ventura, CA
Chaired 2004 Metals in Biology Gordon Research Conference, January 2004, Ventura, CA
Chair 2006 (Vice Chair 2004) Environmental BioInorganic Chemistry Gordon Research Conference.

Publications and Abstracts

Mesoporous Silicate Sequestration and Release of Proteins, Yong-Jin Han, G.D. Stucky, Alison Butler, *J. Am. Chem. Soc.* 1999, *121*, 9897-9898.

Catalytic Activity of Mesoporous Silicate-Immobilized Chloroperoxidase, Y.-J. Han, J. T. Watson, G. D. Stucky and Alison Butler, *J. Mol. Cat. B: Enzymatic*, 2002, *17*, 1-8.

Invited talks at scientific meetings related to the proposal topic:

Molecular Biomimetics Program Review, ONR, November 3-5, 1998, Coolfont, West Virginia.

7th International Symposium on Dioxygen Activation and Homogeneous Catalytic Oxidation (ADHOC-99), July 19-23, 1999, York, England (J. Lindsay Smith, Chair)

2nd Symposium on Biovanadium Chemistry, August 14-19, 1999, Berlin, Germany (joint IUPAC/ GDCh-Gesellschaft Deutscher Chemiker Conference) (Organizers: V. Conte (Padova) and D. Rehder (Hamburg)).

Poster presentations at scientific meetings related to the proposal topic:

"Immobilization of Chloroperoxidase Inside Mesocellular Foam," Yong-Jin Han, Nicole Maier, Galen D. Stucky, Alison Butler, 13th International Zeolite Conference (IZC13), July 8-13, 2001, Montpellier, France (poster: Y. Han) <http://www.izc13.enscm.fr/>

Protein Extraction by Mesoporous Materials, Y.J. Han, D. Margolese, G.D. Stucky, A. Butler, National American Chemical Society meeting, Anaheim, March 1999 (poster: Y. Han).

Mesoporous Silicate Sequestration and Release of Proteins, Alison Butler, Yong Jin Han, G.D. Stucky, Metals in Biology Gordon Research Conference, Jan 23-28, 1999, Ventura, CA. (Poster A. Butler).