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Enzymatic oxidation of volatile malodorous organosulfur compounds in a two-phase reactor

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Abstract
In this work we report the oxidation of volatile organosulfur compounds (VOC) catalyzed by the enzyme chloroperoxidase from *Caldariomyces fumago*. VOC are regarded as atmospheric pollutants due to their unpleasant odor and low detection threshold. Diethyl sulfide, dimethyl disulfide, propanethiol, butanethiol and hexanethiol were found to be substrates for the enzyme in a liquid medium reaction at pH 6, under peroxidatic conditions. Product analysis showed that sulfides were oxidized to their respective sulfoxides whereas thiols were oxidized to the corresponding disulfides. The identified products showed significantly lower vapor pressure than the parental compounds; thus, the products are not considered atmospheric pollutants. A 70-mL two-phase reactor was assembled in order to determine the efficiency of the enzymatic treatment. The liquid phase, consisting of 15% organic solvent and 85% buffer, was contacted with the gaseous phase, consisting of a substrate-enriched air stream. Using dimethyl disulfide as model substrate, we found that only enzymatic oxidation occurred in this system; by controlling the enzyme and peroxide concentration, we found that the substrate is transferred to the aqueous phase where 1 mol of enzyme converted approximately 12,400 mol of substrate, thus highlighting the potential of enzymatic treatment of malodorous gaseous streams.
Key Words: Environmental biocatalysis, organosulfur compounds, chloroperoxidase, peroxidation, volatile substrates.

Resumen
En este trabajo reportamos la oxidación de una serie de compuestos organoazufrados volátiles (COV) catalizada por la enzima cloroperoxidasa obtenida del hongo *Caldariomyces fumago*. Los COV se consideran contaminantes atmosféricos debido a su olor desagradable y a su bajo umbral de detección. El sulfuro de etilo, disulfuro de dimetilo, propanotiol, butanotiol y hexanotiol fueron transformados por la enzima en un medio de reacción acuoso a pH 6 y en presencia de peróxido de hidrógeno. El análisis de los productos demostró que los sulfuros fueron oxidados a sus respectivos sulfóxidos, mientras que los tioles fueron oxidados a sus correspondientes disulfuros. Los productos identificados tienen una presión de vapor significativamente menor que los compuestos originales, por lo que son mucho menos volátiles y por tanto no se consideran contaminantes atmosféricos. Se ensambló un reactor de dos fases de 70 mL de volumen con el fin de determinar la eficiencia del tratamiento enzimático. La fase líquida, compuesta por 85% de amortiguador y 15% de solvente orgánico, se puso en contacto con la fase gaseosa, compuesta por aire enriquecido con el sustrato. Usando disulfuro de metilo como sustrato modelo, encontramos únicamente reacción enzimática en este sistema; al controlar la concentración de enzima y de peróxido en la fase líquida se logró transferir el sustrato a la fase acuosa en donde 1 mol de enzima convirtió aproximadamente 12,400 moles de sustrato, resaltando el potencial de los tratamientos enzimáticos para las corrientes gaseosas con mal olor por COV.
Palabras Clave: Biocatálisis ambiental, compuestos organoazufrados, cloroperoxidasa, peroxidación, sustratos volátiles.

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Introduction

Chloroperoxidase (CPO, EC 1.11.1.10) from *Caldariomyces fumago* is an attractive enzyme for application in several and diverse fields such as the fine chemical and pharmaceutical industry[1], diagnosis[2] as well as the oil-related industry[3] and environment remediation[4,5]. The versatility of CPO is determined by its ability to catalyze different reactions under controlled conditions, including halogenation (chlorination, bromination and iodination), dehydrogenation, oxygen insertion and peroxide dismutation. In addition, low substrate specificity allows CPO to catalyze the transformation of a wide range of compounds of different chemical nature[6]. One of the most interesting catalytic modality of CPO is the peroxygenation activity, which catalyzes the oxidation of olefins and sulfur compounds to generate epoxides and sulfoxides, respectively[1,6]

The broad specificity displayed by CPO towards organosulfur compounds is well established. The enantioselective generation of (*R*)-sulfoxides catalyzed by CPO has been thoroughly studied. The enzyme is able to catalyze the oxidation of β-carbonyl sulfides, cycloalkyl methyl sulfides, alkyl aryl sulfides, dialkyl sulfides and cyclic sulfides to their respective (*R*)-sulfoxides[7-9]. The enantioselectivity is generally favored in the absence of halogen ions and pH near neutrality (pH 6). On the other hand, under acidic conditions (pH 3) and in the presence of chloride, the enzyme catalyzes the oxidation of sulfides to the racemic mixture of sulfoxides[10]. Under these conditions, the sulfoxidation of aromatic and heterocyclic compounds has been reported[11]. Furthermore, significant enzymatic transformation of the sulfur-containing fraction in straight diesel fuel has been demonstrated, thus highlighting the ability of CPO to catalyze the oxidation of chemically diverse organosulfur compounds[12].

Short-chain, volatile organosulfur compounds (VOC) have low odor threshold and very unpleasant odor, characteristic of the putrid odor of rotten egg and vegetables[13]. Exposure to even low concentrations of these compounds leads to dizziness, vomiting, headaches and eye irritation[14]. Thus, these compounds are classified as atmospheric pollutants particularly affecting the area surrounding sources of emission, such as waste water treatment plants, composting sites, paper pulping process and thermal-sludge treatment plants[15].

Considering the low specificity of CPO for sulfur compounds and the environmental problem that VOC represent, it was interesting to study the ability of the enzyme to catalyze the oxidation of short-chain, volatile sulfides and thiols. The results are focused on the kinetic behavior of the enzyme, the identification of products and the operation of a two-phase reactor for the removal of VOC from a gaseous stream.

Materials and methods

Chemicals and enzyme. CPO was produced from *Caldariomyces fumago* UAMH 89362 and purified as reported elsewhere[16] obtaining a preparation with a Rz (A_{398nm}/A_{280nm}) of at least 1.2. Hydrogen peroxide, diethyl sulfide, dimethyl disulfide, propanethiol, butanethiol, hexanethiol and phenyl sulfide were purchased from Sigma Co. Organic solvents and salts were obtained from J.T. Baker.

Kinetic characterization of CPO-catalyzed oxidation of VOC. Biocatalytic oxidation of organosulfur compounds was performed at 25°C in closed vials, to prevent volatilization of the substrates. Kinetic characterization was performed using substrate concentrations of up to 5 mM, due to insolubility of the compounds. The reaction mixture contained 10% acetonitrile, 60 mM phosphate buffer pH 6 and 1 mM H_2O_2. The reaction was started by addition of the enzyme (0.05-0.1 nmol). Substrate conversion was monitored by UV absorbance (typically 220-250 nm in a Perkin Elmer DAD) through reverse phase HPLC (Hypersil ODS 2.1 X 100 μm column from Agilent), using a 30% acetonitrile/70% water isocratic phase. Reduction in the area of the UV signal was used to calculate substrate conversion. Reaction rate was calculated as the ratio of substrate conversion to reaction time. Controls with peroxide and without enzyme were also performed, in order to calculate the non-enzymatic conversion. Kinetic characterization was performed using substrate concentrations of up to 5 mM, due to insolubility of the compounds. Given that saturation could not be achieved in all cases, the value of k_{cat}/K_m was calculated from the slope of the linear section in the initial rate *vs* substrate concentration plot. For the control reactions, in the absence of the enzyme and in the presence of peroxide, the rate constant of non-enzymatic conversion (k_{non}) was also calculated from the slope of rate *vs* substrate concentration plot. Results shown are the average of at least three independent reactions.

Product identification. For product identification, 10-mL reactions were monitored until at least 70% substrate conversion was achieved. The reaction mixture was extracted with methylene chloride, dried through an anhydride Na_2SO_4 bead and analyzed through gas chromatography (Agilent 6890N) coupled to a mass selective detector (Agilent 5973). A nonpolar HP-5ms column (30m x 0.25 mm x 0.25 μm) operating at low temperature (typically 40°C) was used to separate and identify the products.

Reactor operation. A two-phase reactor was assembled as follows. An air stream was passed through a reservoir containing 2 mL of 226.1 mM of dimethyl disulfide (DMDS) in acetonitrile and the resulting DMDS-enriched stream was bubbled at the bottom of a glass vessel containing 70 mL of the liquid reaction medium (15% *tert*-butanol and 85% 60 mM phosphate buffer pH 6). Gas flow was maintained between 40-60 mL/min,