Journal of Molecular Catalysis A: Chemical 424 (2016) 181–193

Contents lists available at ScienceDirect

Journal of Molecular Catalysis A: Chemical

journal homepage: www.elsevier.com/locate/molcata

# Metallo-vesicular catalysis: A mixture of vesicular cysteine/iron mediates oxidative pH switchable catalysis

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ARTICLE INFO

*Article history:*
Received 11 July 2016
Received in revised form 18 August 2016
Accepted 23 August 2016
Available online 24 August 2016

*Keywords:*
pH-switchable catalyst
Multifunctional catalyst
Peroxidase activity
Chloroperoxidase activity
Catalase activity

ABSTRACT

The design of heme and non-heme active centers, which resemble peroxidase enzymes, has recently received a significant attention. Herein, a cysteine-metal complex was designed and encapsulated in a vesicular mixture (1:4; SDS/DTAB) in order to imitate a *chloroperoxidase* (CLP) enzyme via chlorination of thionine at pH 3. This artificial enzyme behaved both as a catalase and CLP at pH 3, and as a peroxidase at pH 1. The shape of the metallo-vesicular catalyst at each pH value was obtained by dynamic light scattering, and was confirmed by transmission electron microscopy (TEM). The different fluidities as interior structure property of catalyst aggregates at pH 3 and 1 were obtained by differential scanning calorimetry (DSC). IR, $^{1}H$ and $^{13}C$ NMR spectroscopies were utilized to investigate the structural properties of the vesicular catalyst. These studies demonstrated that the cysteine/iron (III) center acted as a multifunctional catalyst. In addition, the sulfur and ammonium moieties of L-Cys interacted in the presence of metal ion at pH 1. However, the ammonium side chain was replaced with the carboxylate group at pH 3. Collectively, we engineered two distinct vesicular cysteinate-$Fe^{3+/2+}$ complexes, which functioned as a pH-switchable catalyst.



## 1. Introduction

Chloroperoxidase (CLP) is a versatile heme-based enzyme that behaves differently under various environments. CLP chlorinates substrates at pH 3, and performs as a classic peroxidase by oxidizing substrates at various pH values depending on the types of substrates. It also catalytically decomposes hydrogen peroxide in the absence of an appropriate substrate [1,2]. The active site of CLP contains a prosthetic heme-iron (III) ligated to the thiol group of a cysteine residue. As with classic peroxidases, the CLP active site contains a polar distal pocket [3,4]. These structural features enable the enzyme to carry out multifunctional reactions through the formation of a central intermediate, compound I. Formation of the compound I is common among all peroxidases, and the intermediate is considered to be an active form of CLP produced by cleavage of the O—O bond of the hydrogen peroxide through an acid-base mechanism [5,6].

In the active site of CLP Glu183, which plays a crucial role in pH dependent activity of the enzyme, has multiple actions instead of the two His and one Arg residues present in a classic peroxidase. The carboxylic side chain of Glu takes a proton from incoming $H_2O_2$ and enables the enzyme to form compound I (Scheme 1) [7–9]. Previous studies have shown that complexes of Fe (III) and cysteine adopt various forms that can perform catalytic reactions. In an acidic environment, Fe (III) reduces L-Cysteine to a cystine species that gives rise to distinct $^{1}$HNMR signals [10–12].

Fe (III) has a great potential for carbon bond activation via Fe (III)–H formation. The activation of C—H bond is a significant reaction in modern chemistry, and it is exclusively applied in organic and pharmaceuticals synthesis [13,14]. Iron has a wide range of oxidative state, including $^{+}$IV, $^{+}$V, $^{+}$VII and $^{+}$VIII, all of which are

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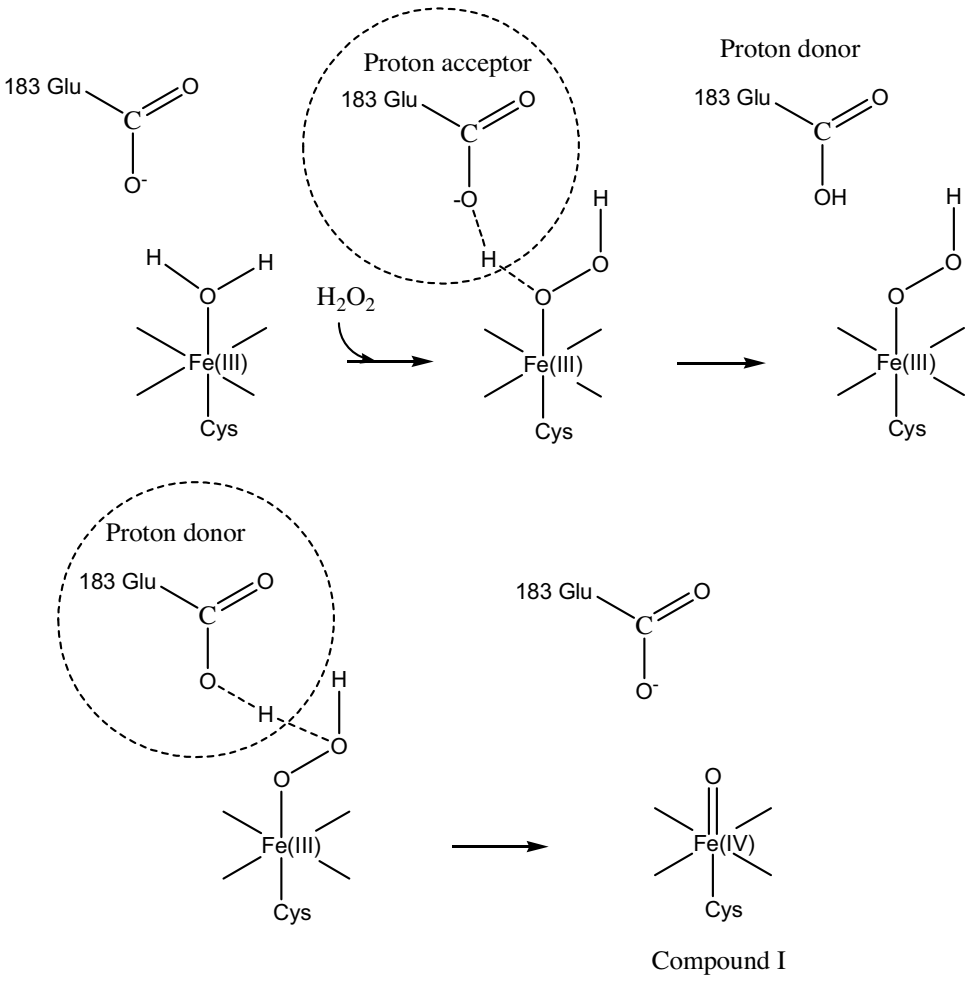
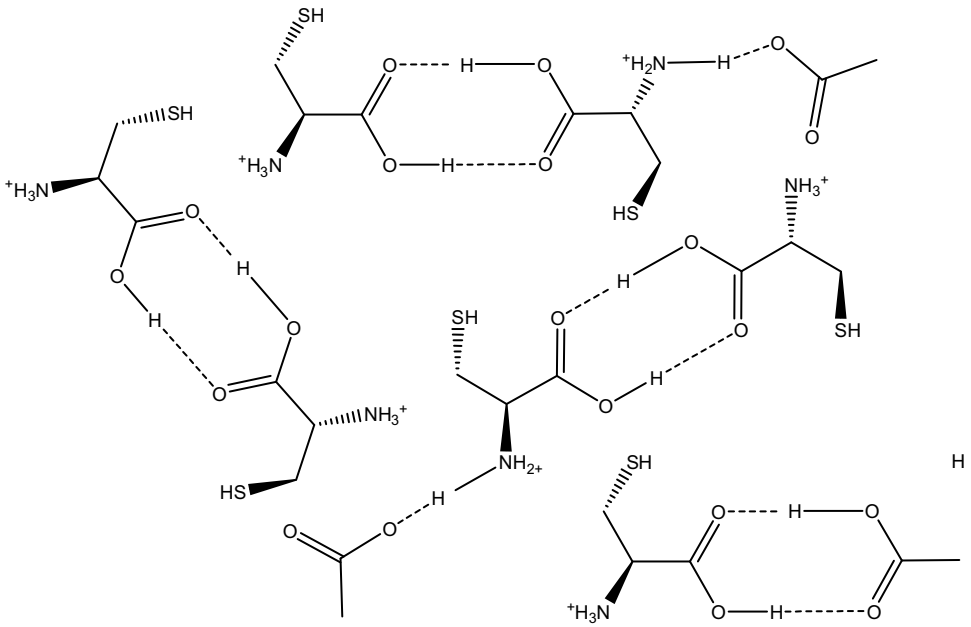
http://dx.doi.org/10.1016/j.molcata.2016.08.023
1381-1169/




**Scheme 1.** Mechanism of formation of compound I in CLP active site.



**Scheme 2.** Hydrogen bond network of cysteine in solution at pH 1 resulting mainly from the dimerization of carboxylic acid side chains.

labile in an aqueous environment, and mostly appear as an oxidant. The rate of carbon bond activation is challenging since the C—H bond is a strong bond [13,15,16]. Fe (II/III), as mentioned above, exhibits an outstanding power in oxidative activation. However, a powerful and robust bio-catalyst that shows activity at a low concentration, as well as improves the rate of transformation, is