Journal of Catalysis 348 (2017) 40–46

Contents lists available at ScienceDirect

Journal of Catalysis

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

Significant electron transfer in heme catalysis: The case of chlorite dismutase

Jing-Xuan Su, Shi-Lu Chen *

Key Laboratory of Cluster Science of Ministry of Education, School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing 100081, People's Republic of China

ARTICLE INFO

Article history:
Received 1 December 2016
Revised 7 February 2017
Accepted 9 February 2017

Keywords:
Chlorite dismutase
Density functional calculations
Electron transfer
Heme proteins
Peracetic acid

ABSTRACT

Electron transfer (ET) is significant in heme catalysis but usually difficult to be characterized. In the present DFT calculations, a transition state for ET has been optimized during the decomposition of peracetic acid (PAA) catalyzed by chlorite dismutase (Cld), using an active-site model based on the X-ray crystal structure of Cld. The Cld-catalyzed PAA reaction is revealed to proceed via a homolytic O—O bond cleavage to transiently form compound II (Cpd II) and acetate radical ($OAc^{\cdot}$), and a subsequent fast ET from Cpd II porphyrin to $OAc^{\cdot}$ leading to compound I (Cpd I) and acetate anion. The second step of ET is rate-limiting. The results highlight the importance of ET in heme chemistry and imply that the mechanisms of heme enzymes may be masked by fast ET even if a key intermediate has been detected (like Cpd I in this case). The comparison with the Cld-catalyzed chlorite reaction further indicates that a heme enzyme may employ different mechanisms for different substrates.

1. Introduction

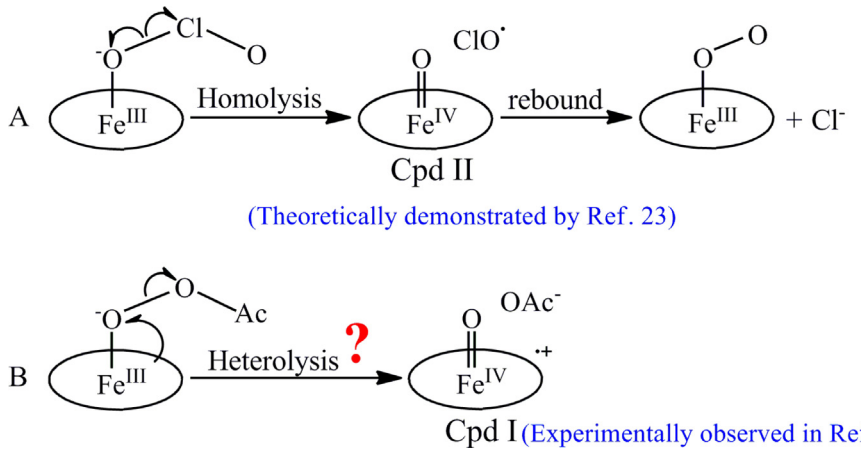
Heme-binding proteins and enzymes are a significant fraction of metalloproteins. They conduct the remarkable diversity of biochemical functions, working in oxidation/reduction, proton/electron transfer, monoxygenation, chlorination, small-molecule sensing, heme trafficking, etc [1,2]. In mechanistic studies, the heme high-valent metal-oxo complexes, such as $O = Fe^{IV}(Por^{\cdot+})$ (Cpd I) and $O = Fe^{IV}(Por)$ (Cpd II), have been frequently predicted to be crucial intermediates [1,3–19]. Furthermore, electron transfer (ET) involving the heme high-valent $O = Fe^{IV}$ species was also often suggested in the catalytic cycles of many heme enzymes [1,3–8]. For example, in some peroxidases, oxygenases, and catalases the heme porphyrin (Por) is oxidized to Por π-cation radical (i.e. Cpd I), and then an electron is transferred to the latter from an amino acid/organic substrate to form Cpd II and an amino acid/substrate radical [4,5,7,8]. Thus, the evidences and characterization of various ET processes in heme are of significance for understanding heme chemistry and interpreting mechanisms of heme-containing systems. In the present work, an ET between Cpd II and acetate radical ($OAc^{\cdot}$), leading to Cpd I and acetate anion (OAc^{-}), was observed in the decomposition of peracetic acid (HOOAc, PAA) catalyzed by heme *b*-dependent chlorite dismutase (Cld). In particular, the transition state for the ET has been optimized.

Cld is responsible for the last step of bacterial perchlorate (ClO_4^-) respiratory pathway and converts toxic chlorite (ClO_2^-) into innocuous chloride (Cl^-) and dioxygen (O_2) [20–22]. The mechanism of the Cld-catalyzed chlorite reaction has been extensively investigated by density functional calculations [23], where a dominant homolytic O—Cl bond cleavage resulting in a Cpd II intermediate, followed by an O—O bond rebound, was revealed (see Scheme 1A). However, in an experimental study using PAA (a non-natural substrate) as the substrate for Cld, Cpd I was observed when pH = 6 (Scheme 1B) [24]. The observation of Cpd I in the Cld-catalyzed PAA decomposition was considered as an indication to a heterolytic dissociation of the peracid O—O bond [24]. By analogy, a heterolytic O—Cl bond cleavage to yield Cpd I was then inferred to be the most likely initial step in the Cld-catalyzed reaction of the natural substrate (chlorite) [24]. This makes the scrutiny into the O—O bond dissociation of PAA in Cld to be crucial and urgent for the interpretation of the Cld reaction mechanisms, and also inspiring for heme catalysis. It is worth mentioning that, in the same experimental study [24], hydrogen peroxide (H_2O_2) was also used as a potential substrate for Cld but it resulted in slow destruction of the heme in Cld. Thus, we only consider the Cld-catalyzed PAA reaction in this paper.

In this work, using unrestricted density functional theory (UDFT) with the hybrid functional B3LYP, we have investigated the peracid O—O bond dissociation of PAA in Cld with an

* Corresponding author.
E-mail address: shlchen@bit.edu.cn (S.-L. Chen).

http://dx.doi.org/10.1016/j.jcat.2017.02.006
0021-9517/



Scheme 1. The decomposition of chlorite (A) and peracetate (B) catalyzed by chlorite dismutase (Cld). Chlorite is a natural substrate while peracetate is nonnatural.

active-site model (Fig. 1) constructed on the basis of an X-ray crystal structure (PDB ID: 2VXH) [25]. The energetics for the PAA reaction and the characterization of the transition states (TS) and intermediates involved are presented. The calculations indicate that the Cld-catalyzed decomposition of PAA employs a stepwise mechanism, where a homolytic O—O bond cleavage takes place leading to short-lifetime Cpd II and OAc•, followed by a fast ET from Cpd II porphyrin to OAc• to form experimentally observed Cpd I [24] and OAc^-. It is demonstrated that the detection of Cpd I intermediate does not always mean an initial step of heterolysis, since a homolysis mechanism may be masked by a subsequent fast ET step. The results render the significance of ET in heme chemistry which may be responsible for crucial catalytic processes monitored difficultly.

2. Computational methods

All calculations were performed using unrestricted density functional theory (UDFT) with the hybrid functional B3LYP [26–28], as implemented in the Jaguar 7.6 package [29]. Geometry optimizations were carried out with the LACVP** basis set, which implies a 6-31G(d,p) basis set [30,31] for the first- and second-row elements and a nonrelativistic effective core potential [32] for the Fe atom. On the basis of the optimized geometries, more accurate energies were obtained by performing single-point calculations with larger diffuse functions-included basis sets, that is, LACV3P+ for Fe and cc-pvtz(-f)+ for the other elements. Using the Gaussian 03 package [33], frequency calculations were performed at the same level of theory as in the optimizations to further confirm the nature of stationary points and to obtain zero point energies (ZPE). To estimate the effects of the ignored protein environment on the calculated energies, solvation effects were calculated at the same theory level as the optimizations by performing single-point calculations on the optimized structures using the self-consistent reaction field method with a Poisson-Boltzmann solver [34,35], as implemented in Jaguar 7.6. The dielectric constant (ε) of the surrounding medium was typically chosen to be 4, which is a standard value that has been used in many previous studies [2,23]. Using an empirical formula by Grimme et al. (i.e. DFT-D3) [36–39], dispersion effects on calculated energies were taken into account in the present enzyme system, where the reaction takes place involving a massive heme cofactor. In the previous study of the Cld-catalyzed decomposition of chlorite [23], dispersion corrections were not included in geometry optimizations but were calculated by performing single-point calculations, which is a reasonable method to consider dispersion especially for the

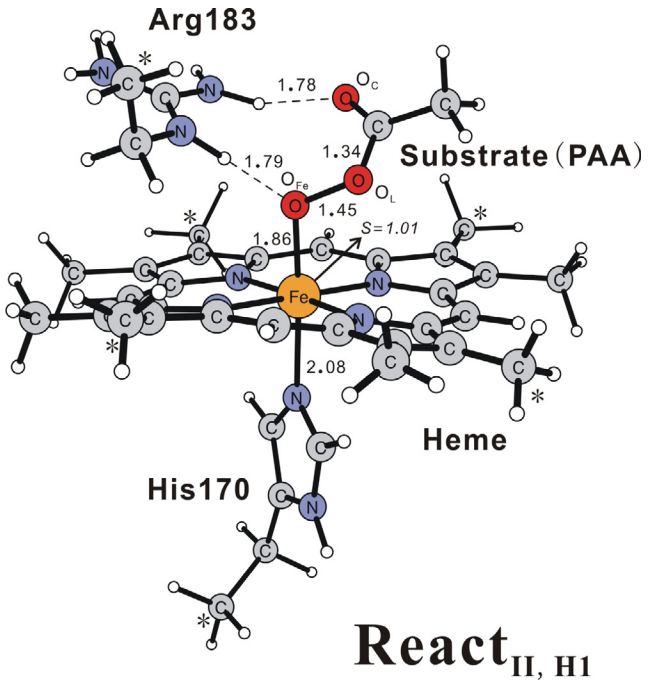


Fig. 1. Optimized structure of the Cld active site with a PAA bound. Asterisks indicate atoms that are fixed to their X-ray positions. All distances are given in angstrom (Å). The unpaired spin population is also shown with an indication of "**S**". O_{Fe} = The oxygen binding to Fe; O_C = The carbonyl oxygen; O_L = The leaving oxygen.

systems with small dispersion effects [40,41]. In this work about PAA, to be compared with the case of chlorite, dispersion effects were considered with the same way. If not otherwise indicated, the energies reported in this paper are corrected for ZPE, solvation, and dispersion effects. The present procedure, termed cluster modeling, has been systematically benchmarked [2,40,42–44] and successfully used to study a large number of metallo-enzyme mechanisms in the past decades by different research groups [2,45–55], including many heme-dependent enzymes where Cpd I and/or Cpd II are key intermediates. The computational methods described here can also be used to optimize the transition state for electron transfer. There have been a lot of successful cases, such as di-manganese complexes [56], class I ribonucleotide reductase (RNR) [57], and class III RNR [58]. The optimizations of transition states for electron transfer have been discussed in more detail in a review by Siegbahn and Blomberg [59].