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Enantioselective oxidation of thioanisole with *Arthromyces ramosus* peroxidase revealed by docking calculations

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Peroxidases are heme proteins that catalyze the oxidation of a wide variety of organic molecules by hydrogen peroxide or organic peroxides. Experimentally it was determined that peroxidases can also catalyze the oxidation of sulfides to sulfoxides in an enantioselective manner. Three mechanisms have been proposed to explain oxygen transfer from the enzyme to sulfide. Docking calculations of the present work showed that thioanisole is able to dock into the active center of ARP. The gained data suggest that sulfoxidation of thioanisole proceeds through the oxygen-rebound mechanism with a 99% yield of S-sulfoxide structures. These data are in excellent agreement with published experimental data.

Key words: docking, peroxidase, enantioselective, sulfoxidation, thioanisole

INTRODUCTION

Most of reactions catalyzed by enzymes in living organisms are stereoselective. The use of enzymes in oxidative conversions is a potentially attractive method for the synthesis of optically active compounds for biotechnology and drug production. Peroxidases are especially attractive biocatalysts for fine chemicals production, since hydrogen peroxide can be used as a cheap oxidant [1].

Peroxidases are heme proteins that catalyze the oxidation of a wide variety of organic molecules by hydrogen peroxide or organic oxide. An oxidation occurs as described in the scheme:

$$E + H_2O_2 \rightarrow \text{cpd I} + H_2O, \quad (1)$$

$$\text{cpd I} + A \rightarrow \text{cpd II} + A^*, \quad (2)$$

$$\text{cpd II} + A \rightarrow E + A^* + H_2O, \quad (3)$$

where E is the enzyme in the resting state (native enzyme), cpd I and cpd II are intermediate active forms, A is a substrate, and A^* is an oxidized substrate (typically radical cation or radical).

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As is depicted in the scheme, the catalytic cycle involves two intermediate forms of the enzyme, cpd I and II (cpd I and cpd II). Cpd I is formed during reaction of H_2O_2 with the ferric enzyme (eq. 1). This form has Fe(IV)=O and contains one electron vacancy on heme. Cpd I is reduced by a substrate molecule to cpd II (eq. 2); it does not contain an electron vacancy on the heme anymore. A second substrate molecule reduces cpd II back to the native enzyme (eq. 3).

It has been shown that peroxidases catalyze the oxidation of sulfides to sulfoxides in an enantioselective manner. Several articles have been published on the catalysis of asymmetric sulfoxidation by horseradish peroxidase (HRP) [1–3], lactoperoxidase (LPO) and *Coprinus cinereus* peroxidase (Cip) [4]. The experimental data outlined in these articles allow to suggest an oxygen-rebound mechanism of enantioselective sulfoxidation. However, there are some evident materials on sulfoxidation by the hydroxyl radical which forms in the active center of peroxidase [5]. In fact, three mechanisms have been proposed to explain oxygen transfer from enzyme to sulfide. In one it is suggested that oxygen moves from cpd I to a sulfide-forming sulfoxide. This is called the oxoferryl or oxene mechanism [1]. In the so-called oxygen-rebound mechanism [1–5] a substrate is firstly oxidized by cpd I to a substrate ra-

dical cation, which forms a complex with compound II. Then the oxygen of cpd II is transferred to the sulfide radical cation, a sulfoxide is released, and the enzyme returns to its native state. The last one involves a step of sulfide radical cation formation, too. It is proposed that a hydroxyl radical (presumably formed during H^+ transfer to oxygen) is released from cpd II, which then reacts with the sulfur radical cation forming the sulfoxide [2, 5].

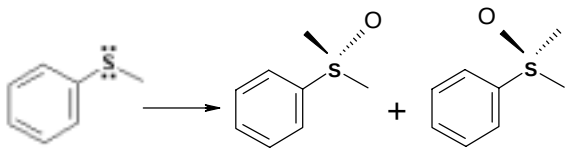
To get a further insight into the mechanism of sulfide oxidation, docking calculations of thioanisole and thioanisole cation radical with *Arthromyces ramosus* peroxidase (ARP) were performed in this work. Three ARP forms were used: native enzyme (ARP-N), its active form (ARP-I/II) and ARP, where the hydroxyl radical is in the active site (ARP-OH).

MATERIALS AND METHODS

Ab initio calculations of electronic structures and the energies of thioanisole (Fig. 1) and thioanisole radical cation were performed using the Gaussian 98 W package [6]. The optimization of geometry was accomplished using HF (Hartree-Fock) theory and the 3–21G basis set. The sulfur atom in thioanisole has two lone pairs, one of which is attacked by the oxygen atom when the oxidation process proceeds. Since there are no parameters to depict lone pairs in the software, docking calculations of thioanisole with lone pairs were not performed. Lone pairs were added in figures just for visualization.

The simulations of substrate docking in the active center of ARP were performed with AutoDock 3.0.5 [7–9]. ARP is identical to *Coprinus cinereus* (CiP) peroxidase; these peroxidases differ only in one terminal amino acid [10]. The crystal structure of native ARP (PDB-ID: 1ARP) [11] was downloaded from the Protein Data Bank. All water molecules in ARP were removed, with the exception of the structural water molecule at the distal side of the heme.

Kollman's united atom charges were introduced into docking calculations. All three forms of ARP (ARP-N, ARP-I/II, ARP-OH) used the same charge scheme, except the distal oxygen charge: the charge of O(H) in ARP-OH form was twice as low as in other forms. Hydrogen for the structural water (ARP-N) and hydroxyl radical (ARP-OH) was not modeled in order to ensure a proper possible oxygen–sulfur interaction, as hydrogen dislocation in the process of 'complexation' had not been modeled with the software. The position of oxygen in ARP-I/II was adjusted to be the average Fe=O bond length of experimentally determined Fe=O bond length distance of cpd I and cpd II of HRP [12], and it was 1.77Å. In ARP-OH the coordinates of hydroxyl radical oxygen are the same as those of the water in the native ARP.



thioanisole *R*-enantiomer *S*-enantiomer

Fig. 1. Structure of thioanisole and sulfoxides

The energy grid maps of atomic interaction were calculated with 0.375 Å spacing and 126 grid points forming a 47.25 Å cubic box, which covered the whole protein with a vast space around. The electrostatic interaction energy grid used a distance-dependent dielectric function of Mehler–Solmajer [13]. The docking was accomplished using the Lamarckian genetic algorithm. The number of individuals in populations was set to 50. The maximum number of energy evaluations of this algorithm is 500000; the maximum number performed was 27000. The number of top individuals guaranteed to survive into the next generation was 1, the mutation and crossover rates were 0.02 and 0.80.

RESULTS AND DISCUSSION

In [14, 15] molecular modeling was performed to rationalize the enantiomeric ratio of sulfide oxidation. However, these investigations lack precision, since the substrates were fixed in the active center of peroxidases at a close distance. Therefore, discrepancies between the experimental data and modeled values were observed. In the present work, modeling without restriction was performed and the docking calculations showed that thioanisole is able to dock into the entrance of the active center of ARP. This is consistent with the experimental observations that ^{18}O-labelled thioanisole oxidation product is produced using ^{18}O-labeled H_2O_2 [14].

In the native enzyme (ARP-N), the aryl ring of all docked thioanisole structures is directed into the active center and is parallel to the heme plane. The alkyl group is directed to the solvent (Fig. 2). In the structure with the lowest docking energy (–6.2 kcal/mol) the minimal distance from the benzene ring to the iron atom is 5.3 Å and to the water molecule 2.9 Å. All structures locate at a distance of about 3.5 Å over the heme.

During oxidation of peroxidase with hydrogen peroxide and cpd I formation, the position of amino acids in the active center practically does not change. This was indicated in experimental studies of HRP [12]. Therefore, the structure of ARP-N and

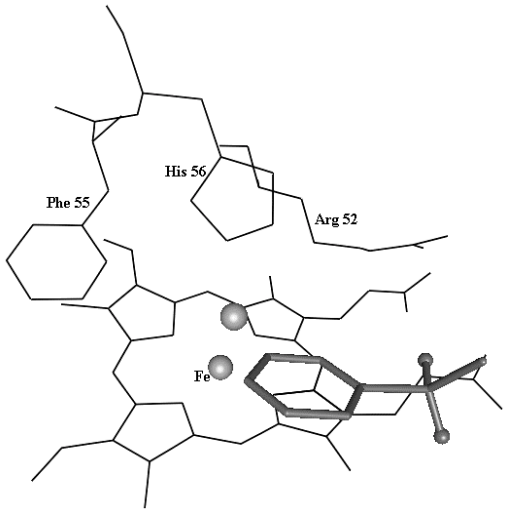


Fig. 2. Docking of thioanisole in native ARP. Sticks with balls at sulfur atom signify electron pairs

ARP-I/II is similar, but the distance between oxygen and iron decreases by 1.19 Å in ARP-I/II. However, a significant difference appears during docking calculations of thioanisole and thioanisole cation radical in ARP-I/II, by which both cpd I and cpd II are treated in this work. Dockings of thioanisole with ARP-I/II are interpreted as a substrate interaction with cpd I, whereas dockings of the thioanisole cation radical with ARP-I/II are interpreted as an interaction between the molecule and cpd II from the point of view of the oxygen-rebound mechanism. Almost all thioanisole radical cation structures dock with their alkyl groups into the active center of ARP-I/II (Fig. 3), forming S-enantiomers with the energy of –8.3 kcal/mol, whereas alkyl groups of the major part of thioanisole are directed to solvent. The dockings of thioanisole in the ARP-I/II form have the same character as the dockings of thioanisole with ARP-N because of the structural similarity of the active center in both forms (mentioned above). The reorientation of the thioanisole radical cation in the active center is determined by a change of charge on the sulfur atom: as it becomes more positive, the sulfur atom electrostatically interacts with ferryl oxygen. Furthermore, the docking energy of the thioanisole cation radical is lower than that of thioanisole, *i.e.* –6.1 kcal/mol (Table). This fact sustains the suggestion that the substrate cation radical complex with ARP forms during the oxidation process and the oxygen-rebound mechanism occurs.

Table. **Docking of thioanisole and thioanisole radical cation in ARP and ARP-OH**

	LDE kcal/mol,	Enantiomer	Distance S—O (Fe= O/ ·OH), Å	Enantiomer yield, %
ARP-I/II				
Th	–6.1	S	2.9	4
Th·$^+$	–8.3	S	2.9	99
ARP-OH				14
Th·$^+$	–7.4	S	2.8	

Th – thioanisole, Th·$^+$ — thioanisole radical cation, LDE – lowest docking energy.

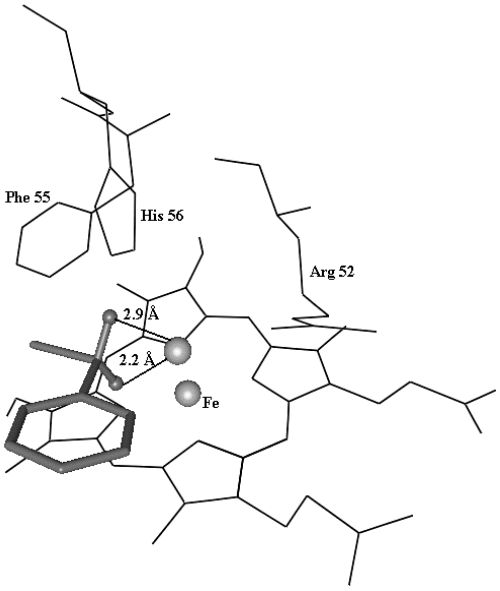


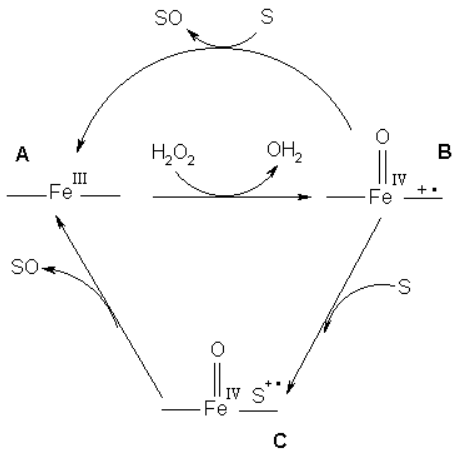
Fig. 3. Docking of thioanisole cation radical in ARP-I/II. The distance is shortest between ferryl oxygen and pro-S electron pair

A comparison of complexes of benzohydroxamic acid (BHA) and salicylhydroxamic acid (SHA) with ARP determined by X-ray crystallographic analysis at 1.6 Å resolution [16] and 1.9 Å resolution [17], respectively with thioanisole radical cation obtained through the docking calculations revealed some similarities. For instance, the aromatic rings of BHA and SHA are positioned at the entrance of the heme pocket, approximately parallel to the heme plane as in the case of thioanisole cation radical docking with ARP-I/II.

Another possible mechanism of thioanisole radical cation complexation with ARP includes the ARP form with hydroxyl radical in the active center, where the hydroxyl radical is formed from cpd II after H^+ transfer to oxygen by a distal group. This hydroxyl radical reacts with the sulfur radical cation [2, 5]. However, our docking calculations show that the docking energy of the thioanisole cation radical obtained in ARP-I/II case is lower than in the ARP-OH form (–7.4 kcal/mol). Furthermore, on-

ly 14% of S-enantiomers were gained in ARP-OH, whereas a maximal yield of 99% of the S-enantiomer and maximal energy were obtained at docking calculations of the thioanisole cation radical with ARP-I/II. These findings show that sulfide oxidation by the oxygen rebound mechanism is more effective than hydroxyl radical attack on the thioanisole cation radical. An experimental investigation of the oxygenation of sulfide showed that the S-sulfoxide could be obtained in a yield of 84% using fungal peroxidase from *Coprinus cinereus* (CiP) [4]. It is very close to the modeled yield of the present work, showing an S-favored enantiomer.

On the ground of the results described above and experimental studies, the mechanism of sulfide oxidation can be summarized (Scheme). The general mechanism of peroxidase starts when the H_2O_2 molecule reacts with the native enzyme (Scheme, A) and cpd I is formed with two oxidative equivalents. In the next step thioanisole can be oxidized by direct oxygen transfer from cpd I (Scheme, B), but the modeling results clearly deny that mechanism of thioanisole oxidation. One electron oxidation occurs with formation of a substrate cation radical and cpd II. The thioanisole cation radical reorients in the active site of CiP/ARP in such a way that its alkyl group is directed to the active center and a complex of cpd II and thioanisole cation radical is formed (Scheme, C). Our modeling data show that formation of that complex is energetically more favorable than the oxygen-transfer from cpd I to thioanisole, and this is in agreement with experimental studies [1–4]. It is supported by the fact that the docking energy of thioanisole with ARP-I/II is 2 kcal/mol higher than the docking energy of thioanisole cation radical with ARP-I/II. After the complex formation the oxygen of cpd II is transferred to the substrate cation radical forming sulfoxide. The oxygenated product is released and the enzyme returns to its native state.



Scheme. ARP catalyzed sulfoxidation mechanism. A – the native enzyme, B – cpd I, C – cpd II and sulfide radical cation complex

CONCLUSIONS

Docking calculations showed that thioanisole is able to dock into the active center of ARP in all its forms. All three possible thioanisole oxidation pathways were examined by docking calculations, and the results suggested that thioanisole sulfoxidation proceeds through the oxygen-rebound mechanism, as was postulated in other experimental studies. The docking results showed that the sulfur atom of thioanisole cation radical docks at close distances to the ferryl oxygen in the active center of ARP. A comparison of thioanisole and thioanisole cation radical docking results revealed that the main substrate reorientation process in the catalytic site is determined by the electrostatic interaction of ferryl oxygen and the sulfur atom, which bears a positive charge.

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TIOANIZOLO KOMPLEKTACIJOS *Arthromyces ramosus* PEROKSIDAZĖJE MODELIAVIMAS: ENANTIOSELEKTYVIOS SULFIDO OKSIDACIJOS TYRIMAS

S a n t r a u k a

Peroksidazės – tai hemą turintys fermentai, katalizuojantys įvairių organinių ir neorganinių junginių oksidaciją. Eksperimentais nustatyta, kad peroksidazės geba enantioselektyviai oksiduoti sulfidus. Pasiūlyti trys oksidacijos mechanizmai, paaiškinantys deguonies pernešimą nuo fermento sulfidui. Šiame darbe buvo atlikti tioanizolo ir tioanizolo katijono radikalo kompleksacijos *Arthromyces ramosus* peroksidazėje (ARP) skaičiavimai naudojant AutoDock 3.0.5 programą. Kompleksacijos skaičiavimų duomenimis, tioanizolas geba kompleksuotis ARP aktyviame centre. Gauti duomenys leidžia manyti, kad oksidacija vyksta vienu iš pasiūlytų mechanizmų, kurio metu susiformuoja tioanizolo katijono radikalo ir fermento kompleksas. Pernešus deguonį nuo fermento sulfido radikalui, susiformuoja S-sulfoksidas. Šiame darbe gauta S-sulfoksido struktūrų išeiga yra 99%. Rezultatai sutampa su publikacijose skelbiamais eksperimentiniais duomenimis.