Intermolecular Forces at Play in the Active Site of Lactoperoxidase

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Introduction

LACTOPEROXIDASE (LPO) is a heme enzyme found in exocrine secretions such as milk, saliva and endodermal mucus. LPO catalyzes the formation of oxidizing oxoanions that act as natural antibiotics in those body fluids.
LPO is a versatile enzyme that converts thiocyanate ions (SCN^-) to hypothiocyanite ions ($OSCN^-$), as well as halide ions (i.e., Cl^-, Br^- and I^-) to hypohalite ions (i.e., ClO^-, BrO^- and IO^-).
These highly oxidizing agents are toxic to germs such as oral streptococci,[1] *Porphyromonas gingivalis*,[2] *Helicobacter pylori*,[3] *Salmonella typhimurium*, HIV, herpes simplex type 1, and the influenza virus.[4,5]

LPO belongs to the peroxidase family of enzymes, which also includes myeloperoxidase (MPO), eosinophil peroxidase (EPO) and thyroid peroxidase (TPO).
LPO, MPO and EPO protect organisms against microbial infections as part of their innate immune system.
MPO and EPO are stored in the granules of white blood cells, whereas LPO is steadily released in the mucus lining of airways, as well as in saliva, tears and milk.[6] In particular, TPO is involved in the synthesis of thyroid hormones.

Table 1. Concentrations of SCN^- and HOSCN Present in Biological Fluids.[6]

Fluid	[SCN^-]	[HOSCN]
Plasma	(10 to 100) µM	N/A
Saliva	(0.3 to 3) mM	(10 to 60) µM
Lung bronchioalveolar fluid	≤ 500 µM	N/A
Upper airway (nasal) ASL fluid	400 µM	N/A

Because of its importance for the immune system, the molecular structure and efficacy of native forms of LPO against various pathogens have been studied for potential applications in medical therapies.[1-6]

In a more advanced approach, chemists seek to design artificial enzymes that mimic the antimicrobial function of LPO because such synthetic catalysts can be modified to vary their specificity for substrates, can be tuned to perform under broader physiological conditions, can have a longer shelf life than a natural enzyme, and can be dispensed in a variety of pharmaceutical forms without the compound losing effectiveness.

To design an artificial enzyme, the pathway by which LPO converts common ions such as chloride, bromide or iodide into germ-killing agents has to be resolved in atomistic detail combining the structural information derived from crystallography, the tool of bioinformatics and the methods of computational chemistry.

As other peroxidase enzymes, LPO and MPO use H_2O_2 to convert its substrates into potent oxidizing agents, according to the following **catalytic cycle**:[7,8,9,10]

STAGE 1. Enzyme activation:

$$\text{Enz–Fe}^{2+} \xrightarrow{H_2O_2 \;\to\; H_2O} \underset{\text{Compound I}}{\text{Enz–Fe}^{3+}\text{–O}^{\bullet} \leftrightarrow \text{Enz–Fe}^{\bullet 4+}\text{=O}}$$

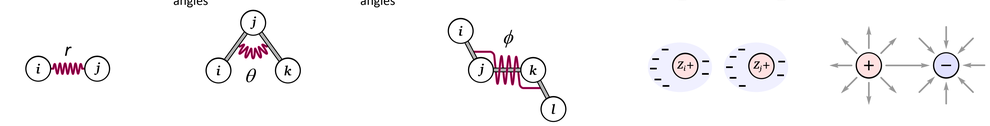
Ground-state

STAGE 2. Two-electron oxidation of a (pseudo)halide:

$$\underset{\text{Compound I}}{\text{Enz–Fe}^{3+}\text{–O}^{\bullet} \leftrightarrow \text{Enz–Fe}^{\bullet 4+}\text{=O}} \xrightarrow{X^- \;\to\; XO^-} \underset{\text{Ground-state}}{\text{Enz–Fe}^{2+}} \quad (X = NCS, Cl, Br, I)$$

Methods

$$E_{tot} = \sum_{bonds} K_r(r - r_{eq})^2 + \sum_{bond\ angles} K_\theta(\theta - \theta_{eq})^2 + \sum_{dihedral\ angles} \frac{V_n}{2}[1 + \cos(n\phi - \phi_0)] + \sum_{i<j}\left[\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right] + \sum_{i<j}\frac{q_i q_j}{\varepsilon r_{ij}}$$



The total energy (E_{tot}) of a large active-site model and its molecular component parts were estimated using the basic molecular mechanics (MM) potential energy function, which consists of:
- terms for atoms linked by covalent bonding (i.e., bond stretching, bond bending and bond torsion),
- terms for atoms not covalently bonded with each other but interacting by van der Waals and Coulomb electrostatic forces.

The parameters (K_r, r_{eq}, K_θ, θ_{eq}, V_n, ϕ_0, A_{ij}, B_{ij}, q_i) in the potential energy function were evaluated with the AMBER force field[17] as implemented in the Gaussian 09 program suite.[18] The AMBER force field has been specially designed to compute three-dimensional structures of proteins and to evaluate the contributions of nonbonding interactions.

The large active-site chemical model consists of the heme group surrounded by the residue side-chains within 12 Å from the iron atom. This structure was extracted from the crystallographic structure 3NYH.[14]

The binding energies of relevant moieties to the stability of the active-site model were computed with the AMBER force field.

$$\Delta E = E(\text{Active-Site} \cdots \text{Ligand}) - E(\text{Active-Site}) - E(\text{Ligand})$$

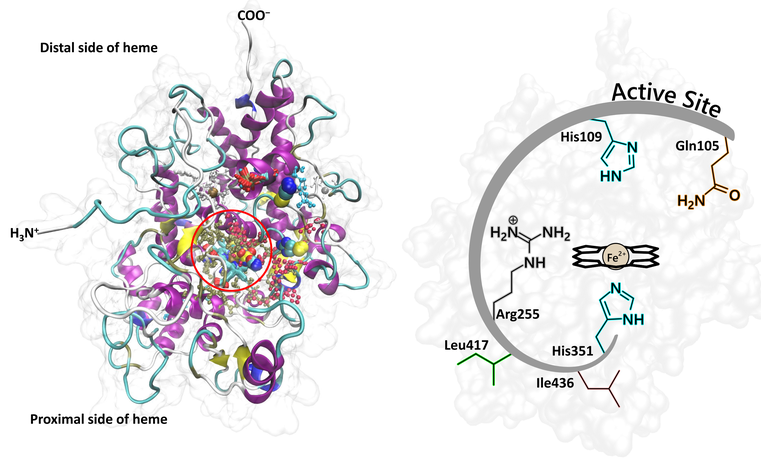
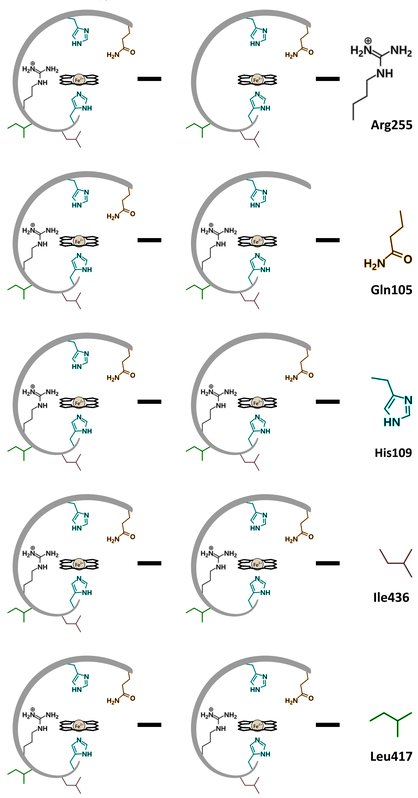


Figure 1. (Left) Representation of LPO's tertiary structure with superimposed active-sites embedded. The active sites (circled) of all the crystallographic structures available in the PDB site are shown overlapped and centered on the heme group. (Right) Schematic representation of the catalytically relevant residues in the active site of LPO.

Results

Figure 2. Graphical schemes of the former equation with various crucial moieties in the active site acting as ligands.
- ΔE_{tot} represents the total energy difference between the whole active site and its parts. Each total energy difference quantifies the association of a part with the whole active site.
- ΔE_{vdW} represents how much van der Waals interactions contribute to ΔE_{tot}.



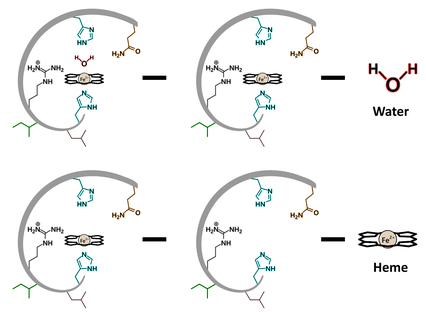
$\Delta E_{tot} = -58.2$ kJ mol^{-1}, $\Delta E_{vdW} = -58.2$ kJ mol^{-1}

$\Delta E_{tot} = -56.3$ kJ mol^{-1}, $\Delta E_{vdW} = -64.8$ kJ mol^{-1}

$\Delta E_{tot} = -36.7$ kJ mol^{-1}, $\Delta E_{vdW} = -41.8$ kJ mol^{-1}

$\Delta E_{tot} = +9.8$ kJ mol^{-1}, $\Delta E_{vdW} = +7.3$ kJ mol^{-1}

$\Delta E_{tot} = -36.7$ kJ mol^{-1}, $\Delta E_{vdW} = -41.8$ kJ mol^{-1}



$\Delta E_{tot} = -10.9$ kJ mol^{-1}, $\Delta E_{vdW} = -10.9$ kJ mol^{-1}

$\Delta E_{tot} = -122.4$ kJ mol^{-1}, $\Delta E_{vdW} = -130.4$ kJ mol^{-1}

Conclusions

- The negative value of the computed association energies indicate the stabilizing nature of the interaction.
- Particularly significant is the slightly repulsive interaction of Ile436 in the active site because of its close proximity to a carboxylate group from the heme.
- The cases where $\Delta E_{tot} > \Delta E_{vdW}$ indicate that the side-chain is subjected to significant bending and torsional strain. This means that the residue is not arranged in its most favorable conformation within the active-site model.
- Consequently, the geometry of the active-site model will have to be optimized in the AMBER force field so that this structure may release the bending and torsional strains and form more stabilizing internal hydrogen-bonding interactions within the active site.
- Ultimately, these force field calculations allowed us to verify that the AMBER parameters were correctly assigned in the active-site model. This detailed assignment is required to set up the quantum mechanics / molecular mechanics (QM/MM) calculations, which will elucidate the detailed catalytic mechanism of LPO at the atomistic level.

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