Nonlinear Analysis: Modelling and Control, 2004, Vol. 9, No. 4, 373–383

Modeling the Enantioselective Enzymatic Reaction with Modified Genetic Docking Algorithm*

A. Žiemys[1,2], L. Rimkutė[1], J. Kulys[2,3]

[1]Vytautas Magnus University, Vileikos st. 8, LT-44404, Kaunas
a3arzi@vaidila.vdu.lt

[2]Institute of Biochemistry, Mokslininkų st. 12, LT-08662 Vilnius

[3]Vilnius Gediminas Technical University, Saulėtekio av. 11, LT-10223 Vilnius

Received: 22.09.2004
Accepted: 04.10.2004

Abstract. The handling algorithms for molecular interaction and docking is of increasing involvement in biological processes modeling. Genetic algorithm, in particular, improves the computation models and leads to more effective and robust calculations. An example of genetic algorithm application for the treatment of enantioselective enzymatic (peroxidase catalyzed) reaction is rendered. The performed modeling revealed the substrate structure influence to the docking in the enzyme active center and provided an explanation to the mechanism of peroxidase-catalyzed asymmetric oxidation reaction. The comparison of modeling results with published experimental data revealed the effectiveness of used algorithm, its suitability for solving problems for enantioselective enzymatic reactions modeling and its relevance to provide the rational design of fine prechiral compounds based targets.

Keywords: peroxidase, enantioselectivity, modeling, genetic algorithm.

1 Introduction

Interactions between biomolecules are fundamental to the obvious majority of biological processes. Based on these interactions, living organisms maintain complex regulatory and metabolic interaction networks that together constitute the processes of life. Understanding of biomolecular interactions is the key in solving

*The research was supported by Lithuanian State Science and Studies Foundation, project No. C-03020.

the biological phenomena. It is well known that protein function is closely related to a three-dimensional structure, much more closely than to the sequence do. Therefore the knowledge of molecular structure and the ability to manipulate the three-dimensional molecular contents reveals new ways of treating our health.

Rapid advances in computational technologies boosted the development of modeling algorithms, tools for molecular interactions and molecular docking itself. These tools are essential for rational design of therapeutic drugs and new synthetic proteins that can cure diseases and improve our health. Such a techniques can be applied to X-ray crystallography, structure-based drug design, lead optimization, virtual high throughput screening (vHTS), combinatorial library design, protein-protein and protein-substrate/inhibitor docking, chemical mechanism studies. Ligand binding is a key aspect of protein function, mediating the ability of proteins to recognize their natural ligands for transport, signal transduction or catalysis, and also the ability to modulate biological function through the discovery of drugs. The mentioned above aspects can be treated with molecular docking. There is a number of software that is successfully implemented in the area of docking studies like DOCK [1], FlexX [2], GOLD [3], LigandFit [4], Glide [5], AutoDock [6].

The software like AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. It has a free-energy scoring function that is based on a linear regression analysis and a large set of diverse protein-ligand complexes with known inhibition constants. The current version of AutoDock search methods now includes Monte Carlo simulated annealing (SA), evolutionary, genetic and Lamarckian Genetic Algorithm (LGA) methods. The last is a big improvement on the Genetic Algorithm (GA), and both genetic methods are much more efficient and robust than SA.

Genetic algorithm is a computation model generally used for optimization. It uses the idea of genetics in biological evolution. An elementary unit of a genetic algorithm, called a chromosome, carries the information about the set of parameters representing a particular instance. In case of molecular docking, the chromosome consists from genes, which each of them describes the translation, orientation and conformation of the ligand with respect to the protein. That set

of genes defines the genotype of the ligand. The genotype defines the particular coordinates of the ligand, which correspond to the phenotype of ligand. Random individuals undergo mutations and crossovers like in natural genetics. Mutations are a crucial part of the algorithm as they allow the creation of radically new solutions. During the mutations some individuals undergo random changes in genes by random amount. Crossover takes place between a pairs of individuals (two chromosomes), which new individuals (new chromosomes) inherit mixed genes from both parents (Fig. 1). A colony or population is a collection of chromosomes which evolves as the algorithm progresses. A selection of the offspring of a generation is based on the fitness of the individual: the individuals having better fitness are let to reproduce, while the individuals having poor fitness "die".

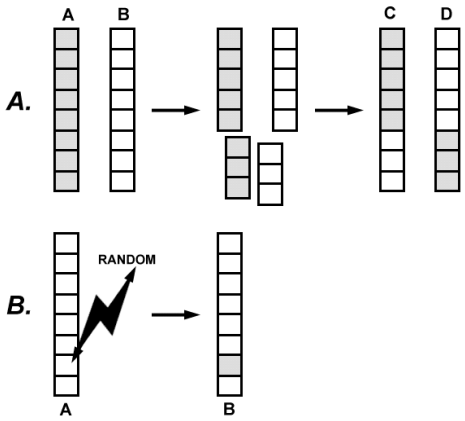

Fig. 1. Crossover (A) and mutation (B) illustration. Each column represents a chromosome (or an individual) and each square – a particular gene.

A modified version of GA is LGA. In LGA GA is optimized for global search and introduced Local Search (LA) method performs local search or global and local at once. That local search method does not require gradient information about local landscape. In addition, local search is adaptive, in that it adjusts the search step size depending upon the recent history of energies [6].

The fitness of the ligand is determined by the total energy of the ligand with the protein. The total energy, or the free energy of the binding, is expressed as:

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{sol} \quad (1)$$

where the first four terms are terms for dispersion/repulsion, hydrogen bonding, electrostatic. ΔG_{tor} models global rotations and translations, ΔG_{sol} models desolvations upon binding and the hydrophobic effect.

ΔG_{vdw} and ΔG_{hbond} can be expressed with general equations:

$$\Delta G_{vdw} = \sum_{ij} (A_{ij} r_{ij}^{-12} - B_{ij} r_{ij}^{-6}), \tag{2}$$

$$\Delta G_{hbond} = \sum_{ij} E(t)(A_{ij} r_{ij}^{-12} - D_{ij} r_{ij}^{-10}) \tag{3}$$

where i and j denotes atoms of ligand and protein, respectively. Coefficients A, B, D dependence on particular pairs of atoms and these are called Lennard-Jones (LJ) potentials. $E(t)$ is a directional weight for hydrogen bonding, which depends on the hydrogen bonding angle, t. A screened electrostatic term is expressed as:

$$\Delta G_{elec} = \sum_{ij} \frac{q_i q_j}{\varepsilon(r_{ij}) r_{ij}} \tag{4}$$

where q is charge, ε is a dielectric constant of a media. ΔG_{tor} term is proportional to the number of sp3 bonds in the ligand. Desolvation calculation is based on atomic solvation parameters, which are introduced into pairwise potential using Gaussian and sigmoid terms [6].

2 Calculations

Ab initio calculations of the electronic structure and the energy of sulfides and sulfides radical cations were performed using the Gaussian 98 W package [7]. The sulfur atom in the investigated sulfides has two lone pairs, one of which is attacked by oxygen atom, when oxidation proceeds (Fig. 2). There are no parameters defining lone pairs in the software. Lone pairs were added by software HyperChem 6.03 after docking for determination of obtained enantiomer type, as it was possible to measure the distances between lone pairs and oxygen.

The simulations of substrate docking in the active center of ARP, HRP and MnP were performed with AutoDock 3.0.5 [8]. The crystal structure of native *Arthromyces ramosus* peroxidase (ARP) (PDB-ID: 1ARP) [9], native horseradish

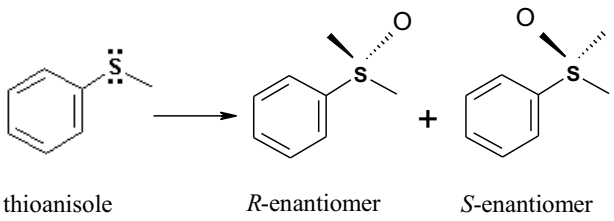

Fig. 2. Structure and stereochemistry of thioanisole.

peroxidase (HRP) (PDB-ID: 1atj) [10] and native manganese peroxidase (MnP) (PDB-ID: 1MNP) [11] was downloaded from the Protein Data Bank. Exploring suggested mechanisms of asymmetric peroxidase-catalyzed oxidation three forms of each peroxidase were prepared: native enzyme (ARP-N), oxidative enzyme ARP-I/II and enzyme with a hydroxyl radical in the active center (ARP-OH). The same tactics was applied to other investigated peroxidases.

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 whole protein with waste space around. The electrostatic interaction energy grid used a distance-dependent dielectric function of Mehler-Solmajer [12]. 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 was 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.

3 Results and discussion

The abstraction of enantiomerically pure compounds has a significant importance as the certain biological processes depend on the particular enantiomer effect. In medicine, for example, certain drugs are more effective in a preferred enantiomeric form and may produce fewer side effects. The elucidation of enantioselective reactions provide useful information, which could be used for improving methods of obtaining enantiomerically pure compounds. Experimental researches showed that certain enzymes, involving heme containing peroxidases, are able

to catalyze asymmetric oxidation of aromatic sulfides. Three mechanisms have been proposed to explain the transfer of oxygen atom from enzyme to substrate sulfur. The docking modeling of thioanisole and thioanisole cation radical with *Arthromyces ramosus* peroxidase (ARP), horseradish peroxidase (HRP) and manganese peroxidase (MnP) was performed to elucidate asymmetric peroxidase-catalyzed oxidation.

During docking procedure structures adopted several clusters over all enzyme surface. The conformations with lowest docked energy found by LGA in the active center of three enzyme forms were analyzed as the "best" docking result is considered to be the conformation with the lowest docked energy. Comparing docking results in all investigated peroxidases forms the lowest docking energy was observed for complexes between enzyme oxidative form (ARP-I/II, HRP-I/II, MnP-I/II) and thioanisole cation radical (thioanisole valency $1^{+\bullet}$) (Table 1). Hence, it can be assumed that these complexes are most favorable and most probable. Analysis of conformations by visualizing the docking results with the help of expressed program revealed the sulfide position favorable for oxygen transfer from enzyme to substrate sulfur in these complexes, where the sulfur atom of thioanisole cation radical resides at particular distance from oxygen in the active center (Fig. 3A). While the substrate position in other peroxidases forms is favorable for oxygen transfer, the docking energy is about 1.0 kcal/mol higher than it is for thioanisole cation radical and peroxidases oxidative form.

Analysis of sulfur orbitals position with regard to oxygen revealed that complexes between ARP and MnP oxidative form and thioanisole cation radical are favorable for S enantiomer as the shorter distance was measured from sulfur pro-S lone pair and oxygen (Fig. 3A). The equal distance was observed between both sulfur lone pairs and oxygen in thioanisole cation radical and HRP oxidative form complex (Table 1). Logically, the oxygen can be transferred to both sulfur lone pairs with the same probability.

There are no crystallographic data about complexes, which were investigated, however, gained modeling data correspond with published experimental researches in which the mechanism of oxygen transfer from peroxidase oxidative form to sulfide cation radical is proposed [13, 14]. The similar possibility of both enantiomer formation obtained in dockings of sulfide cation radical with HRP

oxidative form conforms to moderate enantioselectivity experimentally observed for HRP (Table 1). These results allowed going deeper into peroxidase ability to oxidize sulfides with different structure.

Table 1. Dockings of thioanisole and thioanisole cation radical with all forms of ARP, HRP, MnP. Docking energy, obtained enantiomer and distance between sulfur atom and Fe = O or Fe = ˙OH with difference of distances from sulfur pro-S and pro-R lone pairs to Fe = O or Fe = ˙OH in brackets are advanced. Experimental data from [13]

Enzyme form	Thioanisole valency	Docking energy, kcal/mol	Enantiomer	Distance S–O, Å	Experimental enantiomeric S : R ratio
ARP-N	0	-4.6	-	-	73 : 27
ARP-I/II	0	-5.0	-	-	
	$1^{+\cdot}$	-7.2	S	2.6 (0.8)	
ARP-OH		-6.2	S/R[1]	2.9 (0.3)	
HRP-N	0	-5.2	-	-	60 : 40
HRP-I/II	0	-5.1	S	3.2 (0.9)	
	0	-5.1	R	3.8 (0.7)	
	$1^{+\cdot}$	-6.4	S/R	3.2 (0.0)	
HRP-OH	$1^{+\cdot}$	-5.9	R	2.7 (0.6)	
MnP-N	0	-5.0	-	-	87 : 13
MnP-I/II	0	-5.4	S	3.0 (0.7)	
	$1^{+\cdot}$	-7.6	S	3.0 (0.4)	
MnP-OH	$1^{+\cdot}$	-6.8	S/R	2.7 (0.1)	

[1]The possibility of both enantiomers formation is similar.

The docking calculation of different sulfides with ARP oxidative form was performed to explore the influence of substitutes to docking energy and sulfur orbital orientation. Energetically most favorable complexes between sulfides and ARP oxidative form were considered to be those with the lowest docked energies calculated by LGA. It appeared that energetically and structurally favorable substrates for oxygen transfer from enzyme to sulfur were compounds with substitutes which are hydrogen bond acceptors and those with hydrophobic structure as the 1-methylthionaphthalene (Fig. 3B). The sulfur of these sulfides resided at the particular distance from oxygen in the active center of oxidative ARP form. The sulfur pro-S lone pair of these sulfides is closer to oxygen in the active center than pro-R lone pair, thus the S enantiomer formation is more expected.

There is an experimental conformity of the same gained enantioselectivity for the 1-methylthionaphthalene showed by *Coprinus cinereus* peroxidase (CiP), which is structurally identical to ARP [15]. The orientation of sulfides with hydrogen bond donating substitutes or having cumbersome structure appeared to be structurally non-favorable for oxygen transfer; though the calculated docked energy was low.

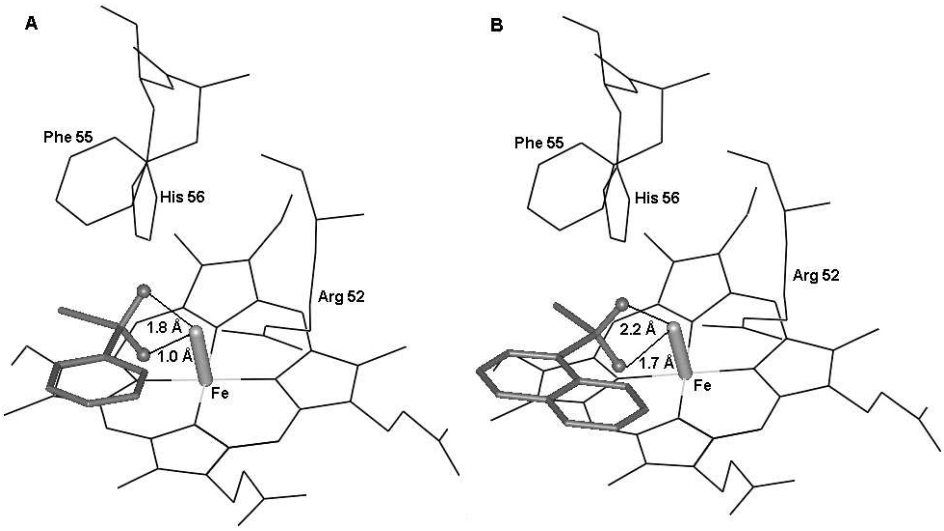

Fig. 3. Docking of thioanisole cation radical (A) and 1-methylthionaphthalene cation radical (B) in the active center of ARP-I/II. The sticks with balls at the end define sulfur lone pairs.

Docking calculations of the same sulfides in HRP oxidative form revealed the similar tendency of the hydrogen bond accepting groups favor to oxygen transfer and the disfavor of the compounds containing hydrogen bond donating groups (the data is not supplied). The sulfur of the latter compounds located at the entrance of the active center in both ARP and HRP, whereas substituted aromatic ring was positioned deeper in the active center (Fig. 4). In this case the simple electron donation from substrate to enzyme is expected in conformity with the experimental study, which show that 4-methylthiophenol behave as a simple phenolic substrate, which react with the oxidative HRP intermediate producing phenoxyl radicals and protons [16].

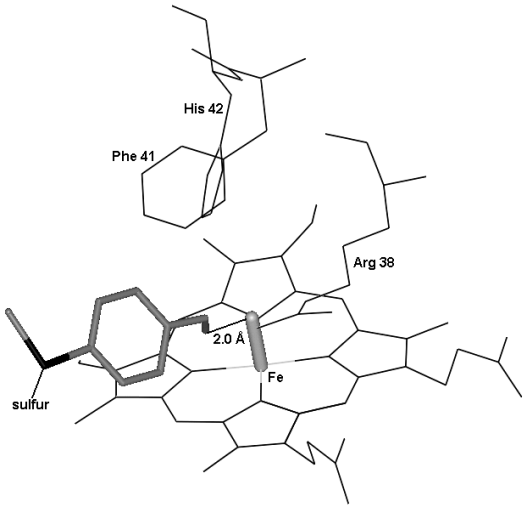

Fig. 4. Docking of 4-methylthiophenol, containing hydrogen bond donating substitute, in the HRP-I/II active center. The substitute form hydrogen bond with Fe = O.

4 Conclusions

The example of an appliance of Lamarckian genetic algorithm to exploration of enantioselective enzymatic reaction was preseneted. The method helped to elucidate the asymmetric peroxidase catalyzed sulfoxidation reaction mechanism and allowed to explain the influence of substrate structure to enantioselectivity. The certain conformity of modeled results to experimental data showed the effectiveness and reliability of the used Lamarckian genetic algorithm. On the ground of performed study the relevance of the method to provide the rational design of fine pre-chiral compounds based on targets can be proposed.

References

1. Kuntz I.D., Blaney J.M., Oatley S.J., Langidge R., Ferrin T.E. “A geometric approach to macromolecule-ligand interactions“, *J. Mol. Biol.,* **161**, p. 269–288, 1982

2. Rarey M., Kramer B., Lengauer T., Klebe G.A. “A fast flexible docking method using an incrememental construction algorithm“, *J. Mol. Biol.,* **261**, p. 470–489, 1996

3. Jones G., Willett P., Glen R.C., Leach A.R., Taylor R., "Development and validation of a genetic algorithm to flexible docking", *J. Mol. Biol.*, **267**, p. 727–748, 1997

4. Venkatachalam C.M., Jiang X., Oldfield T., Waldman M. "LigandFit: a novel method for the shape-directed rapid docking of ligands to protein active sites", *Journal of Molecular Graphics and Modelling,* **21**, p. 289–307, 2003

5. Halgren T.A., Murphy R.B., Friesner R.A., Beard H.S., Frye L.L., Pollard W.T. Banks J.L. "Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening", *J. Med. Chem.,* **47**, p. 1750–1759, 2004

6. Morris G.M., Goodsell D.S., Halliday R.S., Huey R., Hart W.E., Belew R.K., Olson A.J. "Automated Docking Using a Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function", *J. Computational Chemistry,* **19**, p. 1639–1662, 1998

7. Frisch M.J., Trucks G.W., Schlegel H.B. et al. *Gaussian 98,* Revision A.9, Gaussian, Inc., Pittsburgh PA, 1998

8. Morris G.M., Goodsell D.S., Huey R., Olson A.J. "Distributed automated docking of flexible ligands to proteins: parallel applications of AutoDock 2.4", *J. Comput. Aided Mol. Des.,* **10**, p. 293–304, 1996

9. Kunishima N., Fukuyama K., Matsubara H., Hatanaka H., Shibano Y., Amachi T. "Crystal structure of the fungal peroxidase from Arthromyces ramosus at 1.9 Å resolution. Structural comparisons with the lignin and cytochrome c peroxidases", *J. Mol. Biol.,* **235**, p. 331–344, 1994

10. Gajhede M., Schuller D.J., Henriksen A., Smith A.T., Poulos T.L. "Crystal structure of horseradish peroxidase C at 2.15 Å resolution", *Nat. Struct. Biol.,* **4**(12), p. 1032–1038, 1997

11. Sundaramoorthy M., Kishi K., Gold M.H., Poulos T.L. "Preliminary crystallographic analysis of manganese peroxidase from Phanerochaete chrysosporium", *J. Mol. Biol.,* **238**(5), p. 845–848, 1994

12. Mehler E.L., Solmajer T. "Electrostatic effects in proteins: comparison of dielectric and charge models", *Protein Eng.,* **4**, p. 903–910, 1991

13. Tuynman A., Schoemaker E.H., Wever R. "Enantioselective sulfoxidations catalyzed by horseradish peroxidase, manganese peroxidase and myeloperoxidase", *Monatshefte für Chemie,* **131**, p. 687–695, 2000

14. Tuynman A., Vink M.K., Dekker H.L., Schoemaker H.E., Wever R. “The sulphoxidation of thioanisole catalysed by lactoperoxidase and Coprinus cinereus peroxidase: evidence for an oxygen-rebound mechanism“, *Eur. J. Biochem.*, **258**, p. 906–913, 1998

15. Adam W., Mock-Knoblauch C., Saha-Moller C.R. “Asymmetric synthesis with the enzyme Coprinus peroxidase: kinetic resolution of chiral hydroperoxides and enantioselective sulfoxidation“, *J. Org. Chem.*, **64**(13), p. 4834–4839, 1999

16. De Riso A., Gullotti M., Casella L., Monzani E., Profumo A., Gianelli L., De Gioia L., Gaiji N., Colonna S. “Selectivity in the peroxidase catalyzed oxidation of phenolic sulfides“, *Journal of Molecular Catalysis*, **205**, p. 391–400, 2003