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# Homology Modeling of the Mysid Ecdysone Receptor and Docking Simulation with Ecdysteroids

Masashi HIRANO[1], Hiroshi ISHIBASHI[1], Eun-Young KIM[2], Koji ARIZONO[3] and Hisato IWATA[1]

[1]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*
[2]*Department of Life and Nanopharmaceutical Science and Department of Biology, Kyung Hee University, Hoegi-Dong, Dongdaemun-Gu, Seoul 130-701, Korea*
[3]*Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, 3-1-100 Tsukide, Kumamoto 862-8502, Japan*

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**Abstract**—The ecdysone receptor (EcR) belongs to the nuclear-hormone receptor superfamily that functions as a ligand-activated transcription factor. The EcR plays an essential role in arthropod development and reproduction. While the molecular basis for the interaction between the EcR and its ligand has been well investigated in insects, information on the EcR function in crustaceans is limited. In the present study, a three-dimensional model of the ligand-binding domain (LBD) of the mysid EcR was built based on the X-ray crystal structure of the insect EcR LBD bound to ponasterone A and docking simulations of endo- and exogenous ecdysteroids including ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A and tebufenozide were performed. *In silico* simulations demonstrated that these ecdysteroids efficiently docked with the active site of mysid EcR LBD. The relative potencies based on the interaction energy were in the order of ponasterone A > muristerone A > 20-hydroxyecdysone > tebufenozide > ecdysone. These results suggest that this *in silico* assay may be a useful initial screening tool for potential EcR ligands.

Keywords: ecdysone receptor, ecdysteroid, crustacea, *in silico*, docking simulation

## INTRODUCTION

The molting hormones, ecdysteroids play important roles in initiating and regulating molting and metamorphosis in arthropods. Besides the function as a molting hormone, ecdysteroids are also involved in the control of reproduction and embryogenesis (Subramoniam, 2000). Most actions of ecdysteroids are accomplished via the ecdysone receptor (EcR). This receptor is a ligand-dependent transcription factor and forms a heterodimer complex with ultraspiracle protein (USP), which is a homologue of vertebrate retinoid X receptor (RXR) (Yao *et al.*, 1992). The EcR/USP complex binds to the ecdysone response element (EcRE)

with specific DNA sequences, and consequently regulates the expression of ecdysteroid responsive genes, such as *Broad-Complex*, *E74*, *E75*, *DHR3*, *DHR39* and *ftz-f1* (reviewed by King-Jones and Thummel, 2005; Nakagawa and Henrich, 2009).

EcR is a member of the nuclear receptor superfamily and their basic structure consist of five modular domains referred to as A/B (transcriptional activation domain), C (DNA-binding domain; DBD), D (henge region), E (ligand-binding domain; LBD) and F (not well-defined region). In insects such as fruitfly *Drosophila melanogaster* and silkworm *Bombyx mori*, the function of EcR in the transcriptional regulation machinery and its expression patterns during the molting cycles have been well characterized (reviewed by Thummel, 1995; Kamimura *et al*., 1996, 1997). In addition, Billas *et al*. (2003) determined the X-ray crystal structure of EcR LBD from the tobacco budworm *Heliothis virescens* and identified highly flexible ligand-binding pockets for steroidal and non-steroidal ligands. This implies that environmental chemicals may be potential ligands that can trigger adverse effects in the arthropod through the disruption of EcR signaling. However, the molecular basis for the function of crustacean EcR is not fully understood.

The mysid has been put forward as a suitable test organism for assessing endocrine disruptors by the US Environmental Protection Agency (USEPA), the American Society for Testing of Materials (ASTM), and Organization for Economic Cooperation and Development (OECD) (reviewed by Verslycke *et al*., 2004, 2007). However, chemical screening methods based on a specific hormone-regulated mechanism in mysids have not yet been established. Here we report the homology model of mysid EcR LBD as an initial step towards the understanding of the receptor-ligand interaction. The binding affinities of ecdysteroids and non-steroidal ecdysone ligands to mysid EcR were then estimated based on the interaction energy by *in silico* docking simulation.

## MATERIALS AND METHODS

To construct the 3D structure of the LBD of mysid EcR, the homology modeling software MOE (Molecular Operating Environment, Chemical Computing Group Inc., Montreal, QB, Canada) was used. Initially, the coordinate of 1R1K, an X-ray crystal structure of the domain of the EcR/USP heterodimer of tobacco budworm *Heliothis virescens* bound to ponasterone A (Billas *et al*., 2003) was imported from the protein data bank (PDB). The primary sequence of the LBD of mysid EcR was aligned with that of HvEcR to yield the superimposable 3D structure. HvEcR LBD in complex with ponasterone A was used as a template for homology modeling. Finally, the structure of mysid EcR LBD was optimized by AMBER99 force field (Wang *et al*., 2000) with an energy gradient of 0.05. To perform the docking simulation for ecdysteroids including ecdysone, 20-hydroxyecdysone, ponasterone A and muristerone A, and non-steroidal ecdysone agonist, tebufenozide, a chemical library was constructed using the ISIS/Draw (MDL Information Systems Inc., San Leandro, CA), and the chemical structures were rendered and minimized. Before docking, the ligand-binding site was

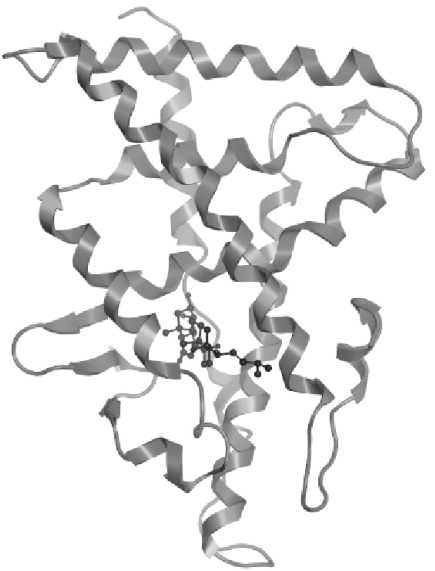


Fig. 1. *In silico* 3D model of mysid EcR LBD bound to ponasterone A. The structural model was generated using the program Molecular Operating Environment (MOE), based on the 1D1K, an X-ray crystal structure of the domain of the EcR/USP heterodimer of tobacco budworm *Heliothis virescens* bound to ponasterone A.

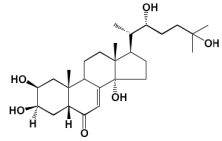
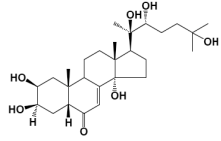
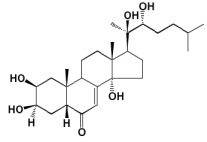
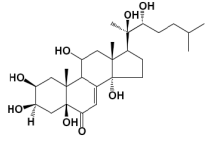
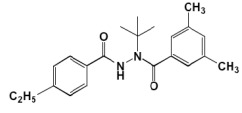
determined using the MOE Alpha Site Finder, and the docking simulation was carried out using MOE-Dock. The energy of the ligand-EcR complex was then refined using MMFF94x (Halgren, 1996) of MOE under the limited conditions in which the side chains of amino acids are fixed. The docking of the ecdysteroids into the receptor was simulated, searching for 30 possible aligned structures. Each docking simulation was evaluated based on the *S*-value, which indicates the interaction energy between the ligand and the EcR ligand binding site.

## RESULTS AND DISCUSSION

In the present study, the 3D model of mysid EcR LBD was constructed using a homology-modeling program of MOE. Ten candidate structures were obtained and the final structure was coordinated (Fig. 1). In the Ramachandran plots, the phi ($\varphi$)/psi ($\psi$) torsion angles were within the favorable region for EcR model. Only 3 residues were identified as anomalies in the stereochemistry of HvEcR. Hence, these amino acid residues were refined using the Protein Geometry embedded in MOE. The root-mean square deviation (RMSD) values of aligned C$\alpha$ atoms with the intermediate structures were 0.335 Å.

To evaluate the binding potentials of tested compounds to the mysid EcR LBD, we performed *in silico* docking experiments for the ecdysteroids and the non-steroidal ecdysone agonist, tebufenozide using MOE-Dock. Results showed

Table 1. *S*-values obtained from docking simulations of the mysid EcR LBD and each compound.

| Compound | Structure | M. W. [a] | *S*-score (kcal/mol) |
|---|---|---|---|
| Ecdysone |  | 464.63 | –13.2 |
| 20-hydroxyecdysone |  | 480.63 | –16.9 |
| Ponasterone A |  | 464.63 | –17.5 |
| Muristerone A |  | 496.63 | –17.2 |
| Tebufenozide |  | 352.47 | –13.3 |

[a]Molecular weight.

that all the chemicals were able to dock well into an active site, indicating lower interaction energies. The *S*-values of ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A and tebufenozide were –13.2, –16.9, –17.5, –17.2 and –13.3, respectively (Table 1). The relative potencies based on the interaction energy were in the order of ponasterone A > muristerone A > 20-hydroxyecdysone > tebufenozide > ecdysone. It has been reported that these EcR agonists have specific binding affinities to insect EcR LBD (Minakuchi *et al*., 2007). In addition, the natural ligand 20-hydroxyecdysone and plant ecdysteroids, ponasterone A and muristerone A are known to be strong activators of EcR/USP complex in insects. Meanwhile, ecdysone and tebufenozide have only modest effects on the EcR transactivation in *Drosophila* and *Daphia* (Baker *et al*., 2000; Kato *et al*., 2007). Our docking simulation data appear to agree well with these studies. From the docking simulation, we speculate that, less than –10 kcal/mol of *S*-values may be an indication for *in silico* screening of chemicals that can interact with the mysid EcR LBD.

## CONCLUSION

By *in silico* docking simulation of selected ecdysteroid compounds with the mysid EcR LBD, we estimated the binding potentials. The relative potencies of these compounds were ranked in the order of ponasterone A > muristerone A > 20-hydroxyecdysone > tebufenozide > ecdysone. We indicated that potential ligands may be deduced from the threshold of the interaction energy. Thus, this *in silico* approach may be applied for the screening of ecdysteriod-like environmental contaminants in crustaceans.

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H. Iwata (e-mail: iwatah@agr.ehime-u.ac.jp)