KP209 Investigation of substrate specificity and regioselectivity based on enzyme and substrate three dimensional structure of CYP1A2 of human and animals.

Mutsuyo WADA[*1], Sari HATTORI[*2], Nozomu KAMIYA[*1], Kohta SAKAI[*2], Tatsuhiro YAMASHITA[*1], Atsushi TOMONAGA[*1]

1) Bio-Chemical research Division, BioIT Business Development Unit, FUJITSU LTD., 1-9-3 Nakase, Mihana-ku, Chiba, 261-8588 JAPAN

2) FUJITSU KYUSHU SYSTEM ENGINEERING LTD., 2-2-1 Momochihama, Sawara-ku, Fukuoka, 814-8589 JAPAN

mutsu@jp.fujitsu.com

1. Introduction

Cytochrome P450 1A2 is the principal cytochrome P450 family 1 enzyme expressed in human liver and participates extensively in drug oxidations. This enzyme is also of great importance in the bioactivation of mutagens, including the N-hydroxylation of arylamines [1]. Prediction of the metabolism by P450 1A2 is important for the safety assessment of the chemicals. Animal models are usually used to predict toxicity in humans, but the problem is that the toxicities are different among species since metabolites are different depending on species.

To analyze substrate specificity and regioselectivity we tried docking of α-NF (α-Naphthoflavone) and well-known 13 substrates to human P450 1A2. At first, we investigated the necessity of the water molecule near the carbonyl group of P450 1A2 which is important molecule in binding of α-NF with the protein [1]. We also discuss the differences of metabolism among human, rat and rabbit by docking using the protein build by homology modeling.

2. Method

The crystal structure of a human P450 1A2-αNF complex (PDB 2HI4) [1] was used in which αNF was removed from the binding cavity and substrates were docked into the same cavity using Molegro Virtual Docker [2]. Homology models of rat and rabbit P450 1A2 was built using SWISS-MODEL automated homology modelling tool with the amino acid sequences of rat (NP_036673) and rabbit (P00187) and the human P450 1A2 structure as the template [3]. Well-known thirteen P450 1A2 substrates from the Fujitsu ADME database [4] were used for the prediction. The energy-minimized coordinate of the substrates were generated using the myPresto cosgene ver.3 [5] and Chem3D Ultra 8.0 (CambridgeSoft corporation). The normal mode vibration analysis of a protein was carried out by the method of Kamiya et al [6].

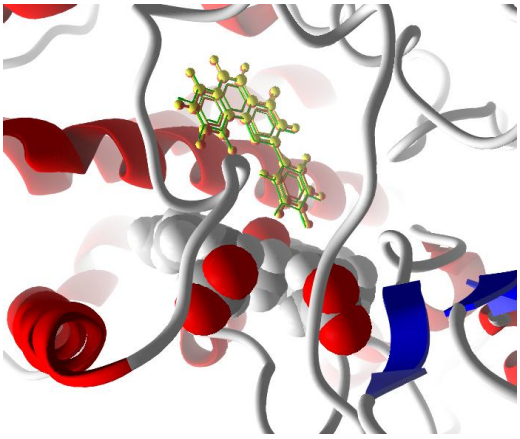

Fig. 1 Predicted and actual location
Yellow: predicted (with water)
Red: predicted (without water)
Green: actual

3. Results and Discussion

1. *P450 1A2-αNF docking analysis and comparison with the observed geometry*

P450 1A2-αNF docking analysis both with and

without water molecules which involves the substrate-enzyme interactions had shown no significant differences between the docking and observed position of the inhibitor (Fig. 1).

2. *Regioselectivity prediction of P450 1A2 metabolism from docking*

To determine the possible metabolic sites of the substrates, the distance and angle between the atoms in a substrate and the heme oxygen (1.8Å perpendicular from Fe) of P450 1A2 and the binding energy (Molegro Virtual Docker docking score) were obtained well-known 13 substrates from docking results. These docking results showed that the values of the parameters were reasonable for metabolic sites on 9 out of 13 substrates, but the parameters could not distinguish metabolic site from not metabolic site.

3. *Comparisons of regioselectivity in different species P450 1A2*

In vivo investigations there indicated that the quantitative differences of metabolic profile in tacrine were clear among human, rat and rabbit P450 1A2. Our docking results showed accurately metabolic sites of tacrine in all these species and it was suggested that the metabolic sites would be predicted from the model used and the distance, angle and energies.

4. *Ragio-selectivity prediction of P450 1A2 metabolism considering protein flexibility by normal mode vibration analysis*

Since in usual docking proteins were treated as rigid, the substrate would not fit for the observed cavity. We are trying to improve the accuracy of the metabolic sites in which the substrates were docked changing cavity shape following normal mode vibration.

4. Conclusion

There is little difference between with or without water molecule docking even if the water is important in ligand and protein interaction. The shortest distance atom in substrate from the oxygen coordinating Hem Fe is observed metabolic sites in 9 of 13 substrates by our docking and the result of interspecies difference analysis of metabolic sites suggests that the possibility of prediction of metabolic profile difference among species using docking and homology modeling.

5. Acknowledgements

We thank Y. Sugihara and Y. Kondo for constructive discussions and comments on this work.

References

1. Sansen, S., et al., *Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2.* J Biol Chem, 2007. **282**(19): p. 14348-55.
2. Molegro Aps Web page : http://www.molegro.com (accessed Sep 4, 2008)
3. Sohl, C.D., et al., *Cooperativity in oxidation reactions catalyzed by cytochrome P450 1A2: highly cooperative pyrene hydroxylation and multiphasic kinetics of ligand binding.* J Biol Chem, 2008. **283**(11): p. 7293-308.
4. Rendic, S. Fujitsu Kyushu System Engineering Ltd. ADME Database. http://jp.fujitsu.com/group/fqs/services/lifescience/asp/adme-database/index.html (accessed Sep 4, 2008)
5. Fukunishi, Y., Y. Mikami, and H. Nakamura, *The filling potential method: A method for estimating the free energy surface for protein-ligand docking.* J. Phys. Chem. B., 2003. **107**: p. 13201-13210.
6. Kamiya, N., A. Ueda, A. Tomonaga, N. Shiobara, and H. Wako, 29th Division of Chemical Information and Computer Science, The Chemical Society of Japan, J18 (2006)