Modelling of Enantioselectivity in *Candida antarctica* Lipase B

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1 Introduction

Enzyme selectivity towards a particular enantiomeric configuration of substrate is necessarily governed by chemical and spatial relationships between the substrate and the enzyme binding cavity. In the case of *Candida antarctica* lipase B experimental evidence shows that enantioselectivity of the enzyme towards the alcohol moiety 3-methyl-2-butanol varies significantly with respect to the chain length of the acyl moiety [2].

However, even high values of enantiomeric ratio correspond to very small differences in free energy, and these small energy differences reflect the difficulty of predicting enantioselectivity in enzyme-substrate systems where the total energy is very large. Here, we present a modelling study that aims to develop a methodology for predicting enantioselectivity, and uses the alcohol moiety 3-methyl-2-butanol with various acyl moieties as substrate molecules for the enzyme *Candida antarctica* lipase B. The experimentally determined values of enantiomeric ratio were used as references.

2 Method and Results

The approach uses molecular dynamics to simulate the motion of systems with modelled transition states that correspond to the reaction intermediates. Thereafter, investigative analyses were performed on ensembles of non-minimised structures. Non-minimised structures reflect the inclusion of the entropic component of the system and so relate the potential energies of the modelled system to free energies.

The analyses were based on potential energies of selected regions within the system. These structural subsets may be classed into three categories, as follows:

1. Structure based selection, which focuses on proximity relationships such as consideration of the substrate and the enzyme binding cavity only.

2. Energy difference based selection, which focuses only on regions in the binding cavity with significant difference in interaction energy between the enzyme and each enantiomer of the substrate.

   Structural subsets corresponding to these first two categories have been the focal points of a previous study where a small number of minimised structures were analysed after a dynamics simulation [1]. It was concluded in that study that energy based subsets were more useful than structure based subsets for predicting enantioselectivity.

3. Function based selection, which aims to focus on key atoms within the system that make up the core structural elements of the transition state. The positions of the remaining atoms in the system contribute either directly or indirectly to the conformational arrangement of these atoms. We consider that a more favourable energetic conformation of the function based subset should relate to a greater likelihood for the reaction to proceed, thereby reflecting higher selectivity.

   The present dynamics study indicates that using a function based subset is the method of choice for predicting enantioselectivity.

References

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[2] Ottosson, J. and Hult, K., Influence of acyl chain length on the enantioselectivity of Candida antarctica lipase B and its thermodynamic components in kinetic resolution of sec-alcohols, (Submitted).