Molecular dynamics simulations of haloalkane dehalogenase
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Chapter 1

Introduction

In the course of this century, industrial activities have created great environmental problems by the production of large quantities of chemical waste. Especially during the last decades awareness of the subject has grown and dumping of possibly hazardous compounds is strongly reduced. But the environment is still suffering from a heritage of xenobiotic substances from the past. One way of attacking the problem is by the use of micro-organisms. One such organism is the bacterium \textit{Xanthobacter autotrophicus} GJ10 \cite{1}. This bacterium is capable of growing on 1,2-dichloroethane, by using this compound as its sole source of energy and carbon. One of the key enzymes involved in the degradation is \textit{haloalkane dehalogenase} \cite{2} a 35 kd (310 residues) protein which catalyses the reaction:

\[
\text{ClCH}_2\text{CH}_2\text{Cl} + \text{H}_2\text{O} \rightarrow \text{ClCH}_2\text{CH}_2\text{OH} + \text{H}^+ + \text{Cl}^-
\]  

Apart from 1,2-dichloroethane, dehalogenase has been shown to catalyse the dehalogenation of a large variety of alkylhalides \cite{2}, haloalcohols and halonitriles \cite{4} although it has a low affinity towards these substrates.

This thesis deals with a computational approach to the study of protein behaviour, with haloalkane dehalogenase as the main topic. It is the aim to find a relation between the dynamics of dehalogenase and its
Figure 1.1: Topology of secondary structure elements of dehalogenase [3]
Figure 1.2: Three-dimensional representation of haloalkane dehalogenase. The main domain is drawn in grey, the cap domain in white. In the center, between both domains, the side chain of catalytic residue Asp124 is drawn in black. Drawn with MOLSCRIPT [5]

function. Particular attention will be given to halide release. All the work presented is based on Molecular Dynamics simulations (MD) in combination with some newly developed techniques. Essential Dynamics analysis (ED) [6] will be applied to investigate large globally correlated motions in proteins. Regions that are more or less rigid will be identified with a method referred to as rigid body analysis. To study the effect of a chloride ion bound in the hydrophobic active site cavity, a polarization term has been added to the force field.

All computational methods will be given attention to in chapter 2. Chapter 3 deals in a more elaborate way with the theoretical foundations of ED. In chapters 4 and 5 the results of simulations of dehalogenase respectively with and without a chloride ion in the active site will be presented. Analysis of these results suggest a conformational change of the molecule which will be closely investigated in chapter 6. Finally, in chapter 7, possible consequences of the obtained results for the kinetics of halide release will be discussed.
The X-ray structure of dehalogenase was first solved at a resolution of 2.4 Å [7] and later at 1.9 Å [3]. Fig. 1.2 shows a three-dimensional representation of the backbone structure. The enzyme consists of two domains. The core of the protein is formed by the main domain (residues 1-155 and 230-310) composed of an eight-stranded β-sheet surrounded by six α-helices. On top of it lies the cap domain (residues 156-229), composed of five α-helices (fig. 1.1). It has this topology in common with other hydrolases classified as α/β-hydrolases [8]. A hydrophobic cavity, situated between both domains, forms the active site. By means of X-ray experiments, the structures of the enzyme-substrate complex, a covalently bound enzyme-substrate intermediate and an enzyme-chloride complex were elucidated [9]. Site-directed mutagenesis studies [10, 11, 12, 13] have revealed that Asp124, Trp125, Trp175 and His289 play a role in catalysis. These results have firmly established a two-step catalytic mechanism, shown in fig. 1.3. After formation of the enzyme-substrate complex a nucleophilic attack of Asp124 on the substrate yields a covalently bound intermediate (step 1). The halide ion produced during this reaction is bound between Trp125 and Trp175. As the next step the intermediate is hydrolysed by a water molecule which is activated by His289 (step 2). In the crystal environment, catalysis still occurs, but no complex between the alcohol product and the enzyme was found [9]. The presence of an enzyme-chloride complex suggested that chloride release was the rate-limiting step of the overall reaction. Halide release has been extensively investigated using stopped flow fluorescence experiments [14]. These studies suggested that halide binding can occur via two distinct routes. In one route, fast halide binding is preceded by a slow enzyme isomerization which was suggested to be a conformational change. In the other route rapid formation of an enzyme-halide collision complex was followed by a slow isomerization. Difference in kinetics between chloride and bromide binding were also observed. Fig 1.4 shows the proposed chloride binding scheme. Kinetic constants are listed in table 1.1. $E_I$ and $E_{II}$ represent both enzyme conformations whereas, $E_{I}.X$ and $E_{II}.X$ their corresponding complexes with the chloride ion. The idea of a con-
Figure 1.3: Proposed reaction mechanism for dehalogenation of 1,2-dichloroethane (see text).
formational change was also supported by a considerable $^2$H$_2$O isotope effect on $k_3/k_{-3}$ and $k_1/k_{-1}$ for both chloride as well as bromide release [14].
<table>
<thead>
<tr>
<th>$k_1$</th>
<th>$3 \pm 0.3 \text{ s}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{-1}$</td>
<td>$&gt; 300 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$&gt; 1450 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_{-3}$</td>
<td>$14.5 \pm 0.5 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_6$</td>
<td>$0.0085 \pm 0.005 \text{ M}^{-1} \text{s}^{-1}$</td>
</tr>
<tr>
<td>$k_{-6}$</td>
<td>$0.66 \pm 0.03 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$K_2$</td>
<td>-</td>
</tr>
</tbody>
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Table 1.1: kinetic data for fig. 1.4 [14]