Molecular dynamics simulations of haloalkane dehalogenase
Linssen, A.B M

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Chapter 5

An extended MD simulation of wild-type haloalkane dehalogenase including a chloride ion bound in the active site

5.1 Introduction

To find out the effect of chloride bound in the active site, simulations including the ion in the active site were performed. Initially, this was done using the usual GROMOS force-field [15], but the interaction of the ion with its hydrophobic environment, containing several aromatic residues, was too weak, so chloride almost instantaneously (within 20 ps) left the active site cleft. In this chapter we describe and analyse an MD simulation in which we have added a polarizability term to stabilize the bound state of the ion.
5.2 Methods

For the simulations the same software and force-field were applied as described in section 4.2, with a polarizability term added (section 2.2). As a starting structure, the X-ray structure at pH 6 and a resolution of 2.1 Å (Brookhaven data bank [36] entry 2DHE) [9, 7, 44] was taken. Except for His289, all histidines were assumed to be electrically neutral. To find out which ring nitrogen was protonated, the X-ray structure was checked for the presence of possible hydrogen bonds by looking for the closest hydrogen bond acceptor. Histidine residues 37 and 54 were protonated at N$_{51}$ positions, residues 102 and 305 at the N$_{52}$ positions. To compensate for the negative charge on the chloride ion, His289 was protonated at both positions making it positively charged. The volume of the periodic box (truncated octahedron) was 174 nm$^3$. The protein possessed a negative charge of -17 e. To obtain an electrically neutral system 17 sodium ions were added as counterions by evaluating the electrostatic potential at the oxygen positions of all water molecules, and replacing those 17 water molecules with the lowest potential by a sodium ion. In total, the system contained 16046 atoms of which 3171 were protein atoms (including the bound chloride ion). Only the chloride ion was treated as a polarizing atom (section 4.2). To decide which atoms should be polarizable, initially all residues that contained at least one atom within a distance of 5 Å (in the X-ray structure) from the chloride ion were taken and all atoms from these residues were treated as polarizable atoms. The residues found were: Glu56, Asp124, Trp125, Phe128, Phe172, Trp175, Phe222, Pro223 and Val226. However, Asp124 had only one atom within 5 Å (O$_{52}$ with a distance of 4.9 Å). One would like to have as few charged residues included as possible, given the fact that the polarization model was designed for describing interaction of ions with hydrophobic residues. For this reason, and because its distance was such that polarization effects are small compared to Coulombic interactions, Asp124 was excluded. The energy of the system was minimized, without restraints, by the steepest descent method for 100 steps. After this, it was simulated for 10 ps using harmonic position restraints on all pro-
tein atoms, with a force constant of 9000 kJ nm\(^{-1}\) mole\(^{-1}\). Then, the position restraints were removed and the system was simulated for 1 ns. Later it was continued for another 500 ps. Analysis of secondary structure elements, rmsd, B-factors and radius of gyration was performed with GROMACS [45].

### 5.3 Results and discussion

![Graph showing root mean square deviation from the crystal structure vs. time.](image)

Figure 5.1: Root mean square deviation from the crystal structure vs. time.

As was already mentioned, originally a simulation of 1 ns was performed. But the rmsd showed a steady increase, which initially led us to believe that the protein was unfolding. To find out how the system would develop further the simulation was continued for another 500 ps. The resulting
rmsd as a function of time is shown in fig. 5.1. After 1 ns the rmsd stabilizes around a value of approximately 0.35 nm to increase again after \( \sim 1.4 \) ns. Close inspection of the trajectory showed that at \( \sim 1.25 \) ns some (charged) residues of the protein in the central periodic box started to interact with residues from the protein's images, i.e. the distance between them became smaller than the large cut-off radius (1.2 nm), and later even smaller than the short cut-off radius (0.8 nm). For this reason we only considered the trajectory until 1.2 ns as being reliable, and restricted our further analysis to this time interval. Fig 5.2 shows the radius of gyration as a function of time. Here we see a substantial increase in the time interval between 600 and 900 ps, after which there seems to be a stabilization. In fig. 5.3 the crystallographic B-factors, as well as the B-factors derived from the simulation are plotted. It can be seen that most of the mean square displacement is concentrated in the region between residues 180 and 210 with distinct peaks at Ser183 and Thr197. There

![Figure 5.2: Radius of gyration vs. time.](image-url)
is also a more narrow peak, with a maximum at Asn14. In fig. 5.4 the secondary structure elements versus time are plotted. Comparison with the previous simulation (fig. 4.1) shows some major differences. Helices 3, 4 and 8 (residues 125-136, 159-166 and 217-227) are less stable in the current simulation. But helix 5 (residues 171-181) appears to be more stable. One might expect that especially in the region between residues 180 and 210 secondary structure elements are unstable, as in this region the largest rmsd is found. Strangely enough however, both helices in this region are highly stable.

For Essential Dynamics (ED) analysis, a covariance matrix was constructed from the $C_\alpha$ coordinates, from the time interval 100-1200 ps. Fig. 5.5 shows the 10 largest eigenvalues. As is to be expected from the large rmsd, they are considerably larger than the ones found in the
previous chapter (fig 4.5). The projections along the first 6 eigenvectors are shown in fig. 5.6. The motion along the first 3 eigenvectors in 3 dimensional physical space is shown in fig. 5.7. The total mean square displacement (msd) was 5.82 nm$^2$ and the sum of the first 3 eigenvalues was 4.01 nm$^2$. This means that fig. 5.7 shows 68% of the total msd. It also shows that most of this motion takes place in the cap domain. Helix 5 (residues 171-181) shifts to the right. The motion of chain segment 180-210 seems to be rather disorganized. To obtain a clearer notion of the type of motion we used the same trajectory for rigid body analysis (section 2.4).
Figure 5.5: The 10 largest eigenvalue in order of decreasing magnitude.

The rigidity scores are shown in fig. 5.8. If we compare fig. 5.8 with the results from the previous chapter (fig. 4.9), we find large differences. The peak with a maximum at residue Thr197 immediately catches the eye. Also fig. 4.9 shows a peak, but not so outstanding. Between Cys150 and Val180, an irregular pattern was found in fig. 4.9. This has turned into a much more regular peak. The peaks in segments 1-3, 23-37, and 81-114 seem to have disappeared. Instead, there is now a region with positive scores with an irregular pattern between residues 253 and 295. If we now subdivide the molecule we find: region A containing residues 184-211, region B containing residues 1-149, 212-252 and 296-310, and region C containing residues 150-183 and 253-295. These regions do not correspond to the ones found in the previous chapter. In fig. 5.10 the new regions are visualized. The regularity of the peaks with maxima Pro168 and Thr197 suggests rigid bodies connected by a hinge. To verify this we performed a least square fit of both regions (skipping residues 181-
Figure 5.6: Projections along the first 6 eigenvectors.

184) from the MD structure after 1.2 ns on the corresponding regions of the crystal structure. The results are shown in fig. 5.9. Compared to the large motions in which both parts of the molecule are involved, they show only little internal motion and there is no clear sign of unfolding. The seemingly disorganized motion in fig. 5.7 is in fact an opening of a helix-loop-helix region between residue 185 and 211. This can be seen more clearly in fig. 5.13 and 5.14. We also had a closer look at the behaviour of the chloride ion. Fig. 5.11 shows the ion and its surroundings in the crystal structure. It is clearly bound between N$_{\delta i}$ of Trp125 and Trp175. Fig. 5.12 shows the same but now after 1.2 ns of simulation. Drastic changes have taken place. Its interaction with Trp125 is weakened (the distance increases from 3.1 Å to 5.1 Å) whereas there are strong interactions with peptide nitrogens from Lys224, Met225 and Val226. Here it should be stressed that Lys224 and Met225 are not taken to be polarizable.
Finally, we also check for a possible tunnel that can act as an exit for the ion, or an entrance for the substrate. This was done by calculating the solvent accessible surface with the software package WHATIF [48]. We found two tunnels that are shown in fig. 5.13. There is a long narrow and curved tunnel leading to the opening created by the motion of the helix-loop-helix region and surrounded by residues Phe190, Pro182 and Phe290. Another shorter tunnel has an opening on the opposite site of the protein, surrounded by residues Lys221, Lys224 and Met225. Fig. 5.14 shows water molecules penetrating the molecule. It can be seen that water is entering through both tunnels.

5.4 Conclusions

It is not uncommon that proteins unfold during MD simulations due to inaccurate force-fields. But when unfolding takes place, in general
secondary structure elements start disappearing. There is no clear sign of this happening in the current simulation. Still, one has to be careful in interpreting the results above, especially because the polarization term as it was implemented, is only a rough approximation; it was originally meant to keep the chloride bound between both tryptophans. Its strong interaction with atoms that were not taken to be polarizable should be regarded with care. However, the observed large motion of the helix-loop-helix, giving rise to an opening, remains interesting. The fact that the helices mostly involved in this motion, remain not only intact, but appear to be highly stable when compared to other secondary structure elements, is certainly unusual. If these motions are assumed to be functional, the question arises how they relate to the reaction path. The chloride ion
could in principle leave the protein via the longer tunnel. This is however not very likely, as it will have to pass along several hydrophobic residues. It is more likely to take the shorter pathway, which has two positively charged residues at it’s opening. The longer tunnel is more hydrophobic and leads directly to the catalytic residues. It could therefore form an entrance for the substrate.
Figure 5.9: Stereoview of Cα backbone regions 150-180 (top) and 185-211 (bottom) taken from the MD structure at 1.2 ns (dotted line). Both regions are fitted to the corresponding regions from the X-ray structure (solid line) (drawn with WHATIF [48]).
Figure 5.10: Regions A (thick dashed line), B (thick solid line) and C (thin solid line), obtained by subdividing the molecule based on rigidity scores.

Figure 5.11: Stereoview of the chloride ion bound in the active site in the X-ray structure, the distances between the ion and $N_{\delta1}$ of Trp$_{125}$ and Trp$_{125}$ are indicated. (drawn with WHATIF [48])
Figure 5.12: Stereoview of the environment of the chloride after 1.2 ns of simulation (top figure) and in the crystal structure (bottom figure). All polarizable residues are drawn (solid lines). In addition, all residues that, after 1.2 ns, have atoms closer to the ion than 5 Å and that were not taken to be polarizable are shown (dashed lines) (drawn with WHATIF [48]).
Figure 5.13: Stereoview of both tunnels leading from the active site to the surface of the protein. The chloride ion is drawn as a dotted sphere with a van der Waals radius. The longer tunnel leads to the opening on the left, surrounded by residues Phe190, Pro182 and Phe290. The shorter leads to an opening on the right-hand side, surrounded by Lys221, Lys224 and Met225 (drawn with WHATIF [48]).
Figure 5.14: Stereoview showing water penetrating the molecule. Water is entering through both tunnels shown in fig. 5.13. This picture was made by calculating the geometrical center of the protein and removing all water molecules with an oxygen distance, from this center, greater then 2.75 nm (drawn with WHATIF [48]).