Chapter 6.

The Aspartate Simulation

6.1 Objectives.

In the Alchemy simulation our classical molecular dynamics (MD) model displayed the sevenfold coordination of Ca\textsuperscript{2+} and the transition to the sixfold coordination of Mg\textsuperscript{2+}. In order to emulate the effects of the E101D mutation in the parvalbumin EF site using MD, it was important that we first showed that the glutamate at position 12 mimicked wild-type metal ion-binding behavior in our model, as it did. Therefore, we next pursued using MD to model the E101D mutation in a simulation called “Aspartate”.

The purpose of the Aspartate simulation was to investigate whether our model would predict that when the residue at EF loop position 12 has been mutated from glutamate to aspartate, it only binds Ca\textsuperscript{2+} in a monodentate fashion, as is seen in the PVEF-E101D/Ca\textsuperscript{2+} crystal structure. It is highly unusual for Ca\textsuperscript{2+} to experience sixfold coordination and unheard of in EF-hand binding sites as sixfold coordination is characteristic of EF-hand Mg\textsuperscript{2+}-binding. Additionally, it was noteworthy to observe the movement of the F helix, the helix C-terminal to the binding loop, since it was observed to move into the loop ~1 Å in the PVEF-E101D/Mg\textsuperscript{2+} crystal structure, and the F helix moved in ~ 0.6 - 0.8 Å in the PVEF-E101D/Ca\textsuperscript{2+} structure. In fact, it seems that the tug-of-war between the attraction of the carboxylate sidechain oxygens for Ca\textsuperscript{2+}, and
their requirement to drag the F helix along with them as they move in towards the metal ion, is a strong determining factor defining the range of coordination spheres that can be achieved in a particular EF-hand site.

6.2 Aspartate protocol.

The protocol for the Aspartate simulation began with the replacement of the glutamate amino acid at residue number 101 with aspartate in the initial coordinate set. This goal was accomplished by first overlapping the PVEF-E101D/Ca$^{2+}$ crystal structure coordinates with the model coordinate set in Quanta97 (Molecular Simulations, 1998). The aspartate coordinates generated in this superposition of structures was then edited into the protein data bank (pdb) coordinate file of the model, and the original glutamate coordinates were deleted. The new coordinates were viewed in the program O (Jones et al., 1991), and the sidechain was rebuilt to prevent bad contacts using the most frequent aspartate rotamer from the database.

Molecular dynamics were run for 25,000 steps, representing 50 picoseconds (ps) of simulation time, with a van der Waals radius for the two bound metal ions that was representative of Ca$^{2+}$. The ShakeH algorithm was employed, and the timestep was 2 femtoseconds (fs). Frames were written every 20 steps, or every 40 fs. The Aspartate simulation was stable, and the root mean square (RMS) deviation from the starting structure never increased above 1.4 Å. The mean RMS deviation value for the simulation was 1.20 Å, with values fluctuating from 1.05 to 1.34 Å (Figure 6-1). The
CD site coordinated Ca$^{2+}$ with normal wild-type pentagonal bipyramid Ca$^{2+}$-binding geometry.

6.3 Results of the Aspartate simulation in the EF site.

There are three other aspartate residues at EF loop positions 1, 3 and 5 of this simulation in addition to the aspartate at position 12 that has been substituted for the glutamate found in wild-type parvalbumin. These aspartate sidechains all coordinated Ca$^{2+}$ at Ca-O bond distances of 2.1 to 2.2 Å, which is tight compared to experimental Ca-O bond distances derived from EF-hand crystal structures (Figure 6-2). These tight bond distances were also observed for Ca$^{2+}$ coordination in the Alchemy simulation, though. In fact, all the ligands other than the mutated glutamate had bond distances in the Alchemy simulation and the Aspartate simulation that were approximately equivalent. Therefore, the short Ca-O bond distances seem to be an artifact of our model and not attributable to the aspartate for glutamate substitution in this simulation. This result is in contrast to the PVEF-E101D/Ca$^{2+}$ mutant crystal structure results where not only the Ca-O bond distance of the substituted aspartate was shorter than the wild-type glutamate bond distance, but the mean Ca-O bond distance was markedly shorter. This was symptomatic of shorter Ca-O bond distances for all of the coordinating oxygens in the mutant EF site, with the exception of the coordinating water molecule. It seems reasonable that experimental results reflected a generally shorter Ca-O distance in the PVEF-E101D/Ca$^{2+}$ mutant because the octahedral
Figure 6-1. Aspartate simulation RMSD from starting coordinates.

Graph of the root mean square deviation (RMSD) from the initial model in the Aspartate simulation. The duration of the simulation was 50 picoseconds (50,000 femtoseconds), and a frame was recorded every 40 femtoseconds, for a total of 1250 frames. The RMSD stays below 1.4 angstroms for the duration of the simulation. The mean RMSD value for this simulation was 1.2 angstroms.
Figure 6-2. Ca-O bond distances in the Aspartate simulation.

(a) ASP 90 OD1 distance to calcium.

(b) ASP 92 OD1 distance to calcium.

(c) ASP 94 OD1 distance to calcium.

Aspartates at loop positions 1, 3, and 5 all exhibit short Ca-O bond distances in the Aspartate simulation, compared to experimental observation. However, they are approximately equivalent to the Ca-O bond distances in the Alchemy simulation, indicating that this is an artifact of the model, and not related to the E101D substitution present in this simulation.
coordination sphere radius is inherently smaller than that of sevenfold pentagonal bipyramid coordination. Although our model does not reflect the general trend for all of the coordinating oxygens to move in on the Ca\(^{2+}\) ion because of the E101D mutation, it does exhibit a shorter Ca-O distance for the coordinating oxygen of the substituted aspartate at position 12 than for the glutamate that is normally found at this position. The Ca-O distance for aspartate 101 in this simulation stayed very steadily in the vicinity of 2.1 Å, while the Ca-O distances for both carboxylate oxygens of glutamate 101 in the Alchemy simulation were from 2.2 to 2.3 Å.

Because the substitution of aspartate for glutamate was performed by altering only the sidechain coordinates, at the start of the simulation the mainchain of aspartate 101 was in the same position usually occupied by the mainchain of glutamate 101. For that reason, the two aspartate 101 carboxylate oxygens were both out of coordination range of the Ca\(^{2+}\) ion at the beginning of the Aspartate simulation. In the starting coordinate file the two aspartate sidechain carboxylate oxygens, O\(_{c20}\) and O\(_{c21}\), are 4.97 Å and 2.94 Å from the Ca\(^{2+}\), respectively. After the first 40 femtoseconds of simulation, these oxygens are 4.72 and 2.74 Å from the Ca\(^{2+}\), and after 80 femtoseconds they have moved in to a Ca-O distance of 2.20 and 3.87 Å (Figure 6-3). At this point, the closest oxygen is definitely coordinating the metal ion, and the other oxygen is not. Throughout the remainder of the simulation, the aspartate at position 12 displays monodentate coordination of the Ca\(^{2+}\) ion (Figure 6-4). Just as in the crystal structure, the F helix that terminates the binding loop cannot move in enough to allow the aspartate to coordinate Ca\(^{2+}\) with both oxygens in our simulation.
Figure 6-3. Aspartate 101 moves in to coordinate Ca\(^{2+}\) within the first 80 femtoseconds of simulation.

In the Aspartate simulation, the starting Ca-O distances for the two sidechain carboxylate oxygens of Aspartate 101 were 2.94 angstroms for the closest oxygen and 4.97 angstroms for the most distant. (a) In the first 40 femtoseconds of simulation, the aspartate sidechain moved in to Ca-O distances of 2.74 angstroms and 4.72 angstroms. (b) By the end of the first 80 femtoseconds of simulation, one of the aspartate sidechain oxygens was within coordinating distance of the Ca\(^{2+}\) ion, 2.20 angstroms. The aspartate never achieved bidentate Ca\(^{2+}\) coordination, however, as the second sidechain oxygen remained about 3.5 angstroms from the Ca\(^{2+}\) ion throughout the simulation.
Figure 6-4. Aspartate 101 exhibits monodentate Ca\(^{2+}\) coordination.

(a) ASP 101 OD1 distance to calcium.

(b) ASP 101 OD2 distance to calcium.

(a) One of the sidechain carboxylate oxygens of aspartate 101 stays too far away from the metal ion to coordinate during the entire Aspartate simulation. This correlates the experimental results from the crystallization of the PVEF-E101D/Ca\(^{2+}\) complex, where aspartate 101 also exhibited monodentate Ca\(^{2+}\) coordination.

(b) The other oxygen coordinates Ca\(^{2+}\) at a distance of about 2.1 angstroms throughout the Aspartate simulation.
The F helix shows obvious movement into the binding site, toward the metal ion during the Aspartate simulation (Figure 6-5). A comparison of the distances of the F helix backbone nitrogens from the Ca\textsuperscript{2+} ion reveals that the entire helix shows a trend to move inward toward the binding site during the first 20 picoseconds of the simulation (Figure 6-6). In order to quantify this movement, the final coordinate file produced by the Aspartate simulation was overlapped with the initial coordinate file using the Kabsch algorithm (Kabsch, 1978). The superimposed structures were viewed in the program O (Jones et al., 1991), and the distance between the aspartate 101 C\textsubscript{a} in the initial coordinate file and the C\textsubscript{a} in the final file was measured to be 0.97 Å (Figure 6-7). This was representative of a somewhat larger movement by the F helix inward toward the metal ion in our simulation than that observed in the PVEF-E101D/Ca\textsuperscript{2+} crystal structure (~ 0.6 - 0.8 Å). However, since the Ca-O bond distances were consistently too short in our MD model, this discrepancy was not surprising.

6.4 Summary.

In conclusion, the Aspartate simulation correlated the experimental result that an aspartate substituted for the glutamate at EF loop position 12 would only be able to coordinate Ca\textsuperscript{2+} in a monodentate fashion. Moreover, the F helix exhibited marked movement in towards the metal ion in our simulation, as it did in the PVEF-E101D mutant crystal structures. However, although the F helix was able to move in somewhat dramatically in the simulation so that one aspartate oxygen was able to bind
Figure 6-5. The F helix moves in toward the metal ion in the Aspartate simulation.

(a) At the onset of the Aspartate simulation, the aspartate 101 sidechain is too far from the Ca$^{2+}$ ion to coordinate. (b) After 560 femtoseconds simulation time, aspartate 101 has moved into the binding site to coordinate Ca$^{2+}$ with one sidechain oxygen, and it has brought the entire F helix along with it. (c) The initial and final coordinate sets for the Aspartate simulation have been superimposed, with the metal ion at the origin of both coordinate sets. The movement inward by the F helix has been illustrated by comparing the initial (i) and final (f) positions of the residue 101 backbone alpha carbon.
Figure 6-6. The F helix backbone nitrogens shift in the Aspartate simulation.

(a) ALA 102 N distance to calcium.

(b) MET 104 N distance to calcium.

The movement of the F helix in the Aspartate simulation can be visualized through the decrease in the distances between the backbone nitrogens of F helix residues and the Ca\(^{2+}\) ion during the Aspartate simulation. (a) Alanine 102 N starts out at a distance of just over 10 angstroms from the Ca\(^{2+}\) ion, and moves in to a distance of about 9.7 within the first 200 frames (8 picoseconds) of the simulation. (b) Methionine 104 N displays a larger movement that doesn't begin to level off until about 500 frames (20 picoseconds), ranging from \(\sim 11.3\) angstroms distant from the Ca\(^{2+}\) to about 10.5 angstroms.
Figure 6-7. The F helix moves 0.97 angstroms during the Aspartate simulation.

The distance between the aspartate 101 Cα(initial) position and the Cα(final) position in the Aspartate simulation was measured to be 0.97 angstroms. This difference in initial and final positions extends throughout the F helix, as is illustrated in this ribbon diagram of the superimposed initial and final structures.
the Ca\(^{2+}\) ion, the aspartate was still unable to achieve a favorable orientation for bidentate Ca\(^{2+}\) coordination. Aspartate is not only shorter than glutamate; it is also less flexible, possessing one less sidechain torsion angle. A modeling experiment, conducted to determine to what degree this lack of flexibility is also a factor in the aspartate’s inability to assume the proper orientation for bidentate ligation of Ca\(^{2+}\), is illustrated in Figure 6-8. The final orientation of the aspartate 101 sidechain from the Aspartate simulation was used in Figure 6-8 to construct a model wherein equidistant Ca-O distances of 2.70 Å were obtained for the two sidechain oxygens by applying rotations around the \(\chi_1\) and \(\chi_2\) torsion angles in the crystallographic visualization program O (Jones \textit{et al.}, 1991). This indicated that the aspartate at residue 101 was sufficiently flexible to obtain bidentate coordination of Ca\(^{2+}\) if the loop could constrict enough to bring the sidechain oxygens closer to the Ca\(^{2+}\) ion.

The noncoordinating aspartate 101 sidechain oxygen Ca-O distance in the Aspartate simulation is quite long, generally between 3.5 and 4.0 Å. This indicates that the sidechain is really stretching to engage in even monodentate coordination of the metal ion. Consequently, monodentate coordination of Ca\(^{2+}\) seems to be the compromise reached in the tug of war between the Ca\(^{2+}\) ion and the F helix.
Figure 6-8. Modeling the aspartate 101 sidechain with equidistant Ca-O bonds.

The orientation of the bidentate glutamate sidechain that occurs naturally in the parvalbumin EF site is illustrated in purple. The glutamate Ca-O distances are 2.27 angstroms for the uppermost oxygen and 2.30 angstroms for the second oxygen. The final aspartate sidechain orientation at the end of the Aspartate simulation is shown in green with Ca-O distances of 2.07 for the coordinating oxygen and 3.18 angstroms for the noncoordinating oxygen. The aspartate sidechain shown in blue is the one that has been modeled using the final orientation at the end of the Aspartate simulation as the starting model. Equidistant Ca-O distances of 2.70 angstroms for each of the sidechain carboxylate oxygens have been obtained by rotation of the side chain torsion angles of the starting model. This indicates that the aspartate at residue number 101 has the flexibility to obtain a bidentate conformation if the loop could constrict enough to bring the sidechain oxygens closer to the Ca$^{2+}$ ion.