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A Nudged Elastic Band Study on the N-terminal Domain Conformational Pathways of Annexin A1 from a Buried State to an Exposed State

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Abstract

Mapping the conformational pathway of biomolecules is a great challenge due to the large size and complexity of biomolecules. The NEB (Nudged Elastic Band) method has been applied to study the reaction pathways for both small organic molecules and small peptides of a few amino acids. In this work for the first time, the NEB method was employed to study the conformational pathways of Annexin A1, a membrane-binding protein of 334 amino acids. The N-terminal domain conformational changes from the buried state within the core domain to the exposed state outside the core domain is a vital step for Annexin A1 to interact with membranes or target proteins. In this work, multiple molecular dynamics simulations using NEB method were performed to simulate the N-terminal domain conformational pathway of Annexin A1. Our results suggested that the N-terminal domain of annexin A1 is removed from the repeat III of the core domain in a sliding motion. The loop region of repeat III covering the N-terminal helix in the buried state does not lift up for the N-terminal to swing out of the pocket; instead, the N-terminal pulls out from the bottom of the core domain. The N-terminal domain linker region (S27-N42) flexibility is critical for the N-terminal domain conformational changes. Our results also suggested a two-step folding process for the helix D in repeat III, M247-V250 folds first and followed by the folding of L251-E254. The results demonstrated that the NEB method could be an effect tool for theoretical studies on conformational pathways of biomolecules.

INTRODUCTION

Annexins are a large family of calcium binding proteins involved in various cellular processes including endocytosis, exocytosis, membrane fusion, membrane microdomain formation, membrane aggregation, membrane repair, calcium ion channel formation, signal transduction, etc.^{1–5} To date there has been no evidence suggesting that annexins direct genome is involved in different biological diseases. However, annexins are indirectly linked to serious human diseases including cancers, diabetes, inflammatory diseases, blood clotting and fibrinolysis, etc.^{6–9} Changes in the annexin expression level can occur under certain

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SUPPORTING INFORMATION

The supporting information includes Table S1 which contains all of the unscaled energy values acquired from the NEB simulations (IMP 1, IMP 2, IMP 3) and the results of the MMGBSA analysis of the molecular dynamics sampling with normal mode analysis (MMGBSA) and without normal mode analysis (MMGBSA-NM).

conditions and these changes can be indicative of different disease pathophysiology. The variety of diseases range depending on the annexin protein and certain diseases can be either up or down regulated with annexin protein expression. For example, Annexin A1 has an anti-inflammatory role due to its connection with COX-2 and PLA₂. The expression and secretion of Annexin A1 is regulated by glucocorticoids and is suggested that annexin A1 can act as a messenger protein linking the glucocorticoid release as regulation of COX-2 and PLA₂. This process is supportive of the prostaglandin pathway.¹⁰ For many of the inflammatory diseases studied in connection to annexin, rheumatoid arthritis and myocardial infarction have been studied in more detail. In experimental studies fragments of Annexin A1 have been shown to protect against myocardial reperfusion which suggests that during inflammatory responses Annexin A1 may have an anti-inflammatory role.¹¹ Besides, Annexins A1, A2, A4, A6, and A11 are shown to be down regulated in prostate cancers.^{6,12} Annexin A2 has been involved in complexes with S100A11 that facilitates membrane repair.

All annexin proteins are composed of two domains, an N-terminal domain and a conserved core domain. The core domain is composed of four repeats, and each repeat contains five helices and approximately 75 residues (Figure 1). The core domain houses calcium binding sites for the protein at the convex side. Calcium binding at the convex side of the core domain serves as a “bridge” for annexin interaction with the negatively charged phospholipids of the membrane. The N-terminal domain has varied length and sequence for each member of the protein family. The N-terminal is very important because it regulates the physiological function of each annexin. Unique N-terminal sequencing is the dictating factor in specific function, and each member of the annexin family tends to have unique physiological functions.

Annexin A1 is a 37 kDa member of the annexin protein family composed of an N-terminal and core domain. Annexin A1 has a long N-terminal domain composed of 41 residues, 17 of which form a flexible linker connecting the two domains. Like other annexin proteins, the core domain is composed of four repeats of 75 residues each. Eight calcium binding sites are present on the convex side of the core domain of Annexin A1. Most of the calcium binding sites have seven ligand contacts at the binding sites through oxygen atoms located in the binding loop and water molecules that are in close proximity. However, AB binding site in repeat 1 and the second AB binding site in repeat 2 have eight ligands contacts.¹⁴ The structures for Annexin A1 in the absence or presence of calcium have been solved using X-ray crystallography techniques. Comparison of the X-ray structures of Annexin A1 indicates that there is drastic change in the N-terminal domain and repeat III of the core domain. In the absence of calcium, the first 12 residues of the N-terminal domain are buried in the repeat III of the core domain. The N-terminal helix replaces the D helix, which in a calcium free state is composed of a flap covering the N-terminal. Upon calcium binding, the N-terminal domain has been ejected from the repeat III, the loop covering the N-terminal in the calcium-free conformation has moved to a position to form the D helix, and the N-terminal is available to interact with other proteins and regulate membrane aggregation. The N-terminal domain conformational changes (Figure 1) from the buried state within the core domain to the free state outside the core domain is critical for Annexin A1 to execute the biological functions.

Previously, molecular dynamics simulations were performed to study the calcium-bridged Annexin A1-membrane interaction and the important interaction between lysine and the membrane was identified at the N-terminal domain.¹⁵ The impact of the N-terminal domain mutations S27E and S27A were also studied for Annexin A1 using molecular dynamics simulation method and the consistent results on calcium binding affinity with experiments were generated.¹⁶ In this work a computational approach was used to study the N-terminal domain conformational change of Annexin A1. To the best of our knowledge a computational study of the conformation change for the N-terminal removal from the core has not yet been done. The NEB (nudged elastic band) calculation has not yet been performed for the proteins of this size. In this work the NEB method was applied to study the N-terminal domain conformational changes of Annexin A1 consisting of 343 residues.

COMPUTATIONAL METHODS

Full-length calcium-free Annexin A1 with N-terminal domain buried within the core domain was obtained from the PDB X-ray structure (1HM6.pdb). There are no crystal structures available for the full-length Annexin A1 with the N-terminal exposed outside of the core domain. Both structures were needed in this work to study the N-terminal conformational pathway from being buried within the core domain to being exposed outside of the core domain. The calcium bound core domain with the N-terminal exposed outside of the core domain was obtained from the PDB X-ray structure (1MCX.pdb) and the missing 41 residues of the N-terminal domain were fused to the core domain using Insight II software. This construction was part of previous molecular dynamics work¹⁵, and was available at the start of this work. Both conformations have a total of 343 residues. These two structures serve as the endpoints (reactant and product) for studying the N-terminal domain conformational pathway in this work.

All computations were performed on a computer cluster of 320 processors at East Carolina University using AMBER's force field (*ff14SB*) in the Amber 16 package. Three simulation were performed on Annexin A1 to determine the N-terminal conformational pathway using the NEB module implemented in the AMBER 16. A total of 32 images (structures) were used on the conformational pathway. For all simulations the implicit water model was used.¹⁷

The NEB method was derived from the plain elastic band method introduced by Elber and Karplus.¹⁸ This method included a spring potential to the energy surface and minimized the total energy for the system. In the plain elastic band method low-energy paths are determined but corners tend to get cut in the energy landscape. Implementation of the NEB method avoids cutting corners by applying spring forces perpendicular to the tangent of the path.¹⁹ The conformation change in NEB is determined using a series of protein structures distributed along the path, which represents the trajectory that the protein undergoes during conformational change.²⁰ The structures located at the endpoints of the pathway are representative of the reactant and product structure. These structures are fixed in space and do not explore the energy landscape. Each structure along the path is connected by springs, which serve to keep the structures from sliding down the energy landscape. If structures were to slide on the landscape, they can slide onto adjacent structures in the pathway. To

keep structures evenly spaced, forces from the molecular potential are condensed along the path. This assists in keeping a smooth conformation change pathway.²¹

It should be mentioned that since the implicit water model parameters are not available for calcium ions in AMBER 16, calcium ions were not included in the simulation system. Although the N-terminal conformational change from being buried within the core domain to being exposed outside of the core domain involved calcium binding on the convex side of the core domain, the initial (N-terminal domain being buried within the core) and final (N-terminal domain being exposed outside the core domain) structures used for NEB calculations do not contain calcium ions. The structures used in this calculation correspond to the calcium free structure of Annexin A1 and calcium bound structure of Annexin A1 with the calcium ions being removed. Furthermore, whether the calcium ions are included in the simulation systems or not, it is impractical to generate the N-terminal domain conformational pathway for Annexin A1 using regular molecular dynamics method without applying an external force to the system; the regular molecular dynamics will take too long to simulate the N-terminal domain changes from being buried within the core domain to being exposed outside the core. In this work, we employed NEB method to investigate the N-terminal conformational pathway of Annexin A1.

For all three simulations the endpoint structures were minimized to reduce steric hindrance. Minimization for the three simulations were performed in 8500, 12000, and 14000 steps, respectively for simulations 1, 2, and 3. Steepest decent method was used over the first 1000 steps, then conjugate gradient method was used for the remainder of the minimization. All simulations included a two-step minimization process, first all atoms except hydrogen were held using a 1 kcal mol⁻¹ Å⁻¹ restraint (**MIN0** in Table 1) and for the second minimization no atoms were held fixed (**MIN** in Table 1). As shown in Table 1, to determine the potential conformation pathway differing spring forces, number of steps, and time steps were used to compare pathways. In all three simulations spring forces were applied to all 343 residues.

After minimization, the systems were warmed for a total of 3.2 ns from 0 to 300 K using Langevin dynamics collision frequency of 1000 ps⁻¹. A spring force of 10 kcal mol⁻¹ Å⁻¹ was used during the heating process for all three simulations. All systems were allowed to evolve at 300 K for a total of 16 ns with the 1000 ps⁻¹ collision frequency. Spring forces of 10–30 kcal mol⁻¹ Å⁻¹ were applied to the systems. After each system was heated, simulated annealing was performed using Langevin dynamics and a collision frequency of 1000 ps⁻¹. Each system was heated from 300–500 K and cooled back to 300 K over 88.32 ns with spring forces varying from 10–50 kcal mol⁻¹ Å⁻¹. After the annealing process the systems were cooled from 300 to 0 K using Langevin dynamics collision frequency of 1000 ps⁻¹ totaling 13.44 ns. Finally, the systems were quenched at 0 K for a total of 1.12 ns. During the cooling and quenching process spring forces of 10–50 kcal mol⁻¹ Å⁻¹ were applied to the systems.

RESULTS AND DISCUSSION

A. Potential Energy Curves

Three simulations (**IMP 1**, **IMP 2** and **IMP 3**) totaling 121 ns were performed to determine the N-terminal domain conformational pathway of annexin A1 from being buried within the core domain to being exposed outside the core domain. This N-terminal domain conformation changes is a vital step required for the completion of many biological functions for Annexin A1. The minimized endpoint coordinates for Annexin A1 were incorporated in the NEB and all 32 images along the conformation pathway were used to map out the reaction pathway and track conformational changes. For each simulation the energy associated with each image, relative to the energy of image 1 (the first point of each curve), is displayed in Figure 2. The unscaled energies from the three independent NEB simulations, and from the MMGBSA results for the 11 selected structures from **IMP 3** are included in the Table S1 from the Supporting Information.

The first simulation (**IMP 1**) for Annexin A1 totaled 14.4 ns. The initial structure with the N-terminal domain being buried inside the core domain (image 1) has a potential energy of -11515.2 kcal/mol and the final N-terminal exposed structure (image 32) has a potential energy of -11519.9 kcal/mol (Table S1). These structures have the lowest potential energy along the conformational pathway. The structure that has the highest potential energy long the pathway is image 14. This structure has a potential energy of -11199.0 kcal/mol. For the first implicit NEB simulation the range in potential energy for the structures along the conformation pathway were 316.2 kcal/mol.

The second simulation (**IMP 2**) of annexin A1 totaled 50.9 ns. Similar to the first simulation, the second simulation was run using a total of 32 images and had the initial and final structures of the pathway as the lowest in potential energy at -11520.3 kcal/mol and -11522.2 kcal/mol respectively (Table S1). In the second simulation the structure with the highest potential energy was structure 14. This is also comparable to the first simulation. Structure 14 had a potential energy of -11285.1 kcal/mol. The potential energy barrier for the second simulation was 235.2 kcal/mol.

The last simulation (**IMP 3**) of annexin A1 totaled 55.88 ns. Similar to the second simulation this one was run with a large number of steps and step size. In the previous simulations (**IMP 1** and **IMP 2**), various spring constants were used at each step. However, for this simulation a spring constant of $10 \text{ kcal/mol}\text{\AA}^2$ was used for all NEB steps. For the third implicit simulation the initial (image 1) and final (image 2) structures of the conformation pathway have the potential energies of -11522.2 and -11525.8 kcal/mol (Table S1). The image with the highest potential energy in the third simulation was 13. This structure had a potential energy of -11247.7 kcal/mol. The range in potential energy values for the third simulation was 274.5 kcal/mol.

Three NEB simulations on Annexin A1 N-terminal domain conformational changes generated similar potential energy curves, as shown in Figure 2. However, the longer the simulation and the smaller the spring force applied to images, the lower the energy barrier going from the N-terminal domain buried state to the N-terminal domain exposed state. The

barriers for **IMP 2** and **IMP 3** are about 80 kcal/mol and 40 kcal/mol respectively lower than that for **IMP 1**. They have similar increases and decreases in energy for corresponding images, and similar image numbers and values sit at the top of the energy curve. This information suggests the similarity in all three simulations. The NEB method used in this work produced structures that seem to be similar based on their energy curves. While some simulations were able to lower the energy, with different parameters the overall conformation change that is occurring in all three simulations may be similar. The investigation of conformation pathways of biomolecules is very challenging, due to the size of the system, however, the similar potential curves generated in this work demonstrated that NEB method has the great potential to map the conformational pathway of biomolecules. To investigate the conformational similarities in more detail cluster analysis, RMSD analysis, and the secondary structure analysis were performed for three NEB simulations.

Although simulations 2 and 3 have lower potential energy barriers than simulation 1, the barrier is still higher than 200 kcal/mol. The large barrier of the calculated N-terminal domain conformational pathway is not unexpected, since the drastic conformational change for the N-terminal domain to be removed from being buried within the core to the exposed state outside the core involved many residues from both core and the N-terminal domains.

To obtain a reasonable estimation for the energy barrier, regular molecular dynamics simulations and MMGBSA^{22–28} calculations were performed for the selected structures from simulation **IMP 3**. 11 structures (images) including image 1 and image 32 were selected at a regular interval from the 32 images of **IMP 3**. The regular molecular dynamics simulations, without any external force applied to the system, were performed for 6.02 ns for each of 11 selected images; total 66.22 ns molecular dynamics simulations were performed for the 11 images. The final simulated structures and the corresponding initial structures used to start the simulation are very similar; the RMSD between the final simulated and the corresponding initial structures range from 1.116 Å to 1.628 Å for the 11 images. Based on the simulations, MMGBSA calculations with the entropy contribution from the normal mode analysis (MMGBSA-NM) and without the entropy contribution were performed for all the 11 selected images of **IMP 3**. The MMGBSA calculated free energies, with and without entropy contribution, were plotted in Figure 2, which indicated an energy barrier of about 88.0 Kcal/mol. Entropy contribution decreased the energy barrier by about 16.0 Kcal/mol. We have verified that after MMGBSA calculation for 11 images, the energy barrier is much lower, and that the conformational pathway remains the same. This confirms that the NEB method is an effective tool to simulate the conformational pathway for substantial conformational changes of large biomolecules. Subsequently, the regular molecular dynamics simulations and MMGBSA calculations performed on selected images from the NEB simulation can generate a reasonable energy barrier for the conformational pathway.

B. Annexin A1 Conformational pathway

Cluster analysis was performed for 32 images along the NEB path for the N-terminal domain to be removed from the core domain. For each simulation the 32 images along the NEB path were clustered into eight representative images. Each representative structure

demonstrates the characteristics of the protein for that stage of the conformation change. The plots of the cluster analysis for all three simulations are shown in Figure 3.

It can be seen from Figure 3 that, for the first simulation (top row **IMP 1**), Clusters 4 and 5 have the most images associated with their clusters. This means at this portion of the conformation pathway there is less structural change going on. It is also interesting to note that these structures are located at the highest point on the potential energy curve. The location of the images for cluster 4 and 5 suggest that these structures are less energetically stable. Fifteen out of the thirty-two structures for the reaction pathway are located in these two clusters. This is just less than half the structures. For the second simulation, there are fewer images represented in the first four clusters, while in the last four there are more images represented. This clustering suggests that the second simulations conformational change might occur quicker for the first half of the pathway than the second. Similar to the first simulation the images located at the top of the potential energy curve are grouped into larger clusters. In the second simulation cluster 5 and 6 are located along the top of the potential energy curve. These clusters incorporate 14 out of the 32 images. For the third simulation, first four clusters have more structures represented than the last four. This suggested that for this simulation there is more similarity to the structures of the first half of the simulation than the last. In this simulation cluster 3 and 4 are located at the top of the potential energy curve. These clusters incorporate 14 out of the 32 images in the pathway.

There are similarities in cluster analysis over all three simulations. In all three simulations structures with the highest potential energy are divided into two clusters. These clusters incorporate more structures than other clusters along the pathway. For all simulations approximately fourteen structures are included in these clusters, which is almost half of the structures along the conformation pathway. The NEB simulations are run using differing parameters and starting structures, so we do not expect to see clusters that are exactly the same. This suggests that the NEB method is consistent over all three simulations. Each simulation used different simulation parameters and produced similar structures along the conformation pathway. Based on the consistency of the data obtained up to this point, we are confident that the conformation changes in all three simulations are the similar, and detailed discussion will be focused on only one simulation. In the remainder of the paper, we will be investigating the conformation change for Annexin A1 using results obtained from the third simulation.

B1. N-terminal domain conformational change—Figure 4 shows the conformational changes that occurred from the N-terminal buried state to the N-terminal domain exposed state of Annexin A1, including an overlapped image of all the clustered structures to clearly illustrate how the N-terminal domain is removed from the core. In Figure 4 (C1) the tip of the N-terminal helix (green) is covered by the loop region of repeat III (red). The N-terminal helix slides down from its previous location in the second clustered structure Figure 4 (C2). The shifting of the N-terminal helix could be facilitated by unfolding of residues D14-E16. The residues D14-E16 have lost their secondary structure and become loops. Loss in their secondary structure will make these residues more flexible than they are as a helix. It is possible that the flexibility of residues D14-E16 are needed to help pull the tip of the N-terminal helix out of the core domain. In the third clustered

structure, Figure 4 (C3), residues D14-E16 have reformed their original helical structure. The N-terminal helix is shifted even further down from the third repeat. In cluster 3 (C3) there is unfolding of residues M2-E5. At this point the N-terminal helix is almost fully removed from the N-terminal domain. Secondary structure change of the first few residues in the N-terminal helix might be required to fully remove the N-terminal helix from the core domain. Helical shaped structures tend to take up more space than structures with loop-like conformations. When the N-terminal is a helix it is more ridged and is not able to slide out of the pocket easily. Losing some helical structure right before its removal from the core domain may assist the tip of the N-terminal to come out of the pocket, by making it more flexible. After the N-terminal is removed from the third repeat of the core domain the N-terminal continues to shift away from the core.

In Figure 4 (C4–C5) the N-terminal continues to move away from the core domain and the N-terminal helix begins to straighten. In previous structures, the N-terminal was bent due to its association inside of the core domain. Once free, this helix is able to straighten itself out. In the 5th clustered structure (C5) there is also refolding of the N-terminal helix at residues M2-E5. The N-terminal has completely removed itself from the core domain and reforms its helical structure. This process of refolding suggests that once fully removed from the core the end of the helix can now refold. This structure also has folding of residues S33-V35 in the N-terminal linker region. This linker is very flexible and is able to move around during the simulation. In this process it is possible that its movement puts certain areas of the linker in a proper conformational orientation to fold. That could be an explanation why this area of the linker has slight folding. There is no experimental evidence explaining why folding in the linker could occur. In the structure of the 7th cluster, the folded portion of the linker region loses its structure and goes back to a loop conformation. The N-terminal helix straightens out even more in this structure. In the final 8th clustered structure (C8), the N-terminal helix is fully straightened and resembles the structure with the N-terminal domain exposed outside the core domain.

Overall the N-terminal removal from the third repeat of the core domain is vital for further protein and membrane interaction. Here we were able to see that the N-terminal gradually pulls itself out of the third repeat. There are also some changes in secondary structure for the N-terminal during this process which seems to aid in proper removal of the N-terminal. Without flexibility of the linker region connecting the N-terminal and core domain this process would not occur properly, or at all. Previously, experimental studies^{29,30} have demonstrated that mutations at the N-terminal domain of Annexin A1 may change the function of Annexin A1. The S27A mutant of Annexin A1 had the same calcium dependence for Annexin A1-induced phospholipid vesicle aggregation, while the S27E mutant of Annexin A1 had a higher calcium concentration requirement and lower maximal extent of phospholipid aggregation. Our previous computational studies¹⁶ have demonstrated that the S27E mutation at the N-terminal domain changed the flexibilities of the N-terminal domain and its relative position to the core domain, and thus may change the Annexin A1 induced membrane aggregation process.

B2. Core domain conformational change—The most notable change in the core of Annexin A1 is the formation of helix D in repeat III. The detailed process for this

conformation change has not been investigated. Here we provide insight into a detailed process showing how conformation change in the third repeat can occur. When examining the overlapped Annexin A1 clustered images in Figure 4, there is less visible change in the core domain than in the N-terminal helix. However, there is noticeable conformation change occurring in the third repeat of the core domain.

In the first three clustered structures of Annexin A1, Figure 4 (C1–C3), the portion of the third helix involved in folding is initially a loop. This area, shown in red, is undergoing movement along the conformation pathway. In **C1** this loop is overlapping with the tip of the N-terminal helix which is in the location where helix D will form. As the N-terminal helix is removed from the third repeat, the loop begins to compact itself and create more waves in the loop. Comparing **C1** to **C3** reveals a noticeable difference in the orientation of the loop. Orientation of the loop in this fashion can put it in a proper orientation to form helix D. In **C2** there is folding of A206-R211. These residues are located on helix A of repeat III. Initially these residues are loops connecting helices A and B, but early in the conformation pathway these residues fold making helix A longer. These residues remain folded so it can be assumed that these residues are needed for proper conformational change to occur in the remainder of the core domain. The first set of residues to start the formation of helix D in repeat III are M247-V250. These residues start forming the bottom of the helix. As soon as the N-terminal helix is removed from the binding pocket, the loop of repeat III is not blocked by the N-terminal and can form the helix. The second step of folding for helix D, are residues L251-E254 (**C5**).

To make sure all folding steps were identified, all 32 structures were examined. In all three simulations folding of helix D in repeat III is a two-step process. This conformation change is consistent over all three simulations. In the remainder of the structures Figure 4 (C7–C8) the core domain does not undergo any major conformational change. RMSD and secondary structure analysis were performed to see if any other changes occurred over the conformation pathway.

RMSD analysis. RMSD (Root-Mean-Square Coordinate Deviation) analysis looks in closer detail at each residue and compares structural alignments between two cluster structures. In Figure 5 RMSD of two cluster structures are given, to show changes in certain areas of Annexin A1 core domain over the entire conformation pathway. Spikes in RMSD at the binding site located in repeat II at residue 130 are present most prominently in comparisons of **C1** and **C2**, and **C4** and **C5**. These two RMSD plots have the largest peaks for this area. Residue 130 is a site II binding loop for calcium ion in repeat II. There is also movement for residues 190–200. These residues are part of the linker connecting repeat II and III. This region is flexible and its proximity to repeat III helix D formation may be a result in its increased movement.

The area of the protein undergoing largest conformational change is located in repeat III around residue 250. Residues surrounding helix D also have changes in RMSD through the conformation change. It is unreasonable to assume that only folding of helix D will occur. Residues in repeat III will have some cooperative changes to ensure that the protein will fold properly. In repeat IV there are changes in RMSD for calcium binding site II in the area

around residue 290. **C1** vs **C2** has the largest spike for this area of the core domain. However, there are smaller spikes and fluctuations occurring in **C2** vs **C3**. RMSD differences for this binding site could be caused by movement of the residues or conformational change.

By performing RMSD analysis we were able to determine that more changes are occurring rather than just the formation of helix D in repeat III. Some of these fluctuations were not noticed by visual investigation. Further analysis is needed to determine what kind of change is occurring. If there is any kind of secondary structure change occurring in the core domain, we can detect that by performing secondary structure analysis. This will help investigate which regions of the protein are moving slightly or which areas are having conformational change.

Secondary structural analysis.: The third repeat of annexin A1 has drastic structure change for residues M247-E254. These residues participate in folding to form helix D. To determine if other areas of the core domain are undergoing a cooperative secondary structure change, we have investigated the helical percent of each residue of the core for all clustered structures. Overlapping the helical percent data gives insight into areas of the protein that are undergoing structure change. It can be seen from Figure 6, the residues with the most structure change are M247-V254. Overlapping the data for all eight clusters shows that this area is cluttered with variations in helical percent. The secondary structure plot expresses that **C4** starts to form the helix D of repeat III first and continues to form until all residues have formed the helix. In this plot, regions of the protein that have no secondary structure change will not show fluctuations as all helical percent and will be the same.

Areas of the core domain that seem to have structure change other than forming helix D of repeat III, are areas where calcium binding would occur. In Figure 6, these binding sites fluctuate in the amount of secondary structure change that is occurring. For example, in the first calcium binding site of repeat I there is fluctuation in secondary structure between two residues, while in the first calcium binding site of repeat III there is change over four residues. The first calcium binding site of repeat III has increase in helical structure from residues 208–212 over the first seven clusters, then in cluster eight there is loss of most of the secondary structure. For some binding sites there are fluctuations in secondary structure.

Overall, the majority of change in secondary structure for the protein is occurring in the third repeat. However, over the course of the conformation change secondary structure change is occurring at calcium binding sites. This indicated that calcium binding sites changes may be correlated with the repeat III helix D of M247-V254 formation and correlated with the removal of the N-terminal domain from the repeat III. This also confirmed that calcium binding is the cause of the N-terminal domain to be ejected from the core domain. In the calcium free Annexin A1 the N-terminal domain is buried within the core domain, while in the calcium bound Annexin A1 the terminal domain is exposed outside the core domain. This study provides valuable information for future investigations using point-mutations to modify the function of Annexin A1. So far, no mutation studies at repeat III M247–254 have been reported yet. The point mutations at the repeat III M247–254 may change the rigidity of the sequence M247–254 and change its folding process, and thus may affect the removing

process of N-terminal domain from the core domain. Subsequently, the point mutations at repeat III M247–254 will change Annexin A1 functions in membrane binding.

CONCLUSION

In this work, three simulations using NEB method were performed to track the conformational change of Annexin A1 from the N-terminal buried state to N-terminal free state. The conformation pathway for all three simulations were compared, and due to structural consistency, the conformation change for all three simulations were concluded to be consistent. Based on our results the N-terminal comes out of the core in a “sliding” motion. There is no evidence that the loop region of the third repeat is swinging away from the helix allowing it to come out. Residues D14-E16 of the N-terminal helix lose secondary structure, possibly allowing for bending of the N-terminal and proper removal from the core. After removal from the core the N-terminal helix reforms its helical structure at D14-E16.

In the core domain residues M247-V250 start helical formation of helix D in repeat III. The last residues to complete helix D formation are L251-E254. Over all three simulations we were able to see this two-step process for helical formation. Other conformation changes were noticed at the calcium binding loops of the convex side of the core domain.

Overall, we were able to determine a possible mechanism for Annexin A1 conformational change from the N-terminal buried state to an ejected state. In detail we see the N-terminal remove from the core domain in a sliding motion and repeat III of the core domain fold to form helix D in two steps. Based on our results we concluded that the NEB method is reasonable to use for proteins of this size (343 residues) and confirm the ability to obtain consistent results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
The N-terminal domain (in yellow) conformational pathway from the buried state within the repeat III (in red) of the core domain to the exposed state.

Figure 2.

The energy plots of three NEB simulations, the regular MD simulations, and the MMGBSA analyses for the selected 11 images. IMP 1: green, circle; IMP 2: violet, square; IMP 3: red, triangle-up; MMGBSA: maroon, cross; MMGBSA-NM: black, plus.

Figure 3.
Clustered analysis of three NEB simulations for the N-terminal domain to be removed from the buried state to the exposed state (IMP 1: circle, IMP 2: square, and IMP 3: triangle).

Figure 4.
Representative cluster images along the NEB pathway of the N-terminal domain from the buried state to the exposed state

Figure 5.
RMSD of different clusters going from the N-terminal buried state to the N-terminal exposed state.

Figure 6. Secondary structural helical percent for each of the four repeats of annexin A1 core domain. The plots for different clusters are overlapped (Cluster **C1**: black, **C2**: cyan, **C3**: green, **C4**: blue, **C5**: turquoise, **C6**: brown, **C7**: orange, **C8**: magenta). The calcium ion binding sites are indicated with Ca^{2+}

Table 1.

NEB Simulation Parameters for all Three Implicit Water Simulations. Differing Parameters were Used for the Minimization Cycles, Number of Steps Performed, Time Step (DT), and Spring Force (k).

	IMP 1	IMP 2	IMP 3
MIN0: CYCLES	3500	6000	7000
MIN: CYCLES	5000	6000	7000
HEAT			
NUM STEPS	40,000	40,000	40,000
T (fs)	0.5	0.5	1.5
k (kcal/molÅ²)	10	10	10
EQUILIBRATION			
NUM STEPS	100,000	100,000	200,000
T (fs)	1.0	1.0	1.5
k (kcal/molÅ²)	20	30	10
ANNEALING			
NUM STEPS	420,000	900,000	800,000
T (fs)	0.5	1.5	1.5
k (kcal/molÅ²)	50	20	10
COOLING			
NUM STEPS	120,000	120,000	120,000
T (fs)	1.0	1.0	1.5
k (kcal/molÅ²)	50	50	10