Simulation and modeling of synuclein-based "protofibril" structures: as a means of understanding the molecular basis of Parkinson’s disease

I F Tsigelny, Y Sharikov, M A Miller and E Masliah

Abstract. The protein \( \alpha \)-synuclein (aS) is a 140 amino acid cytosolic protein of unknown function expressed abundantly in neurons and is associated with both environmentally induced and inherited forms of Parkinson's disease, the neurological disorder caused by progressive death of dopaminergic neurons in the substantia nigra. Here we demonstrate that aS aggregation leads to protofibril formation that accompanies aS binding to membranes. The specific surfaces of aS that bind to membranes are likely developed during the folding maturation process, where the 3D combinations of amino acids that are bound to these surfaces are very specific. Using molecular modeling and simulation technique, we elucidate the folding pathway of aS into protofibril structures and its configurations that bind to membranes. These membrane-binding surfaces can be targeted with pharmaceutical intervention for dissolution.

1. Introduction
The protein \( \alpha \)-synuclein (aS) is a 140 amino acid cytosolic protein of unknown function expressed abundantly in neurons, and is associated with both environmentally induced and inherited forms of Parkinson's disease, the neurological disorder caused by progressive death of dopaminergic neurons in the substantia nigra. Evidence for the involvement of aS in disease progression comes from the association of Parkinsons with \( \alpha \)-synuclein overexpression (caused by genomic triplication of the encoding genes) and with inherited forms that with three specific mutations in the aS gene (A53T, A30P, and E46K). Both cases are characterized by development of aggregated protein inclusions (Lewy bodies) in the cytosol and on the membrane of surviving neurons. The connection between development of Lewy bodies and dopaminergic neuron apoptosis is still unclear. For a number of years, Parkinson’s disease progression was thought to reflect the impact of massive fibril accumulation, which eventually prevents normal cell functions. Recent evidence suggests that a small intermediate “protofibril” structure may actually be the toxic species (see [1, 2] and references in them). It is therefore important to examine the mechanism of aS aggregation and binding of these aggregates to the cell membrane, as a means of understanding the biochemical and biophysical mechanisms leading to onset of the disease.

Here we demonstrate that aS aggregation leads to protofibril formation that accompanies aS binding to membranes. The specific surfaces of aS that bind to membranes are likely developed during the folding maturation process, where the 3D combinations of amino acids that are bound to these surfaces are very specific. Using molecular modeling and simulation technique we elucidate the folding pathway of aS into protofibril structures and its configurations that bind to membranes. These membrane-binding surfaces can be targeted with pharmaceutical intervention for dissolution.

1.1. Unstructured proteins dynamics and membrane interactions

Well-known proteins and peptides such as aS, beta-amyloid, and prion that are responsible for a number of neurodegenerative diseases have one common feature – all of them are naturally unstructured proteins that do not have any stable conformation. To understand their pathology, one
must develop tools to assess the behavior of possible conformations that molecule occupies in solution, as well as the potential for interaction of each conformation with the surface of the cell membrane.

The experiments we conducted take the view that these proteins exist in solution, while changing conformations. From time to time, the unstructured protein makes contact with the surface of the membrane, in whatever conformation it currently occupies. The protein will remain in contact with the membrane for a time that is defined by the energy of interaction between the membrane and the protein. If the energy of interaction is weak, the protein molecule will dispatch from the surface of the membrane back to the solution after a relatively short time. This cycle of binding and release will continue, and the protein will sample many conformations as the process goes on. If a protein has a favorable membrane binding conformation, the protein will remain on the surface of the membrane longer. Ultimately, if the time of residence on the membrane is long enough, the protein may start penetration into the outer layer of the membrane. In this period of time, the membrane may also stabilize the conformation of protein, by providing specific regions of hydrophobic and hydrophilic structure.

In the altered environment near the membrane surface, additional molecules of the same protein may come in contact with the first molecule located on the membrane and create dimers. Depending on the energetics of this interaction, these can propagate into higher-ordered multimers with time. The process is complex and does not have clearly defined steps, boundaries, or even initial molecular conformations to work with. Solving this problem is intractable (with current computational resources) if one wishes to conduct molecular dynamics simulations of all possible conformations and all possible interactions. Accordingly, we chose to address this problem by a sparse-sampling methodology that relies on representative “snapshots” of the system at various points in time and in various time regimes, rather than on complete end-to-end simulations of the entire process.

2. Modeling and Simulation

1.2. Computational approach to unstructured proteins

For study of protein aggregation (oligomerization) on the membrane with possible organization of pores, as well as its subsequent penetration into the membrane, we would require the following methods:

- Prediction of membrane-contacting surfaces of proteins
- Molecular dynamics simulation of protein molecules in water and on the membrane
- Molecular docking of the protein molecules on the membrane
- Molecular dynamics simulation of protein oligomers in the membrane

We have created a set of programs and workflows to address this challenge.

2.2. Membrane contacting regions recognition

Figure 1 shows the first block program, called MAPAS [3], to assess how strongly the protein interacts with the membrane (membrane-contacting potential). It contains scoring functions developed using methods of modeling of amino-acids disengagement and Poisson-Boltzmann free-energy calculations. The MAPAS program initially calculates all possible membrane-contacting surfaces of a given protein and then calculates the membrane philicity score and predicts the best membrane-contacting surface. This program was tested on a number of known membrane-contacting proteins from the PDB and showed good correspondence to the experimental data.
2.3. Initial molecular dynamics in water environment and shape clustering

A set of molecular dynamics (MD) runs for unstructured proteins is a necessary step to define possible conformations that are more stable than others in solution. The MD simulations are conducted on the IBM Blue Gene computer and take quite a long time, but these are a necessary step in the entire modeling mentioned above. The calculations are described further in [4]. Because aS has no strongly favored low-energy conformers, we had to create a strategy for selection of possible "temporarily" stable conformers. We used a shape-clustering method to select a representative set of aS conformations obtained during MD simulations (we will refer to them as "conformers") during MD in water solution, and we evaluated their tendencies to associate with a phospholipid bilayer. To the input of the clustering program we submit all conformers from MD runs. The clustering program selects most populated clusters (figure 2). The core representatives of each cluster are used for farther docking on the membrane.

Figure 2. Most populated 20 clusters.
This way we select most common conformations of the protein (in this case aS). From a statistical point of view, these have the highest probability of existence through the time of the MD run. In the case of unstructured proteins we cannot use more stringent criteria because there are very small differences between the energies of these conformers.

2.4. Selection of conformers for further molecular docking on the membrane
We next selected from among the statistically most represented conformers to identify those conformers that are most likely to interact strongly with a phospholipid bilayer. These properties were calculated using the program MAPAS. We then selected the conformers with the highest membrane-contacting scores to examine for docking and possible formation of higher level aggregates at the membrane surface. Figure 3 shows the representative conformer cluster with the highest membrane contacting score.

Our simulation experiments show that aS dimers at the membrane can be divided into two groups. The first group adopts conformations at the membrane surface are energetically favorable for further propagation into ringlike structures (we call these “propagating dimers”). Propagating dimers support the oligomerization with subsequent protein molecules on the membrane. This process continues until a ringlike oligomer structure is organized on the membrane. We want to emphasize that at all steps of oligomerization the process can be stopped by some specific “nonpropagating” docking. In this situation no ringlike structure would be created. The second group does not adopt conformations that present a docking surface with sufficiently low energy of interaction to bind a third aS molecule. These forms do not proceed to form ringlike structures (we call these “nonpropagating dimers”).

We note that docking of unstructured proteins on a lipid bilayer is completely different from docking simulations conducted in solution. First, the bilayer is not homogeneous but has regions of very high and very low polarity that are in close proximity. Second, contact between the bilayer and the protein excludes a significant part of the protein surface from the docking process. A key postulate of our work is that the outcome of a docking event between unstructured proteins is radically different when it occurs simultaneously with a membrane surface than when it occurs in solution. We believe that only through the membrane interaction can “propagating dimers” of aS form. We have already seen that docking to a bilayer excludes a significant part of protein surface from docking with other proteins.

Figure 3. Conformation of aS after 4.5 ns molecular dynamics (green) in the best membrane-contacting position selected by MAPAS (pink – membrane). This conformation had the best score of membranephilicity and has been selected for farther docking experiments on the membrane.

The entire surface facing the membrane on figure 3 is excluded from further docking. It is exclusion of this region that makes possible the formation of propagating dimers, by excluding a region that has a strong tendency to form nonpropagating dimers.
Figure 4 illustrates how the membrane-contacting surfaces of aS are changed during molecular dynamics. Black ellipses enclose the parts of the molecule that contact the membrane. Evolution of these surfaces is conducted through MD runs. Our program assesses each of the snapshots to select the conformers for the largest membrane-contacting surface (that is scored not by the surface of contact but by the surface of contact with the membrane by the specified amino acids). The important point of the proposed mechanism is that the protein has to have sufficient “membranophilic” surface to exist on the membrane for enough time to contact other protein molecules to create a “core” propagating dimer that would have enough energy of interaction with the membrane to start a process of membrane penetration. Oligomerization will continue through the binding of subsequent molecules to this core dimer.

Figure 4. Evolution of aS membrane-contacting surfaces during molecular dynamics.

2.3. Molecular docking and protein oligomerization
A simple schematic representation of the stages of the organization of a pore in the membrane is shown in figure 5. In the initial stages (A) a protein molecule contacts the membrane and undergoes some conformational changes (B,C). These changes are simulated using MD of protein-membrane system. The resulting conformers are used for docking that can be done using various programs, for example, HEX, DOT, the Docking module of Insight (Accelrys), and (D, E). The final simulation is conducted with the ringlike oligomer in the membrane. This system is located in the water box, and unrestrained MD is conducted.
Figure 5. Scheme of pore organization in the membrane by aS molecules: A. Initial approach of aS, B. Contact of aS with the membrane, C. Conformational changes of aS due to this contact, D. Docking of the next aS molecules in the upper layer of the membrane, F. Interaction of aS molecules with the specific regions of phospholipids (red) and cholesterol (green), start of the oligomer penetration to the membrane with the damage of the phospholipids layer in the middle of the ringlike structure, G. Organization of the phospholipids elimination from this region with the pore organization.

After organization into a ringlike oligomer on the membrane, simulations suggest that the aggregated protein starts to embed more deeply into the membrane, eventually penetrating to the other side of the membrane. This provides a clear mechanism for the toxicity of aS in small aggregates (protofibrils) noted above. The ring structures created by aS self-assembly in simulations possess a central opening that would create a pore in the membrane. This could quickly lead to collapse of membrane ion gradients and cell death. For example, the concentration of calcium outside the cell is much higher than inside the cell, so this concentration difference-driven influx will be significant. Calcium can start the caspases-based cell death cascade that leads to the neuron death (a main factor in Parkinson’s disease). An important consideration is whether the process of oligomerization can be separated at very earlier stages. One process may go toward the formation of ring-like structures (protofibrils) and the other toward the large fibrils. The last process may actually be less harmful to the cell than the first one.

From the point of practical application of the abovementioned theoretical studies, it is very important to understand the mechanism of oligomerization and penetration of the oligomer through the membrane. At all stages (schematically shown on the figure 5) the molecular contacts that are responsible for continuation of the pore organization process can be elucidated.
Figure 6. Phospholipids and cholesterol molecules that have direct contact with the αS molecule on the initial stages of penetration (selected from the entire membrane molecular set). The inset shows the entire picture of initial membrane-protein interactions.

Our studies of the αS interactions identified specific putative contacts for αS aggregation/self assembly at the membrane surface. Preliminary results show that interfering with these putative contacts can decrease the aggregation of αS. The results are already being used for drug design.

References

[1] Moore D J, West A B, Dawson W L and Dawson T M 2005 Molecular pathophysiology of Parkinson disease Annu. Rev. Neurosci. 28:55-84

[2] Bertoncini C W, Jung Y S, Fernandez C O, Hoyer W, Griesinger C, Jovin T M and Zweckstetter M 2005 Release of long-range tertiary interactions potentiates aggregation of natively unstructured \{alpha\}-synuclein Proc. Natl. Acad. Sci. USA 102:1430-1435

[3] Sharikov Y, Walker R C, Greenberg J, Kouznetsova V, Nigam S, Miller M A, Masliah E and Tsigelny I 2008 MAPAS: a tool for predicting membrane-contacting protein surfaces Nature|Methods 5:119

[4] Tsigelny I F, et al. 2007 Dynamics of alpha-synuclein aggregation and inhibition of pore-like oligomer development by beta-synuclein FEBS J. 274:1862-1877