Investigation of the Binding Propensity between Alpha-2-Aacroglobulin and Amyloid-Beta Peptide Using Molecular Docking Software

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RESEARCH QUESTION

What is the binding propensity between alpha-2-macroglobulin monomer and amyloid beta monomer?

INTRODUCTION

Energy Landscape Theory

The folding of proteins is an incredibly complex process and still eludes many biochemists to this day. These large bioorganic molecules are flexible and can take the form of various "conformational structures" (Zheng, 2015). Given a 100-residue peptide chain and assuming that there are only two conformations that each residue can form, there are 2^{100}, or 10^{30}, possible conformations of the single molecule (Stefani, 2008). This was called Levanthal's paradox, the idea that it would take an astronomical amount of time for a peptide chain to fold and reach it's final conformational structure. The energy landscape theory was proposed to explain that the native folded structure of proteins is based on energy stability (Onochue, Wolynes, 2004).

The energy landscape represents the free energy of the system. The local minimums of the landscape represent relatively stable structures. In order for peptide chains to consistently fold into the proper, functional, native structure, the native structure must be energetically favorable. Thus, it is assumed that the lowest minimum in the global energy landscape, represents the free energy of the native structure of the protein.

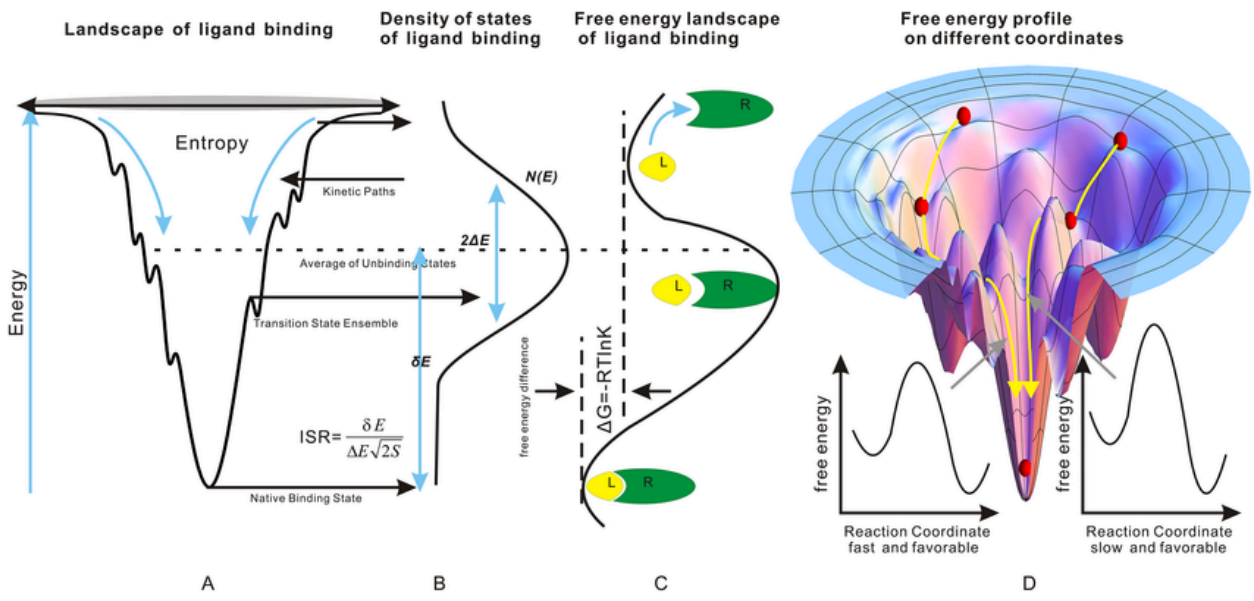

Figure 1. Diagram A is a two-dimensional representation of the free energy binding landscape. Diagram D is a three-dimensional free energy landscape. Obtained from Zheng, 2015.

Amyloid-beta Peptide Structure and Aggregation Mechanism

Amyloid plaque has been identified as one of the physiological manifestations of Alzheimer's disease and is suspected of being one of the pathological molecules causing the disease. The mechanism behind the formation of amyloid plaque is the aggregation of amyloid-beta to form fibrils (Nelson, 2005). The peptides form cross-beta sheets with a hydrogen-bond backbone. Van der Waal forces and London Dispersion forces help to stabilize the amyloid fibril filaments (Nelson, 2005). The high density of hydrogen bonds is key to the rigidity and flexibility of these fibrils and makes clearance difficult. This accumulation of plaque interferes with the synaptic connection between neurons; if neurons do not receive signals, they die. Therefore, there are multiple therapeutic strategies being developed to try to prevent the reduction of signals between neurons. Drugs used to increase dopamine, a common neurotransmitter, are the most prevalent among those therapies; however, these drugs are only

able to slow the symptoms of the progression of Alzheimer's (Kumar et al., 2016). There are currently no effective therapies for prevention let alone cure for Alzheimer's.

Amyloid-beta (a-beta) peptide are a byproduct of the inaccurate cleavage of the APP protein. This a-beta peptide, also called a-beta monomer, exhibits a cross-beta sheet structure (Nelson, 2005).

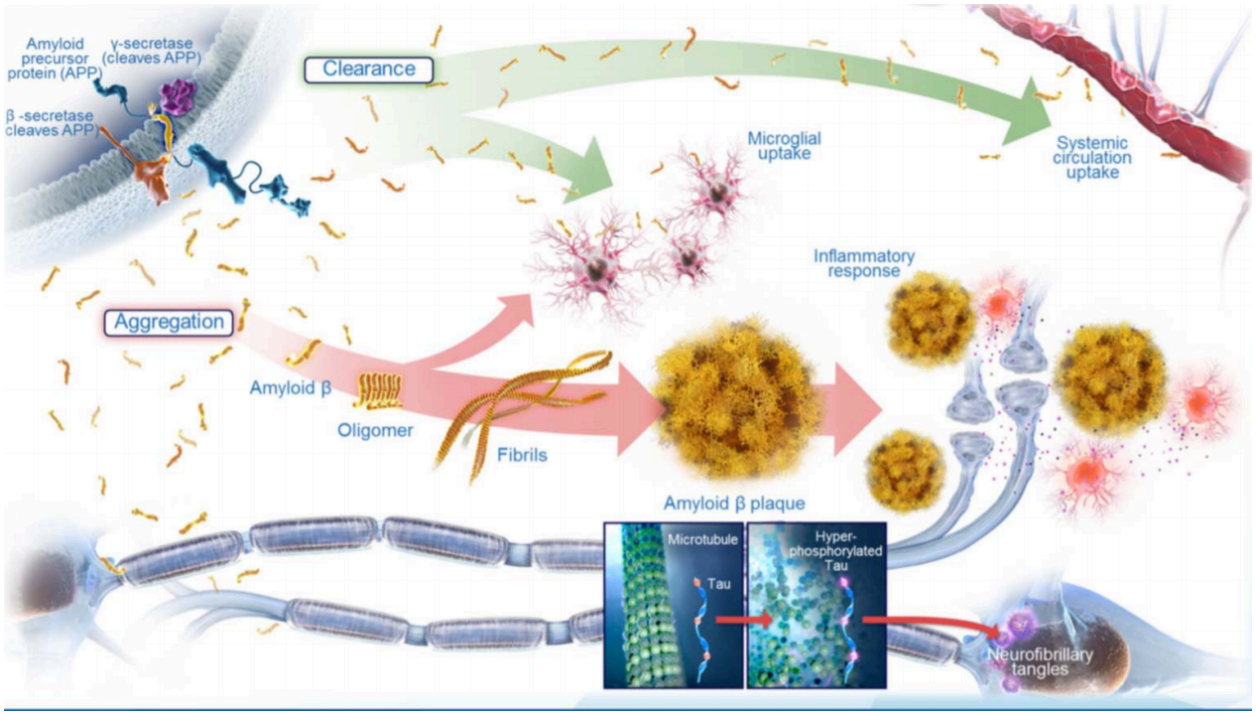

Figure 2. This diagram describes the mechanism behind how amyloid-beta peptides are formed and the pathway they follow within the microbiological environment. The monomers become oligomerized and continue to aggregate into high-ordered fibral structures.

This investigation focuses on the binding interaction between a-beta monomers and alpha-2-macroglobulin, and does not analyze the interaction of oligomers nor fibrillar aggregates. A-beta monomers have been established as being highly cytotoxic due to their interference with various receptor sites within the micro-neuro-environment (Stefani, 2008). They damage multiple ion channels (Margulis et al., 2013). Amyloid fibrils are stable, relatively inert and benign structures (Stefani, 2008). This is due to their comparatively lower active

surface area to interact with or harm other proteins than the smaller monomers (Kim et al., 2016). Recent studies have even established that amyloid fibrils have antimicrobial properties, suggesting that fibrillation may be an evolutionary development to defend against microbial infections (Kagan, 2011).

Protein Misfolding and Molecular Chaperones

Increased pressure, temperature, or changes in pH impact proteostasis, causing the proteins to misfold or unfold (Stefani, 2008). The function of proteins is highly reliant on the structure of the proteins; any deviation will change the structure and ultimately the function of the protein. Proteins are integral for conducting the metabolic processes that keep life going. Any loss or change in function could drastically change quality of life or even lead to death.

As discussed before in the energy landscape theory and how it applies to the folding sequence of a peptide chain, there are numerous conformations. And as seen by the energy landscape graph, there is not one singular energy point to which the entire system converges to. There are other local minimums. The consensus is that these other local minimums represent, misfolded, nonfunctioning, but stable conformational structures of the peptide chain (Stefani, 2008).

Molecular chaperones, also known as chaperonins, are proteins that bind to misfolded proteins and facilitate either the refolding or disaggregation of proteins. Alpha-2-macroglobulin is a tetramer protein that's composed of four basic monomers and has been found to lower concentrations of a-beta deposits by chaperone activity (Cater, 2019).

Chaperones present a novel therapeutic option for Alzheimer's and other degenerative diseases that stem from protein aggregation or misfolded proteins. Most age-related diseases are

due to the misfolding of proteins and the aggregation of these proteins which interfere with biochemical reactions. This method of using molecular chaperones to mitigate misfolded proteins could advance further to an effective treatment method for age related diseases.

Ligand Docking

The calculations for docking ligands utilizes a search algorithm to find what it thinks is the most likely orientation and distance a small molecule, ligand, will bind to a receptor site based on the free energy of the system (Goodsell, 1996). When making these calculations, there is a trade off between speed and accuracy. The most sophisticated algorithms are the ones used for molecular dynamics, which attempts to simulate the entire interaction between the two molecules based on the movement of every atom of the system (Durrant, Mccammon, 2011). However, the accuracy and precision of this process also means that the simulation is incredibly computationally expensive and time consuming. Therefore, docking algorithms seek to streamline the process by providing enough calculations and science to properly elucidate the binding pose of a small molecule within a reasonable amount of time. To do this, the docking algorithm searches the free binding energy landscape in order to determine the conformational pose and location of the ligand on a receptor site. Theoretically, this conformation would be the most stable and therefore most likely to result of the reaction.

AutoDock Vina was used in this investigation to conduct the molecular docking calculations. The algorithm was designed top be a faster, more efficient alternative to the previous version, AutoDock 4.2, by using a stochastic algorithm rather than a genetic algorithm (Trott 2009). The genetic algorithm used by AutoDock 4.2 thoroughly analyzes the entirety of the free binding energy landscape in order to determine the conformational structural complex.

Whereas, the stochastic algorithm reduces the computational load by randomly sampling the energy landscape. The computer then takes "steps" (samples) in the direction where the free energy is most negative, the deepest point in the landscape (Trott, 2009). The stochastic docking algorithm trades accuracy for speed.

For this investigation, the aim was to use the AutoDock Vina program to determine the binding pose of the ligand, a-beta peptide, on the a2m receptor site.

METHODOLOGY

Preparing the Molecule Files for Docking

UCSF Chimera was the software used to graphically visualize the molecules and perform the preparations for the docking calculations; the online documentation was used as reference (Chimer's User Guide). The molecular files were obtained from the Protein Data Bank. These .pdb files contain the all the atoms and their three-dimensional coordinates based on x-ray crystallography and nuclear magnetic resonance studies (RCSB Protein Data Bank). Chimera uses these coordinates to build the three-dimensional molecule.

Once both the ligand and receptor protein files were loaded into the environment, solvent molecules are removed to prevent interference with the docking. The partial charges of hydrocarbon bonds are removed due to the fact that hydrocarbon bonds nearly have no difference in charge. Then incomplete or truncated side chains are fixed. Then hydrogens are added to ensure that the environment has a physiologically reasonable pH. Finally, the charges are assigned to each of the atoms within the ligand and the receptor protein. The files are saved as .pdbqt files (-qt indicates a pdb file with charges). The molecules are then ready to by docked.

In the final preparation steps, the a-beta peptide was designated as the ligand and a2m was designated as the receptor. The “gridbox”, or search volume, was determined based on the literature citation of the a2m receptor site (Jenner, 1998).

As stated before, docking algorithms give up accuracy for speed by making approximations in the calculations and by using a modified search function. In order to reduce the impact of these approximations, the program was set with the highest exhaustiveness setting of eight. The assumption was made that the pose with the lowest free energy would have the most stable binding conformation.

DATA PRESENTATION

Following the docking calculations, AutoDock Vina provided the following table of poses and their calculated free energy and

Poses / Orientation	Score (kcal/mol +/- 2.85 kcal/mol)	RMSD l.b.	RMSD u.b.
1	-6.3	0.000	0.000
2	-6.0	45.384	50.238
3	-5.9	12.057	17.287
4	-5.9	24.491	28.099
5	-5.8	35.341	40.533

Table 1. Vina calculations produced five poses, or positions, of the ligand with the lowest free energy within the search volume.

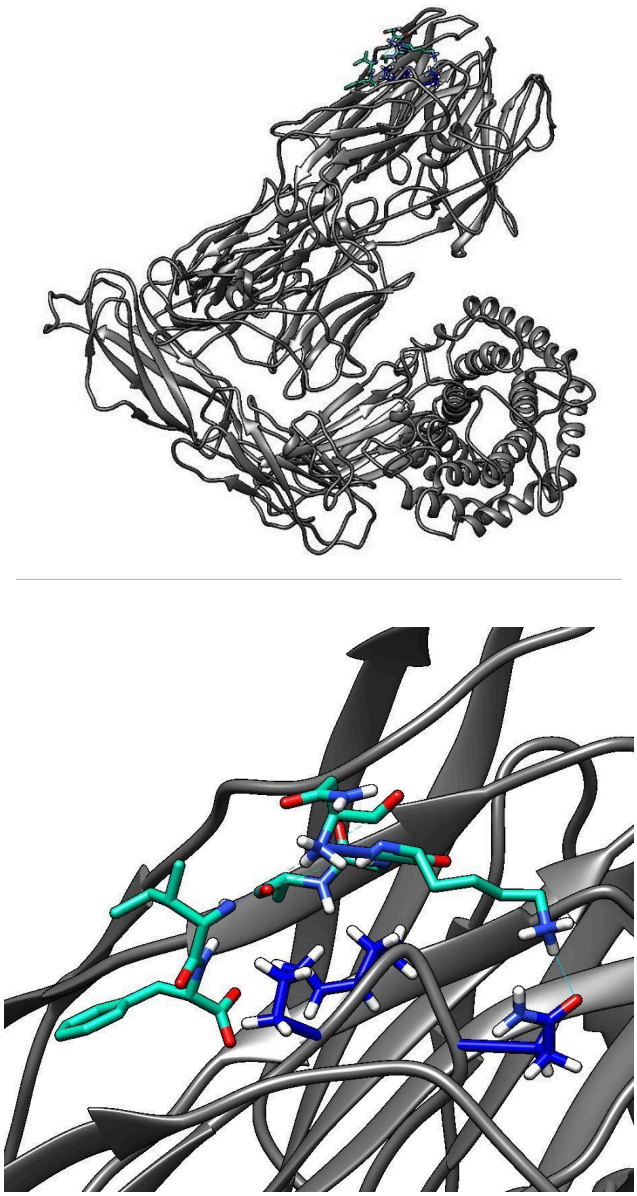

Figure 3. These images are of the a-beta peptide (colored structure) docked onto a2m (grey ribbon model) of pose 1. The image on the right is zoomed out to show the entire final complex. The image on the left is zoomed in to show to peptide chain pose after being docked onto the a2m receptor site.

ANALYSIS

Docking pose 1 was chosen for analysis due to the fact that this pose was calculated to be -6.3 kcal/mol with a standard uncertainty of +/- 2.85 kcal/mol (Trott, 2009).

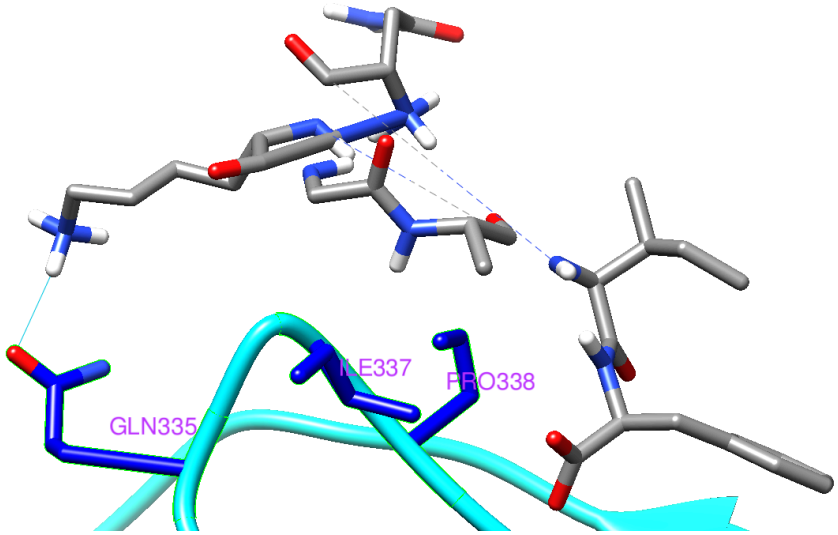

Figure 4. Close up image of docking pose 1. The grey structure is the a-beta peptide chain above while the blue structure on the bottom is a part of a chain of a2m. The dotted lines represent hydrogen bonds. The a-beta peptide is bent due to conformational calculations and manipulations. The side chains of the residues that are directly interacting with a-beta are labeled as follows from the left to the right: GLN335, ILE337, and PRO338. Hydrogens bonded to the carbon chain were removed to provide clearer viewing of the docking interaction.

The docking results suggest that the peptide chain binds to the receptor through direct interactions with the glutamine , isoleucine, and proline side chains. The methyl group on the end of the peptide chain is hydrogen bonding to the glutamine side chain.

DISCUSSION

The residues within 0.2 angstroms were chosen because hydrogen bonds and electrostatic interactions are sensitive to both angle and distance. Thus the side chains that would interact with the greatest electrostatic attraction would be those closest to the ligand. The massive uncertainty was due to the general accuracy of AutoDock Vina.

EVALUATION

There were many limitations to the design. The ligand was not modified to have rotatable bonds and therefore was not considered flexible. The high uncertainty of the binding energy was due to the high inaccuracy of the program. Despite the high inaccuracy, the low RMSD value suggested that the conformational change in the molecular structures was lowest for pose 1. Although the free binding energy cannot reliably be determined, the binding pose determined by the program is likely to be the actual binding conformation.

Improvements

Given more time or more computational resources, the following paths can be taken to further investigate the function and structure of a2m: molecular dynamic simulations, virtual screening of possible bindings agents, fragment-growth based method of drug development,

The docking algorithm is limited in the fact that it excludes solvents and other ions. Water, ions, and other proteins exist in the microenvironment of the cell. Additionally, docking analyses only exhibit a frozen conformational complex between the ligand and receptor. In reality, the ligand and receptor would be flexible and the structures would be fluctuating moment to moment as the distribution of charges changes.

Future analyses of the interaction between a2m and a-beta will be conducted using molecular dynamic software. Molecular dynamic are constructed with more sophisticated algorithms and render the bonding in real time. Molecular dynamic simulations are also capable of treating the structures as flexible, including all the possible torsion angles of both the ligand and receptors.

Another path for further investigation is the use of virtual screening where libraries of ligand are screened, docked and ranked based on the stability of their conformations. This creates a set of possible molecules that can realistically bind to the receptor site.

CONCLUSION

- Based on the pose with the greatest free energy and smallest deviation which was calculated AutoDock Vina program, amyloid-beta peptide binds to alpha-2-macroglobulin by the following residues: GLN335, ILE337, and PRO338.
- Further investigation
    - Flexibility in the ligand and receptor will be considered by including rotatable bonds.
    - Molecular dynamic software will be considered to develop a more accurate depiction of the active interaction between the a-beta peptide chain and the a2m receptor.
    - Virtual screening will be performed to find the molecule with the greatest conformational bind to a-beta peptide.

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