Molecular Mechanism of β-amyloid Inhibition By Inositol

by

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Contents

Chapter 1

Introduction

1.1 Amyloid disorders

Many diseases, most of them neurodegenerative, have been linked with the presence of amyloid: Prion-related diseases, Parkinson's, Huntington's disease, Type II diabetes etc.

1.1.1 Alzheimer's Disease

1. More than a hundred years have pass since Dr. Alois Alzheimer first presented the connection between the presence of neuronal plaques and the clinical symptoms of presenile dementia characteristic of Alzheimer's disease (AD).

2. Today, AD is known to be the most common cause of dementia in persons of age 65 or older. With the increasing longevity of our population, AD is approaching epidemic proportions with no cure or preventative therapy available.[1]

3. The discovery of amyloid plaque deposits in brains of deceased dementia patients led to the formulation of the amyloid hypothesis, which posits that amyloid aggregates initiates the pathogenesis of AD and that the other pathological symptoms such as neurofibrillary tangles are secondary.

1.2 Amyloid: formation and mechanism of toxicity

1. Finding a treatment for AD and other fatal neurodegenerative diseases motivated many biochemical and biophysical studies of the amyloid state.

2. In vitro, a variety of proteins and peptides, folded or intrinsically disordered, have been shown to be able to aggregate to form amyloid fibrils under certain solution conditions. Currently, it is thought that amyloid fibrillar state may be the globally stable folded state for all proteins.

3. Amyloid fibrils are formed via a complex aggregation pathway. Initially, monomers aggregate to form soluble oligomers which exists in equilibrium with amyloid fibrils. Some of these oligomers are on-pathway to fibril formation, while others themselves may be end-points of the aggregation pathway. Amyloid fibrils, typically the visible

endpoint of aggregation, have a characteristic cross-β, while oligomers may be structurally disordered and are morphologically distinct from fibrils.

1.2.1 Structure of Fibril

- Decades after the initial discovery of plaque deposition in dementia patients, Aβ, the central protein component of neuronal plaques, was synthetically produced in the laboratory. In vitro, Aβ42 precipitates out of solution almost immediately.
  - Describe the molecular structure of Aβ amyloid fibrils
    - Define the cross-β structure [Show a schematic here].
  - Briefly mention the techniques that can be used to obtain structural information of amyloid fibrils.

1.2.2 Structure of Non-fibrillar oligomers

Due to their structural disorder and their insolubility, structural determination of oligomers have posed challenging experimentally.

1.2.3 Kinetics

Amyloid fibrils have been observed to form via a two-step nucleation-polymerization process. In the nucleation phase, energetic barriers of aggregation must be overcome to form the initial aggregation nucleus or seed. Following nucleation, free monomers bind to the nucleated aggregates and polymerize into mature fibrils.[2]

1.2.4 Toxicity

- Multiple lines of research have identified oligomers as a likely causative agent for neuronal cell death. By contrast, the monomeric and fibril forms are thought to be less toxic than oligomers. It is hypothesized that soluble oligomers may cause toxicity by perturbing the integrity of cellular membranes through binding and disrupting the lipid bilayer (perhaps by making them ion permeable).[3]
- Include a paragraph about amyloid formation and lipid membranes (?)

1.3 Amyloid Inhibition: A promising treatment for amyloid disorders

- In this section, I will provide an overview of some of the challenges to overcome when developing a small molecule therapeutic for Alzheimer's disease. Furthermore, using this information, I will motivate why inositol is an exciting avenue to explore.
  - scyllo-Inositol is able to cross the blood brain barrier. It has high bioavailability. Because it is not broken down in the gut, it can be taken orally.
  - Inositol is not toxic to the human body. Inositol is used in signaling pathways.

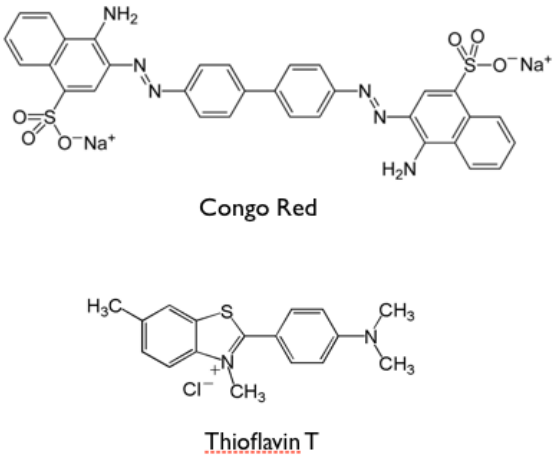

Figure 1.1: Amyloid binding dyes Congo Red and Thioflavin T

- Briefly mention non-small molecule putative therapies which also acts via amyloid inhibition. The focus of this thesis will be on small-molecule amyloid inhibition.

1.3.1 Molecular mechanisms of amyloid inhibition by small molecules

1. Amyloid inhibition as a treatment for Alzheimer's disease and related amyloid disorders. Amyloids are attractive drug targets. Small molecules may be one effective way to develop a treatment for amyloid disorders because they have the potential to be able to treat the underlying disease. Through in vitro screening, many small molecules have been found to effect the amyloid aggregation pathway. Some were demonstrated to inhibit amyloid fibrils, where as others were shown to arrest or reduce oligomer formation.

   (a) Pharmacological perspective of the challenge of developing an Alzheimer's drug. In order to effectively treat Alzheimer's and other neurodegenerative diseases, small molecule drug candidates must pass the blood brain barrier at sufficient concentrations for inhibition. This is difficult to achieve.

   (b) In vitro screening has led to the discovery of a large number of small-molecules which were found to affect the amyloid aggregation pathway. Many of these small molecules are thought to act by directly binding to amyloidogenic peptides and aggregates.

      i. Thioflavin T and Congo red are dye molecules used to identify the presence of amyloid. Both molecules bind to mature amyloid fibrils and have been shown to affect fibril formation.(Fig. 1.1)

      ii. Polyphenols, is a large group of natural and synthetic molecules. (−)-epigallocatechin-3-gallate, curcumin, and a polyphenolic grape seed extract, known for their anti-oxidant properties, were recently discovered to be capable of affecting amyloid formation.(Fig. 1.2)

   (c) Small molecule inhibitors share common chemical features and groups. They are typically planar in geometry, have many aromatic rings, and polar functional

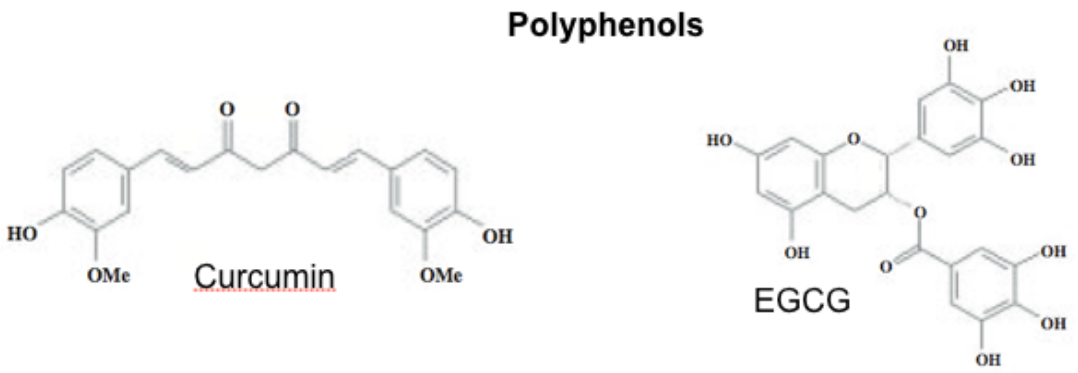

Figure 1.2: Polyphenols

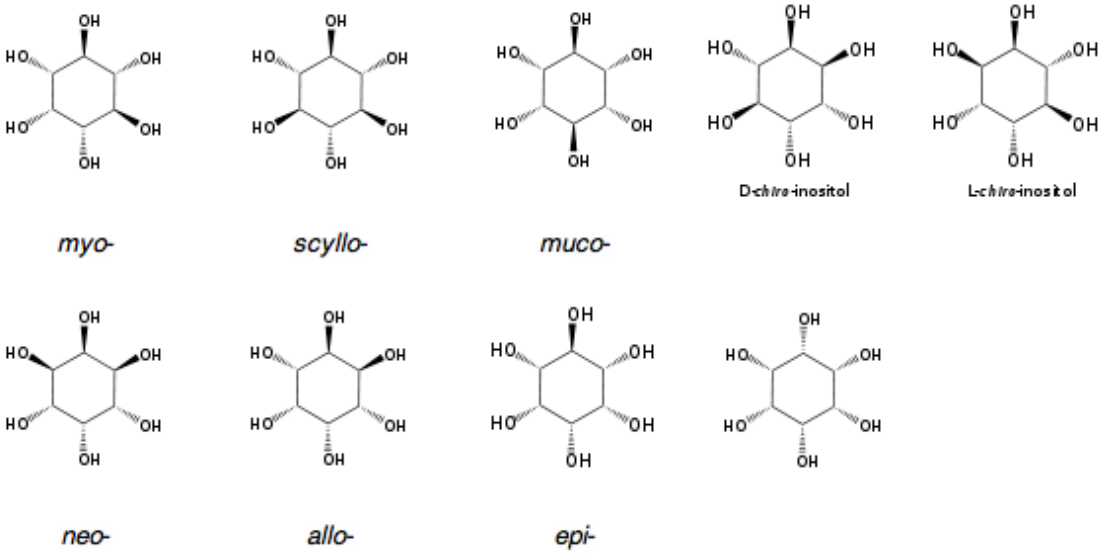

Figure 1.3: Inositol stereoisomers

groups (hydroxyl groups) around the edge of these aromatic rings.

(d) Mechanism of action. Some small molecules inhibit fibril formation, where as others may prevent oligomerization, but not fibrillation. A high concentration is often required to observe activity (micromolar to millimolar), which suggests that they may be non-specific inhibitors. EGCG, one such polyphenol, is known to have the lowest IC50.

i. Molecular mechanism of binding of dye molecules. Thought to bind flat on on the surface grooves of amyloid fibrils where they interact with hydrophobic groups exposed at the surface.

1.3.2 Inositol molecules

(e) Inositol stereoisomers.(Fig. 1.3) Role of inositol in the human body.

i. *myo*-inositol. Use the physiological role of myo-inositol as a lead to transition into the

(f) Where is inositol found. Present in human body tissues. Myo- is present in certain grains, grape fruit, but scyllo- is only found in small quantities in food sources.

(g) Role of inositol in amyloid inhibition. Include the background on how inositol was discovered as an amyloid fibril inhibitor.

i. In vitro and In vivo studies (mouse)

ii. Include some data on human clinical trials (?)

1.3.3 Analogy to Sugar-protein binding

Experimental techniques to study sugar-binding modes

Sugar Binding modes

1.4 Protein-ligand interactions

1.4.1 Forces involved in binding

- Protein-ligand non-covalent interactions that are important for ligand binding and recognition
  - Electrostatic interactions. Polar (hydrogen bonding) and charge-charge interactions
  - Nonpolar (hydrophobic) interactions
    - Van der Waals

1.4.2 Binding equilibria

- Enzyme and its putative ligand typically bind specifically (high affinity binding). We want to optimize binding specificity to increase the efficacy of the putative drug, and decrease adverse side effects (toxicity) in the human body.
- The dissociation constant, K_d, is a measure of the affinity of a ligand for its binding site on the host protein. Pharmacologically, it can be interpreted as the concentration at which 50% of the drug is bound to the protein. In experimental studies, K_d is often used to quantitatively screen for potential drug candidates.
- Binding equilibrium

$$[Protein \cdot Inositol] \rightleftharpoons [Protein] + [Inositol] \tag{1.1}$$

- The binding free energy of a ligand to a protein is directly related to its dissociation constant, K_d, the equilibrium constant of the above reaction

$$K_d = f_{ub} \frac{[Protein]\,[Inositol]}{[Protein \cdot Inositol]}, \tag{1.2}$$

- Experimental techniques for estimating K_d

- What experimental techniques are used to estimate binding affinity? (May need to study up on this)
- Isothermal titration calorimetry (ITC) is a technique which can be used to measure energetics of ligand binding to peptides.

Bibliography

[1] Kaj Blennow, Mony J De Leon, and Henrik Zetterberg. Alzheimer's disease. *Seminar*, 368:387–403, 2006.

[2] Regina M Murphy. Peptide aggregation in neurodegenerative disease. *Annu Rev Biomed Eng*, 4:155–174, 2002.

[3] Dominic M Walsh and Dennis J Selkoe. A beta oligomers - a decade of discovery. *J Neurochem*, 101(5):1172–1184, 2007.

Chapter 2

Methods

- Molecular dynamics simulations are a useful tool to study the structure, dynamics, and interaction of biomolecules. MD simulations employ an empirical mathematical function to describe the atomic interactions in a molecular system, and together with classical laws of Newtonian mechanics, atomic trajectories of motion are generated. Thermodynamic and kinetic properties can then be extracted as time averages from these trajectories and used to make a number of predictions that are often experimentally challenging to observe or measure.

- MD simulation studies have been useful in studying many existing fundamental problems of biology and biochemistry, including protein dynamics and function, protein folding, biomolecular self-aggregation, and protein-ligand binding.

2.1 Methodological Details

- Why is MD correct? Describe the fundamental assumptions of MD. Here, I want to give the readers who aren't familiar with the methodological details of MD a sense of the rigorousness of MD.

- The Born-Oppenheimer approximation: electronic and nuclear motions are uncoupled, and therefore can be treated separately.

- MD does not account for the movement of electrons. Although electrons are not taken into account in MD simulations, their presence is implicitly accounted for via the use of potential energy functions. Atomic nuclei can be treated as classical particles.

- Application of an empirical force field can be used approximate atomic interactions in the system. A force field typically has many parameters which need to be calculated. One approach to do this is to fit to quantum mechanical calculations. Often, force fields are iteratively improved by predicting experimentally observable quantities for small compounds, and adjusting the fit based on comparisons of these computationally predicted quantities with experimental measurements.

- There many different force fields (AMBER, Gromos etc), each differing slightly in the potential energy function and its parameterization. In this thesis we performed all of our simulations using the force field OPLS-AA/L.

- Force field potential energy function

$$\begin{aligned} E = \sum_{bonds} k_b(b-b_0)^2 + \sum_{angles} k_\theta(\theta-\theta_0)^2 \\ + \sum_{dihedrals} k_\chi(1+cos(n\chi-\delta)) + \sum_{impropers} k_\gamma(\phi-\phi_0)^2 \\ + \sum_{nonbonded} \frac{q_1 q_2}{er} \\ + \sum_{nonbonded} \epsilon[(\frac{r_{min}}{r})^{12} - 2(\frac{r_{min}}{r})^6] \end{aligned} \tag{2.1}$$

2.2 Challenges and limitations of MD simulations

- MD is computationally challenging because of limitations in length and time scales.
  - Length scale. Large systems are too complex to obtain statistics and quantitative predictions.
  - Time scale. Relevant biochemical reactions such as protein folding happens on the time scale of milliseconds, hours, and days. Currently with MD simulations, we are routinely able to approach the microsecond time scale, massive computing power is still insufficient to observe phenomena on the millisecond timescale.
  - Obtaining convergence. Explain why this is difficult, in particular for systems with disordered peptides.
- Limitations in the accuracy of current force fields.

2.3 Application of MD: structure-based drug discovery

- A broad application of simulations of proteins is to computationally design drugs and combining that with structure-based drug design. In recent years structure-based computer modeling of protein-ligand interactions have become a core component of modern drug discovery.
- Current drug discovery platform. Typically, the first step in drug discovery is to identifying a target, a putative binding site. Then, solve the X-ray crystal structure of the target.
- Ligands which may act as potential drugs typically have high binding affinity to the binding site. The goal is to find high specificity inhibitor of a protein (usually an enzyme). The binding free energy is an important quantity which can be used to evaluate how well a ligand binds. One method of estimating binding affinity is by using computational docking methods, where the binding affinity is typically estimated without taking into account of protein dynamics. Although docking is computationally less expensive, it is inaccurate for identifying true drug candidates because binding often involves crucial changes in protein conformation.

- With computer hardware becoming faster and cheaper, MD simulation and modeling can be used to rapidly prototype experimental ideas – for example, one can perform computational alchemy, that is, "mutate" residues to test various hypotheses. Furthermore, simulations may be used to determine whether a chemical change will produce a more potent drug candidate.

- Currently state of the art computational binding studies take into the account of change in protein conformation. MD simulations is an effective method, where the protein and drug is allowed to relax and freely move about in the system.

- However, in the case of understanding a specific binding reaction (eg. when developing an enzyme inhibitor), the ability to observe the relevant binding events is a low probability event on the timescale achievable in our simulations. Therefore, it is impractical in this case to solely apply brute-force sampling techniques to determine binding free energies.

  - Methods used to determine binding free energies using simulations:
    * Thermodynamic perturbation[1]
      · thermodynamic integration
      · free energy perturbation

2.4 Review of MD studies of amyloid inhibition by small molecules

- In recent years, molecular dynamics simulations have been intensively used to investigate the molecular basis of the structure and stability of amyloid fibrils.

- MD simulations of Congo red binding have been done with the protofibril-like crystal structure composed of the segment GNNQQNY.{Wu, 2007 #621}

- A recent simulation study of an N-methylated peptide with Aβ16-22 models of amyloid aggregates has provided insight into the possible mechanism of action of peptide inhibitors of amyloid formation.{Soto, 2007 #597} This peptide inhibitor was shown to preferentially bind monomers to form dimers, possibly acting to inhibit fibril formation by sequestering monomers. However, peptide-based inhibitors have poor pharmacological profiles as they are actively broken down by proteases in the stomach and are difficult to transport across the blood-brain barrier. In addition, these peptide inhibitors specifically target Aβ and thus do not have the potential to treat multiple amyloid diseases.

2.5 Thesis objectives and rationale

2.5.1 Challenges of amyloid inhibition

- The protein-ligand binding model developed to understand enzyme inhibition cannot be directly applied to understand the molecular mechanism of amyloid inhibition by small molecules.

  - Amyloid inhibitors are found to be very weak binders. How do non-specific inhibitors act as a drug? And how do we approach this with MD simulations?
  - Because the Aβ amyloid aggregate pathway encompasses a variety of species, some of which has no folded structure, a single conformation cannot be assumed for binding. Furthermore, structural information of amyloidogenic species lags behind those of enzymes, which tends to be globular proteins amenable for X-ray crystallography. This means that the putative binding sites are not known.
  - The structural disorder of the peptides involved poses a challenge for obtaining converged properties from MD simulations.

- Aβ peptides are completely disordered. We also do not know what the binding site looks like, where it is located on these structures.
- To date, few studies have attempted to provide statistically meaningful results pertaining to general mechanisms of protein self-aggregation and amyloid formation. Furthermore, despite the abundance of MD studies of Aβ, few studies have systematically examined the mechanism of action of small molecule inhibitors of amyloids
- In AD, there is the added challenge of the drug being able to cross the brain barrier, while remaining non-neurotoxic. What kind of drugs cross the BBB? Typically hydrophobic drugs.

2.5.2 Study Design and Rationale

- Here describe in detail how I designed my study to circumvent the challenges presented by the amyloid inhibition problem, and the limitations of MD simulations. At this point, clearly explain and discuss my study design and rationale. (Fig. 2.1)
- Beginning with the simplest model systems for an amyloidogenic peptide, the alanine dipeptide, we systematically examine binding of inositol with systems of both increasing sequence and structural complexity.
- We exploit conventional MD simulation techniques because simulation approaches used for understanding enzyme-ligand binding is not applicable.
- Instead, we use conventional MD simulations and repeats of independent simulations to determine the binding modes, and binding equilibria of inositol with amyloidogenic peptides and aggregates of Aβ.

2.6 Thesis Organization

The first chapter introduces the thesis in the context of the field. The second chapter introduces the main methods used in the work in the thesis. Chapters 3, 4, and 5 are the results of simulations of inositol with amyloid like peptides and aggregates. Chapter 6 shows work of the general applicability of our methods developed throughout this thesis to MD simulations to understand protein - carbohydrate binding. Chapter 7 provides discussion, suggestions for future work, and perspectives.

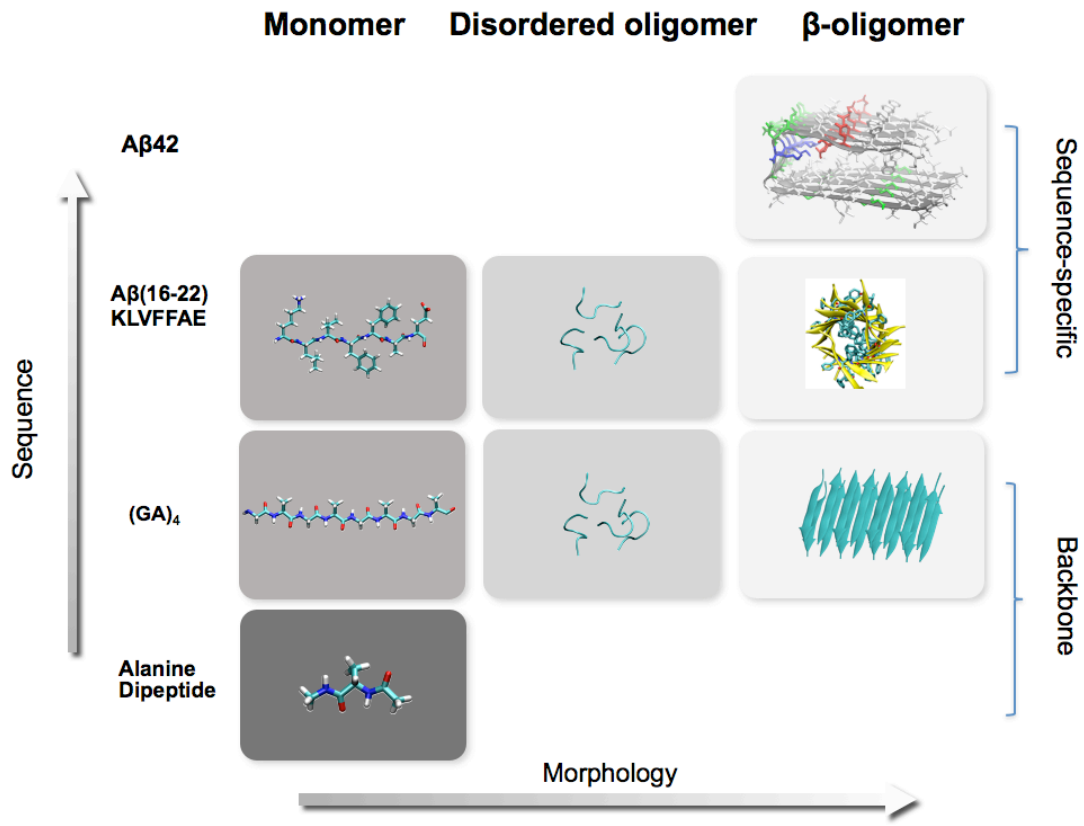

Figure 2.1: Shows the progression from small, model systems to larger and structurally more complex systems involving the full-length Aβ42 peptide.

Bibliography

[1] Michael K Gilson and Huan-Xiang Zhou. Calculation of protein-ligand binding affinities. *Annual review of biophysics and biomolecular structure*, 36:21–42, 2007.