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Natural polyphenols as inhibitors of amyloid aggregation. Molecular dynamics study of GNNQQNY heptapeptide decamer

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ABSTRACT

Amyloid-like fibrils had been associated with many fatal diseases, and the rational design of the fibrillization inhibitors holds the great promise of finding the prevention and treatment options. The understanding of the mechanisms by which the small molecules can inhibit the aggregation plays the key role in such design. Here we present the results of MD simulations that provide the atomistic details of the process, by which the small molecules may destabilize the ordered amyloid oligomers formed by the model hexapeptide. We select a heptapeptide fragment (GNNQQNY) from Sup-35 yeast prion protein, which is capable to form both amyloid fibrils and microcrystals. Atomic-resolution structures of its crystals were reported by Eisenberg et al. (Nature 447:453, 2007). We analyze several MD trajectories describing the evolution of the decamer fragment taken from this crystal structure, both by itself and in the presence of myricetin and kaempferol (two naturally occurring polyphenols, found to be strong and weak aggregation inhibitors). While the decamer of GNNQQNY demonstrates remarkable stability of its structure after 2 ns simulation, myricetin disturbs the aggregation. The simulations show myricetin interacts with the β-sheet due to polar interactions with side chains of the peptide weakening the interstrand hydrogen bonds, wrapping the β-sheet and disaggregating the outer layer. Both backbone to backbone and side chain to side chain hydrogen bonds are lost, and the β-sheets are moving away from each other. This leads to the loss of backbone H-bonding and eventual separation of one β-strands from the outer layer. We also test several AMBER force fields and implicit solvent models for their ability to keep the decamer of GNNQQNY aggregated. The RMSDs of decamer of GNNQQNY with force field 99SB and implicit solvent models of igb2 and igb5, were maintained at less than 4 Å.

1. Introduction

Many proteins, when placed in appropriate conditions, can misfold and aggregate into a fibril agglomerates, called amyloids. These fibrils were found to have characteristic X-ray diffraction pattern and are often identified by staining with Congo red [1,2]. Amyloid-like fibrils had been associated with many fatal diseases, including Alzheimer's disease, type II diabetes mellitus, and the transmissible spongiform encephalopathies, and prion diseases [3], which affect millions of people throughout the world. The mature fibrils, and, more importantly, soluble oligomers had been recently shown to have a key role in the cytotoxic nature of amyloidogenic proteins [4]. Currently, there are no approved therapeutic agents directed against the formation of fibrillar assemblies. Over the past few years, significant research effort was made toward developing of therapeutics and chemical probes that inhibit these specific protein–protein interactions [5,6]. Apart from small molecules, a number of peptidomimetic aggregation inhibitors have been developed, based on the core fragments of amyloid peptides. Many of these inhibitors are assumed to interact with the protofibrillar and fibrillar species through formation of the main-chain β-sheet hydrogen bonds. Presumably, their inhibition mechanism is based on capping the β-sheet extension. In contrast, small molecule non-peptidic inhibitors have also been studied, including small aromatic molecules such as phenol red, polyphenols etc. These non-peptidic molecules might exhibit different interaction patterns and inhibition mechanisms compared to the peptidomimetic inhibitors. Study on the difference of the two types of inhibitors should provide more insight into the amyloid inhibition mechanisms leading to rational design of inhibitors [6]. Molecular simulations are becoming an important part of the rational design strategy. They are based on a model representing the amyloid and involve analysis of MD trajectories.

Decades of investigations of the structural properties of amyloids have established that all the fibrils have a common structural motif, called cross-β spine that consists of the β-sheets parallel to the fibril

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axis, with their extended protein strands perpendicular to the axis [7]. Due to the non-crystalline and insoluble nature of the amyloid fibril, it is difficult to obtain atomic-resolution structures with traditional experimental methods. However, some short peptides are capable to form both fibrils and microcrystals that are sometimes found together in solution [8]. Recently, several crystal structures of a hexa- and heptapeptides, identified as fibril-forming segments of the known pathological proteins were reported [2]. These microcrystals have similar diffraction patters to the fibrils and are capable to serve as nucleation sites for the fibril growth. These evidence indicate that atomic-resolution structures of these crystals make a good model for amyloids and can be used to investigate the mechanism of amyloid formation and disaggregation by molecular modeling methods.

One of the crystal structures determined in the work [2] was that of the heptapeptide fragment (GNNQQNY) from Sup-35 yeast prion protein. This short fragment displays many of the common characteristics as a full-length Sup-35, including cooperative kinetics of aggregation, fibril formation, and binding of the dye Congo red [8]. Molecular dynamics simulations for the pairs of β-sheets containing 3 to 50 strands of the heptapeptide GNNQQNY cut out from the crystal structure indicate that while the cross-β spine architecture is preserved, the β-sheets are twisted in the absence of the crystal packing [9]. The pair of β-sheets taken from GNNQQNY crystal structure is shown on Fig. 1. This fragment demonstrates the cross-β spine architecture with the β-sheets separated by a dry, non-hydrated interface. Each β sheet is formed by parallel strands. In addition to the classical hydrogen bonds between backbone atoms, the β sheets are stabilized by hydrogen bonds between the matching polar side chains (so-called the polar zipper). At the dry interface, the side chains of residues N2, Q4 and N6 are also tightly interdigitated with the corresponding residues of the opposing sheet (structure is known as steric zipper) [2,8].

Fragments of GNNQQNY crystal structure present an attractive target for theoretical studies of aggregation and disaggregation. A number of theoretical works explored the early aggregation behavior of the GNNQQNY sequence [10–12]. Zheng et al. [12] performed simulations of various oligomers of the original peptide and its mutants to study its stability and dynamics in the explicit solvent. Different sizes and arrangements of the aggregates were studied. The results showed that the stability of the oligomer increased dramatically with the increase in the number of strands, suggesting that the even a small nucleus can serve as a seed for the fibril formation. The

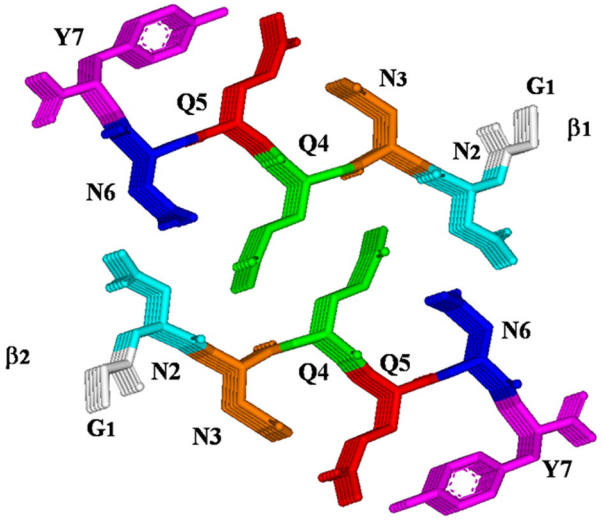


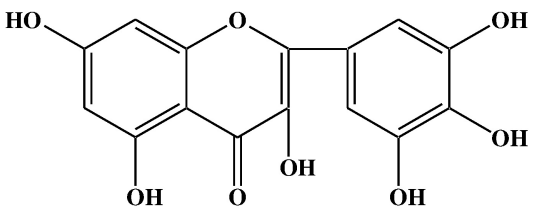
Fig. 1. The atomic presentation of the GNNQQNY oligomer; sandwich of 2 beta sheets formed by 5 heptapeptides each. Intersheet steric zipper is formed between the side chain of the residues Asn-2, Gln-4 and Asn-6 of the β1 sheet and those of the same residue of β1 sheet.

MD simulations also show that within sheet, the driving forces to associate and stabilize are interstrand backbone–backbone and side chain–side chain hydrogen bonds, whereas between sheets, shape complementary by dry polar steric zipper via the side chains of Asn-2, Gln-4, and Asn-6 holds the sheets together. Moreover, mutant simulations showed that the correct geometrical matching of the side chains via intersheet interaction plays an important role in determining the stability of oligomers. In another study Gsponer et al. [13] found that the parallel β sheet arrangement is favored over the anti-parallel one because of stacking interactions of the tyrosine rings and hydrogen bonds between amide groups. The disaggregation mechanisms of the GNNQQNY fibrils and its aggregation pathways were investigated by high-temperature molecular dynamics simulation in explicit solvent by Wang et al. [14]. Hexamer and dodecamer models, both with two parallel β-sheets separated by a dry side chain interface were adopted in their computational analysis. Landscape and kinetics analyses also indicate that the parallel β-sheets form earlier than the dry side chain contacts during aggregation.

Apart from the understanding the amyloid fibril formation, study of the small aggregates may help to understand the role of oligomers in amyloid diseases. There are several hypotheses suggesting different mechanisms for amyloid toxicity [15,16]. Some recent experimental results focus the attention on soluble oligomeric aggregates, which were shown to be more toxic than fully formed mature amyloid fibrils [17,18]. The mounting body of evidence reveals a strong correlation between the cognitive deficit in mice and the amount of soluble oligomers assembled from the monomeric constituent peptides [19–21]. Compounds that inhibit aggregation, fibrillization, and/or plaque formation may be capable to reduce this toxicity and thus display therapeutic effect. Various small molecule aggregation inhibitors have been recently discovered [16]. The well known example is Congo red, a sulfonated dye that specifically binds to amyloids and is commonly used histological dye for amyloid detection. It was reported to inhibit fibrillization and neurotoxicity of Aβ [15]. Unfortunately, this dye cannot cross the blood-brain barrier and is carcinogenic if given orally, thereby hindering its therapeutic use [15]. Natural polyphenolic compounds found in teas, berries, fruits, spices, and plants were known to have antioxidative, and anti-inflammatory activities. Recently their significant effects on fibril formation, nucleus formation/extension and disaggregation were also discovered [22–24]. In this study we focus on myricetin (Scheme 1), which was reported to be the most potent among the polyphenolic group of compounds.

There have been several theoretical attempts to study the interactions between current inhibitors and oligomers at the atomic level [21,25–27]. Using all-atom molecular dynamics simulations with explicit solvation model, Wu et al. [26] have identified and characterized two specific binding modes of Congo red molecules to protofibrils formed by GNNQQNY. Two potential inhibition mechanisms of disrupting β-sheet stacking were identified and suggested to use for the development of non-peptidic amyloid-specific inhibitors.

Convertino et al. [25] used MD with implicit solvent to investigate the influence of two planar aromatic compounds, 9,10-anthraquinone (AQ) and anthracene (AC), on the early phase of the aggregation of the Aβ heptapeptide segment H14QKLVFF20, the hydrophobic stretch that promotes the Aβ self-assembly. The simulations show that AQ interferes with β-sheet formation more than AC. In particular, AQ



Scheme 1. Structure of myricetin.