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Effect of D23N mutation on the dimer conformation of amyloid β-proteins: *Ab initio* molecular simulations in water

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ABSTRACT

The molecular pathogenesis of Alzheimer's disease (AD) is deeply involved in aggregations of amyloid β-proteins (Aβ) in a diseased brain. The recent experimental studies indicated that the mutation of Asp23 by Asn (D23N) within the coding sequence of Aβ increases the risk for the pathogeny of cerebral amyloid angiopathy and early-onset familial ADs. Fibrils of the D23N mutated Aβs can form both parallel and antiparallel structures, and the parallel one is considered to be associated with the pathogeny. However, the structure and the aggregation mechanism of the mutated Aβ fibrils are not elucidated at atomic and electronic levels. We here investigated solvated structures of the two types of Aβ dimers, each of which is composed of the wild-type or the D23N mutated Aβ, using classical molecular mechanics and *ab initio* fragment molecular orbital (FMO) methods, in order to reveal the effect of the D23N mutation on the structure of Aβ dimer as well as the specific interactions between the Aβ monomers. The results elucidate that the effect of the D23N mutation is significant for the parallel structure of Aβ dimer and that the solvating water molecules around the Aβ dimer have significant contribution to the stability of Aβ dimer.

1. Introduction

Aggregations of amyloid β-proteins (Aβ) have been considered to play a key role in the mechanism of molecular pathogenesis of Alzheimer's disease (AD), which is a serious dementia, accompanied by senile plaques in a diseased brain [1]. The proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases produces Aβs [2], and the γ-secretase cleave APP at its several alternative sites to produces Aβs of varied lengths. The most abundant Aβs contained in the senile plaques are Aβ40 and Aβ42 [2,3], each of which has 40 or 42 amino acid residues, respectively. Aβ42 was found to aggregate more rapidly and comprise a major component of the senile plaques in a diseased brain [4–6]. These plaques cause a death of neuronal cells in a diseased brain [7]. Since Aβ peptides have several hydrophobic amino acid residues, Aβs form strong aggregates in water due to the hydrophobic interactions between these residues, leading to a fibril formation of Aβs [8]. Accordingly, it is expected that compounds having a strong binding affinity to Aβ can inhibit the Aβ aggregation and be a potent inhibitor for the amyloidogenesis and pathogenesis of AD.

With respect to the fibril structure of the wild-type Aβs, numerous structural studies [9–20] based on solid-state nuclear magnetic resonance (SSNMR) or electron paramagnetic resonance (EPR) measurement have revealed that the most of the fibrils are stabilized in a common conformation with parallel β-sheets. In contrast, the recent SSNMR measurements on polymorphic samples of fibrils formed by the Asp23-to-Asn (D23N), or Iowa mutant of Aβ (D23N-Aβ) have revealed that a large fraction of the D23N-Aβ fibrils contain antiparallel β-sheets [21,22]. The D23N mutation causes a familial, early-onset neurodegeneration involving extensive cerebral amyloid angiopathy [23]. It is thus likely that the antiparallel β-sheets of the D23N-Aβ fibrils exert distinct pathogenic effects.

The experimental studies [21,24] based on electron microscopy, X-ray diffraction and SSNMR spectroscopy have revealed that the D23N-Aβ40 forms fibrils considerably faster than the wild-type Aβ40 (WT-Aβ40) without a lag phase. In addition, SSNMR measurements indicate that only a minority of the D23N-Aβ40 fibrils contains the parallel β-sheet structure commonly found in the WT-Aβ40 fibrils, while the majority of the D23N-Aβ40 fibrils have the antiparallel β-sheet structures. These antiparallel structures were found to be thermodynamically metastable with respect to conversion to the parallel structure under typical conditions [22]. However, it is not elucidated yet why only the D23N-Aβ40 fibril forms the antiparallel β-sheet structure.

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In addition, the reason why the WT-Aβ40 fibrils have only the parallel β-sheet structure is not clarified yet. In the SSNMR analysis for the WT-Aβ40 fibrils [22], it is predicted that the electrostatic interaction between Asp23 of a certain Aβ and Lys28 of the neighboring Aβ can stabilize a parallel conformations of fibrils (PDB ID: 2BEG [25] and 2LMN [26]). In contrast, in the antiparallel conformation, since Asp23 and Lys28 are separated at least 9.6 Å in the direction of the fibril axis, the attractive interaction between these residues is weak. As a result, the antiparallel conformation of the WT-Aβ fibril is less stable than the parallel conformation. By introducing the D23N mutation into Aβ40, the charged residue Asp is replace by the noncharged residue Asn, so that the electrostatic attractive interactions between Aβ40 monomers in the parallel conformation fibril are weakened. As a result, the stability of the parallel conformation is expected to become similar to that of the antiparallel conformation [22]. Consequently, it can be possible for the antiparallel conformation of D23N-Aβ40 fibril to appear as a metastable conformation.

In order to elucidate the effect of the D23N mutation on the Aβ dimer conformations at atomic and electronic levels, we here investigated stable structures for the solvated dimers composed of the wild-type or the D23N mutated Aβ, by use of classical molecular mechanics (MM) and *ab initio* fragment molecular orbital (FMO) [27–35] simulations. In addition, based on the electronic properties of the dimers evaluated by the FMO calculations, we highlighted the residues of Aβ contributing mainly to the dimer formation and elucidated the effect of the D23N mutation on the specific interactions between Aβ monomers. The results are useful for predicting the difference in the initial stage of Aβ aggregations between the WT-Aβ and the D23N-Aβ.

2. Details of molecular simulations

2.1. Optimization of Aβ dimer structures in water

As the initial structures of Aβ dimers with a parallel or an antiparallel conformation, we here employed the PDB structures of the parallel Aβ fibrils (PDB ID: 2LMN) [26] and the antiparallel Aβ fibrils (PDB ID: 2LNQ) [22], respectively. The PDB structure with the parallel conformation [26] has two hexamers of WT-Aβs, and we adopted the two centrally-located C and D chains of the first hexamer as the initial structure of the parallel WT-Aβ dimer. On the other hand, the PDB structure with the antiparallel structure [22] is for the octamer of D23N-Aβs, and its D and E chains located around the center of the fibril were adopted as the initial structure for the antiparallel D23N-Aβ dimer. In the PDB structure for the parallel WT-Aβ fibril [26], the positions of the 1–8 residues of Aβ are missing, while the 1–14 residues are missing in the antiparallel PDB structure [22]. To conform in size between the two structures, we cut out the 9–14 residues of the parallel structure to produce the parallel WT-Aβ(15–40) dimer, because the 1–14 residues are likely to be partially or fully disordered [13–15,22,25,26]. Finally, the N- and C-terminals of Aβ are terminated by the acetyl and the NH_2, respectively.

In addition, by use of SWISS-MODEL [36], the Asp23 residues in the parallel WT-Aβ(15–40) dimer were mutated by Asn to produce the initial structure for the parallel D23N-Aβ(15–40) dimer, while the Asn23 residues in the antiparallel D23N-Aβ(15–40) dimer were mutated by Asp to produce the antiparallel WT-Aβ(15–40). In this paper, we refer the parallel and the antiparallel WT-Aβ(15–40) dimers, and the parallel and the antiparallel D23N-Aβ(15–40) dimers as parallel WT-Aβ, antiparallel WT-Aβ, parallel D23N-Aβ and antiparallel D23N-Aβ dimers, respectively.

These four types of Aβ(15–40) dimers were solvated in a water box of the size of the twice of the Aβ dimer. In fact, the number of solvating water molecules for each dimer is 6271 (parallel WT-Aβ), 6322 (antiparallel WT-Aβ), 6309 (parallel D23N-Aβ) and 6247 (antiparallel D23N-Aβ), respectively. The solvated structures were fully optimized by the classical MM method implemented in the molecular simulation program package GROMACS Ver.4.5.3 [37]. In the MM optimizations, we used the FF99SB force field [38] in combinations with the TIP4P-Ew water model [39], because the previous replica exchange molecular dynamics (MD) calculations [40] shown that this combination of the force field and the water model produces an ensemble of configurations for solvated Aβ(21–30), being in good agreement with the NMR data. The threshold value of the energy-gradient for the convergence of the MM optimization was set to 0.0001 kcal/mol/Å.

2.2. Ab initio FMO calculations for Aβ dimers in water

The electronic properties for the solvated Aβ dimers were investigated by the *ab initio* FMO method [27–35], to elucidate which amino acid residues in Aβ are important for the stability of the solvated Aβ dimers. In the FMO calculation, the target molecule is divided into units called “fragment”, and the electronic properties of the target molecule are estimated from the electronic properties of the monomers and the dimers of the fragments. The specific interactions between the fragments can be investigated from the interaction energies obtained by the FMO calculation.

In the present FMO calculations, we investigated the relative stability between the parallel and the antiparallel conformations for the Aβ(15–40) dimer, with the solvating water molecules considered explicitly. We chose the 765 water molecules solvating nearest to the Aβ(15–40) dimer in the MM-optimized structure of the solvated dimer. These water molecules exist within a 6 Å distance from the surface of the dimer. The C- and N-terminals of Aβ(15–40) were terminated by the acetyl and the NH_2 groups, respectively. These groups were considered as the 14th and the 41st residues of Aβ(15–40) in the FMO calculations. Each residue of Aβ(15–40) and each water molecule were assigned as a fragment, because this fragmentation enables us to evaluate the interaction energies between the Aβ residues and between the residue and the solvating water molecules. Total number of the fragments for the solvated Aβ(15–40) dimer is 821, and total charge is assigned 0 and +2 for the WT-Aβ dimer and the D23N-Aβ dimer, respectively.

The *ab initio* MP2/6-31G method was employed to investigate accurately the π–π stacking, NH–π and CH–π interactions between the residues of Aβ(15–40). We used the FMO calculation program ABINIT-MP Ver.6.0 [41]. By considering water molecules explicitly, we attempted to elucidate the influence of solvating water molecules on the stabilization of the dimer conformation. In addition, to elucidate which amino acid residues of Aβ(15–40) contribute to the stability of the dimer conformation, we investigated the specific interactions between the fragments by the FMO method at an electronic level. It is noted that the interaction energies between the charged amino acid residues are overestimated, because the FMO calculations are preformed in vacuum with solvating water molecules considered explicitly.

3. Results and discussion

3.1. Optimized structures of solvated Aβ dimers

As shown in Fig. 1a, in the solvated structure of the parallel WT-Aβ(15–40) dimer, Asp23 of monomer-A and Lys28 of monomer-B form a salt-bridge between the monomers. The previous SSNMR analysis for the WT-Aβ40 fibrils and the MD simulations for the WT-Aβ39 dimer [14,26,42–44] indicated that Glu22/Asp23 and Lys28 form a salt-bridge both in a monomer and between