Analysis of interaction characteristics between amyloid β and lecanemab by HDX-MS

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Background

Soluble amyloid-β (Aβ) aggregates including protofibrils (PFs) have been reported to be more toxic than insoluble fibrils[1,2]. Detailed structural characteristics of the soluble Aβ aggregates have not been clearly established, since it was difficult to directly analyze their detailed tertiary structure. Recently, anti-amyloid antibodies targeting these soluble Aβ aggregates have been developed.

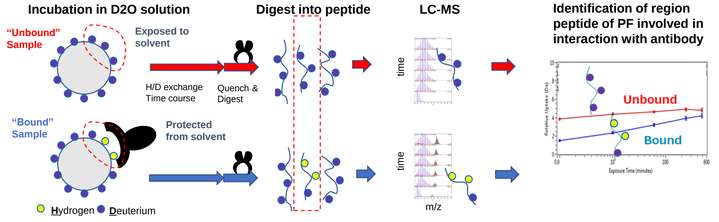
Lecanemab is a humanized anti-Aβ monoclonal antibody with preferential binding to Aβ protofibrils and soluble oligomers and, to lesser extent, insoluble fibrils[3]. Molecular basis for the binding preference to PF has not yet been elucidated.

Here, we investigated structural characteristics and dynamics of Aβ PFs and its change upon binding to lecanemab in solution using hydrogen deuterium exchange mass spectrometry (HDX-MS).

Material & Methods

Aβ (1-42) PFs used were prepared in vitro. The hydrogen/deuterium (H/D) exchange status for Aβ (1-42) PFs and monomeric Aβ (1-40) were studied by HDX-MS System (Waters) in phosphate buffered saline (PBS) to explore the characteristics of their regional structural dynamics. Next, the effects of the anti–Aβ PF antibody lecanemab on H/D status of Aβ (1-42) PFs and monomeric Aβ (1-40) were studied to explore the structural basis for the characteristics of antibody- Aβ interaction and specificity against Aβ PFs. The analysis was performed in n=3 except the point of 10min of PF, that was n=2. The statistical significance was analyzed by *p* value from Welch's *t*-test and global $\Delta\overline{HX}$ significance threshold at α=0.01 [4].

HDX-MS - Technology for characterization of binding region and conformational change upon protein-target interaction -

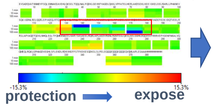


Analysis

Heat map

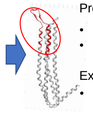
- Direct (unbound)map: native H/D exchange profile
- Difference map : difference of H/D profile between two states (e.g. bound-unbound, etc.)

Ex. Difference map(bound-unbound)



3D mapping

Check three-dimensional location: proximity location indicates conformational epitope



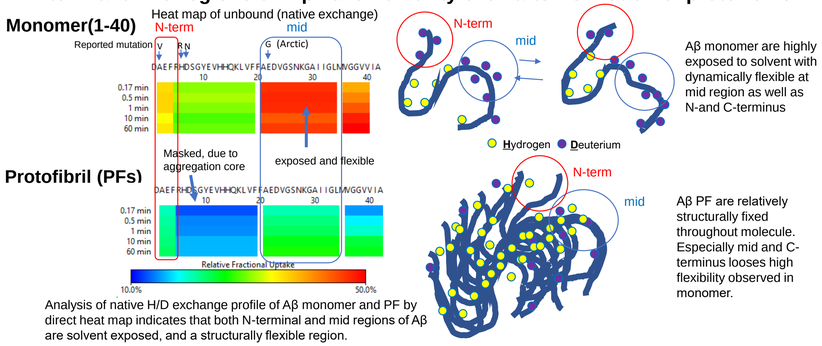
Protected region indicates:
- Binding region
- Conformational change

Exposed region indicates:
- Conformational change

Results

Aβ (1-42) PFs were more protected from H/D exchange throughout the molecule than monomeric Aβ (1-40), though the N-terminal and the mid region of Aβ still showed relatively higher H/D exchange in the molecule. Upon interaction with lecanemab, the mid region of Aβ (1-42) PFs was also protected further in addition to the N-terminal region, and its protection level of Aβ (1-42) PFs was higher than monomeric Aβ (1-40). The higher protection from H/D exchange both on N-terminal and mid regions of Aβ PFs is considered to be characteristics of interaction between Aβ (1-42) PFs and lecanemab.

N-term and mid regions of Aβ have flexibility even after formation of protofibrils



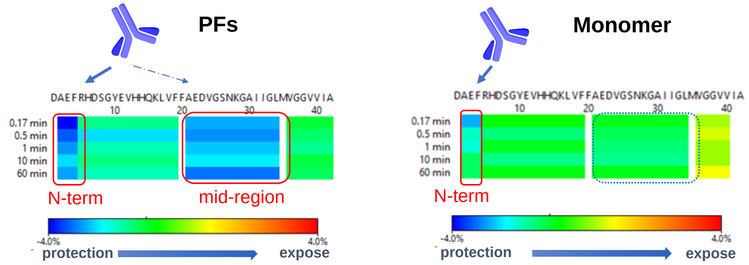
Aβ monomer are highly exposed to solvent with dynamically flexible at mid region as well as N-and C-terminus

Aβ PF are relatively structurally fixed throughout molecule. Especially mid and C-terminus looses high flexibility observed in monomer.

Analysis of native H/D exchange profile of Aβ monomer and PF by direct heat map indicates that both N-terminal and mid regions of Aβ are solvent exposed, and a structurally flexible region.

Both N-term and mid region of Aβ (1-42) PFs would be effector region for lecanemab

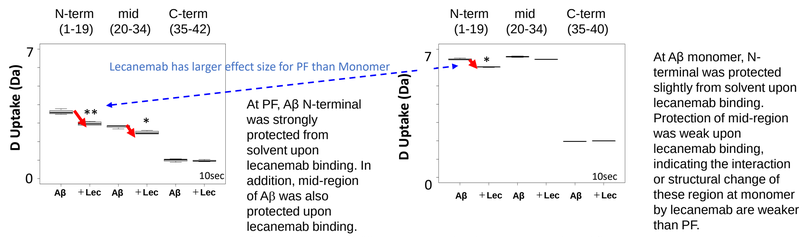
Heat map of differential (bound -unbound)



N-terminal and mid region of Aβ(1-42) within the examined PF aggregates were protected from solvent exchange upon interaction with lecanemab suggesting that regions are involved in the interaction with lecanemab or that they are structural changed region upon the interaction.

N-terminal region of Ab monomer was identified as a major interaction region for binding to lecanemab as reported previously. Smaller effect of mid-region compared to PF was also detected in the Ab monomer upon lecanemab binding.

Both N-term and mid region of Aβ (1-42) PFs would interact with lecanemab

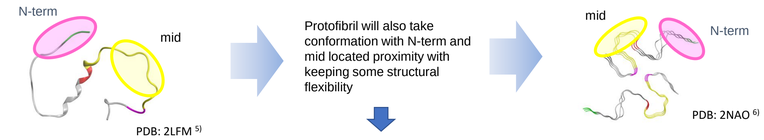


At PF, Aβ N-terminal was strongly protected from solvent upon lecanemab binding. In addition, mid-region of Aβ was also protected upon lecanemab binding.

At Aβ monomer, N-terminal was protected slightly from solvent upon lecanemab binding. Protection of mid-region was weak upon lecanemab binding, indicating the interaction or structural change of these region at monomer by lecanemab are weaker than PF.

*p<0.05, **p<0.01 vs Aβ only. Significance of difference were analyzed as Hageman, T.S. et al [4].

3D mapping for monomer and fibril estimated possible conformational epitope by N-term and mid region of Aβ (1-42) PFs against lecanemab



N-term and mid are occasionally located at proximity with dynamic flexibility in structural equilibrium

Possible conformational epitope* composed from N-term and mid region

N-term and mid are located at proximity with possibly less flexibility

*Epitope composed from multiple regions separated in sequence but located in proximity in 3D structure.

Conclusion

- In the examined Aβ PF structure, mid region of Aβ showed relatively high structural flexibility in addition to the N-terminal region which has been reported previously.
- N-terminal and mid regions of Aβ(1-42) within the examined PF aggregates are protected from solvent exchange upon interaction with lecanemab.
- The present data indicated the possibility of (1) lecanemab interaction with a potential conformational epitope generated by both N-terminal and mid region of Aβ, and/or (2) lecanemab binding to N-terminal region causing a structural change in the mid region of Aβ in the examined PF structure.
- Future studies are planned to further investigate the possibility of lecanemab interaction to a confirmational epitope in addition to its binding to N-terminal region.

Reference

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