【Grant-in-Aid for Transformative Research Areas（B）】

Supramolecular behavior and dynamic capture of meta-aggregates

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Research Area Information	Number of Research Area : 23B304　Project Period (FY) : 2023-2025 Keywords : amyloid, neurodegeneration, high-speed AFM, molecular evolution, G-quadruplex

Purpose and Background of the Research

●Outline of the Research

Proteins play a role in the biological functions through 3D structure. However, taking incorrect 3D structure can lead to the accumulation of insoluble aggregates (amyloid fibrils) both inside and outside cells, resulting in various diseases. Over 50 types of amyloids have been reported so far, and neurodegenerative diseases caused by their accumulation in the brain, in particular, pose significant challenges in an aging society due to their intractable nature.

While drug discovery research targeting oligomers (Fig. 1, left) has been pursued, definitive success has not yet been achieved in therapeutic strategies. This is primarily due to two main reasons: (1) Most neurodegenerative diseases are complex and multifactorial, involving the onset of multiple amyloids, and (2) Compounds developed based on *in vitro* aggregation mechanisms of amyloids may not necessarily succeed in clinical settings.

Originally, amyloid aggregation was explained by a template-dependent mechanism based on monomeric structures *in vitro*. However, it has become evident that in the biological environment, amyloids aggregate together with other biomolecules that co-localize. Against this backdrop, there's a strong need to consider the contributions of biomolecules other than individual amyloids to amyloid aggregation and to understand the supramolecular behavior of **meta-stable** co-aggregates from a holitic perspective (**meta-perspective**).

Hence, the need to understand the interaction between amyloids and co-localized biomolecules, as well as their pathological propagation mechanisms, prompted a reevaluation of atypical oligomers composed of heterogeneous co-aggregates, which are now defined as "**MetaAggregates**" (Fig. 1, right). This research aims to elucidate these aspects.

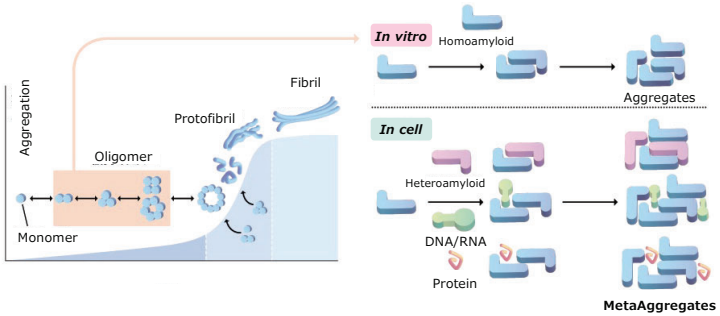

Figure 1　**MetaAggregate** model in neurodegenerative diseases

●MetaAggregate analysis by high-speed AFM

Until now, thioflavin method developed by Dr. Hironobu Naiki at Fukui University in the 1980s has been the gold standard as an amyloid analysis. However, due to the lack of analyzing methods for MetaAggregates, the focus is first on addressing this issue. The powerful analysis tool, high-speed atomic force microscopy (high-speed AFM), which enables the simultaneous visualization of the structure and dynamics of single molecules, is used to tackle the question of how MetaAggregates behave within the biological environment (expansion in XY direction or fluctuation in Z direction) (Fig. 2).

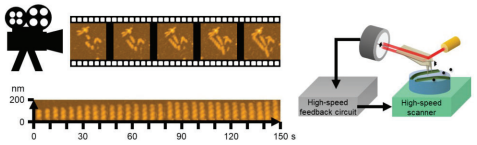

Figure 2　High-speed AFM for MetaAggregate dynamics

Expected Research Achievements

●Advancing the field of amyloid supramolecular chemistry

By elucidating the structure and dynamics of MetaAggregates involving ① heteroamyloid, ② nucleic acid structure, and ③ protein through co-localization and biomolecular condensation, we aim to develop new leads for drug discovery.

① Amyloid and heteroamyloid

While unraveling the mechanisms of **dementia-relevant MetaAggregates**, we aim to clarify their toxicity, leading to establishment of biomarkers for neurodegeneration and to develop disease-modifying therapies (Fig. 3).

② Amyloid and nucleic acid structure

Pioneering a new concept that centers around the "**G4-MetaAggregates**", originating from RNA guanine quadruplexes (G4RNA), we propose a novel therapeutic approach that underscores the core of neurodegenerative disease onset (Fig. 4).

③ Amyloids and protein

Focusing on amyotrophic lateral sclerosis (ALS), whose pathology RNA binding proteins (RBPs) are related to, we aim to search for "**ALS-MetaAggregates**", via *ex vivo* molecular evolution (Fig. 5).

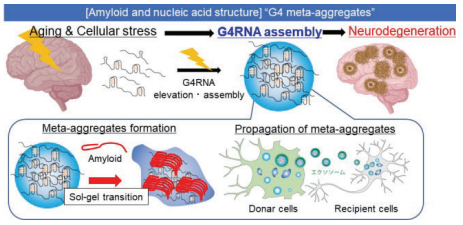

Figure 4　MetaAggregate triggered by RNA structure

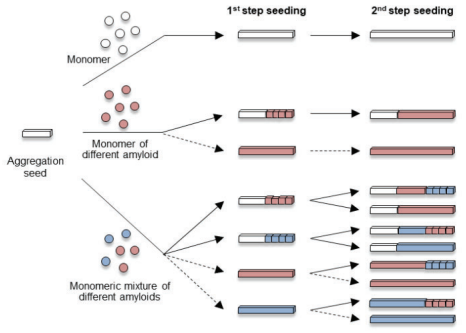

Figure 3　MetaAggregate model of heteroamyloids

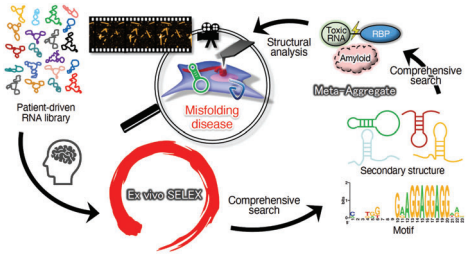

Figure 5　Comprehensive search for MetaAggregates

Homepage Address, etc.
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