Amyloid Oligomers

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Amyloid oligomers are considered to be potential targets for the development of therapeutic strategies for a wide range of neurodegenerative diseases. However, due to the low-populated, transient, and heterogeneous nature of amyloid oligomers, they are hard to characterize by conventional bulk methods. The development of single molecule approaches provides a powerful toolkit for investigating these oligomeric intermediates as well as the complex process of amyloid aggregation at molecular resolution.

Keywords: Amyloid Oligomers ; Single molecule fluorescence detection ; protein aggregation ; neurodegenerative disease

1. Introduction

Cellular proteostasis is one of the key problems in biology and has been explored for decades. Within the complex cellular environment, newly synthesized polypeptides may be prone to misfold under certain conditions. Failing to eliminate misfolded proteins results in their accumulation and self-assembly into a variety of aggregates, such as the β-rich fibrillar structures termed amyloid [1][2][3]. The misfolding and aberrant aggregation of proteins and peptides into amyloid fibrils is associated with many human diseases, including the most common neurodegenerative diseases Alzheimer's and Parkinson's as well as type 2 diabetes [4][5]. In addition, the ability to form amyloid is found to be a common or generic property of polypeptide molecules and amyloid structures execute special biological functions in a range of cellular processes in living organisms [6][7]. Amyloid aggregation is a complicated process involving the transition from soluble monomers to insoluble aggregates. Although great effort and progress has been made to reveal the structures and assembly mechanisms of the β-sheet-rich amyloid fibrils [8][9][10][11][12], much evidence indicates that the oligomeric intermediates formed during the amyloid fibril formation process rather than mature fibrils are toxic to cells and are the major pathogenic agent in neurodegenerative disease [13][14][15][16]. Moreover, these oligomeric species with a wide range of sizes and distinct conformations are also crucial intermediates of amyloid aggregation. Therefore amyloid oligomers have attracted great interest during recent years and are considered to be potential targets for the development of therapeutic strategies [17].

It is desirable to study the structure, quantity and properties of oligomers during the process of amyloid aggregation. However, due to the low-populated, metastable and transient nature of amyloid oligomers, only limited information can be obtained using conventional bulk techniques such as the thioflavin T (ThT) fluorescence assay, circular dichroism spectroscopy (CD), dynamic light scattering (DLS), electron microscopy (EM) and nuclear magnetic resonance (NMR) spectroscopy, given that the oligomeric species are vastly outnumbered by monomeric and larger aggregate populations [18][19][20][21][22][23]. Fluorescence microscopy-based single molecule techniques have advantages over conventional biochemical and biophysical methods and can characterize individual protein molecules, allowing exploration of the highly heterogeneous and dynamic amyloid oligomers. By obtaining information at the single molecule level, species that represent only a small proportion of the system can nevertheless be detected, instead of being hidden and averaged as in the case of ensemble experiments, thus providing insight into the process of amyloid aggregation in unprecedented detail.

There are two primary experimental configurations for single molecule fluorescence detection, namely confocal microscopy and total internal reflection fluorescence (TIRF) microscopy. In the confocal setup, a collimated laser beam is focused by a high numerical aperture objective onto a diffraction-limited femtoliter volume. Fluorescence-labeled molecules at picomolar concentration in solution can then be excited to give fluorescence bursts when diffusing across the focal volume. In the TIRF setup, the laser is reflected by the bottom of the coverslip and the evanescent wave excites the fluorescence-labeled molecules that are immobilized or adsorbed on the surface of the coverslip. In contrast to the short observation time in the solution confocal setup (<1 ms), the surface-immobilized fluorescent molecules can be observed for longer time periods (seconds to minutes) before photobleaching. Under both setups, single-molecule Förster resonance energy transfer (smFRET) can be performed, in which a protein of interest is site-specifically labeled with a donor-acceptor dye pair and the distance change between the labeling sites is represented by the FRET efficiency. For most commercial dyes, FRET efficiency is sensitively dependent on the donor-acceptor distance in the range of 2–10 nm,

thus is particularly suitable for investigating protein folding, structural transitions, and the populations and dynamics of specific protein conformations [24][25]. The confocal setup can also be employed for fluorescence correlation spectroscopy (FCS) which is based on the fluorescence fluctuations originating from the fluorescent molecules diffusing across the focal volume [26][27]. By analyzing the auto-correlation curves of fluorescence fluctuation, quantitative information such as the concentration, diffusional coefficient and dynamic properties of molecules can be obtained [28].

2. Characterization of Amyloid Oligomers by Single Molecule Fluorescence Detection

In order to detect the oligomeric species formed during amyloid fibrillization by smFRET, monomers are labeled with donor and acceptor fluorophores, mixed in equal amounts and then incubated to initiate aggregation. The formation of oligomers gives FRET signals that can be detected as coincident bursts in donor and acceptor detection channels when the oligomers diffuse across the focus even in the presence of an excess of monomers [29][30][31][32]. Two-color coincidence detection (TCCD) is an alternative detection mode, which similarly to smFRET is based on a confocal setup but is particularly suitable for samples with low intermolecular FRET efficiency. For TCCD the protein molecules are labeled with two spectrally separated fluorophores that are excited by an overlapped dual-color laser beam [33]. The fluorescence bursts occurring in only one emission channel are counted as monomers, while simultaneous fluorescence emission in both channels is counted as oligomers [34]. The oligomer concentration can be determined by converting the oligomer proportion referenced to a standard sample with known concentration. It is also possible to obtain information about the size and conformation of oligomers by analyzing the fluorescence intensity and FRET efficiency of oligomer bursts. During single molecule experiments, samples need to be diluted to the picomolar concentration range which may cause dissociation of unstable oligomeric species. In order to avoid this, fast-flow microfluidic techniques can be combined with single molecule detection, by which the acquisition time can be shortened from hours to minutes and so reduce the opportunity for oligomers to dissociate after dilution [35][36].

Amyloid oligomers can also be studied by TIRF imaging using either a single-color laser or coupled with TCCD. The samples are deposited onto the coverslip and excited by single-color or two overlapped lasers of different wavelengths. The intensity and colocalization of fluorescence in the images can be analyzed to give detailed information about the composition, size and morphology of the oligomeric species present [37]. An alternative to covalently labeling the target proteins with organic fluorophores, is to directly observe the unlabeled amyloid aggregates by TIRF imaging in the presence of a structure-specific dye such as ThT or pentameric formyl thiophene acetic acid (pFTAA) [38][39][40][41]. This can avoid exogenous effects introduced by covalently-linked dyes and photobleaching in conventional imaging. However, the resolution of traditional far-field optical imaging is around 250 nm due to the optical diffraction limit, far beyond the scale of amyloid oligomers and aggregates which are usually in the range from a few nanometers up to tens of nanometers. The development of super-resolution imaging techniques, either based on stochastic optical reconstruction microscopy (STORM) or stimulated emission depletion microscopy (STED), has now greatly improved the optical resolution to below 20 nm, allowing the exploration of the morphology and properties of amyloid aggregates on the nanoscale [42][43][44][45][46].

Over the past decade, with the development of ultrasensitive single molecule fluorescence techniques, amyloid oligomers have been thoroughly and quantitatively characterized, despite their low-populated, dynamic and heterogenous nature. The thermodynamic and kinetic properties of amyloid oligomers measured in vitro can be extrapolated to physiological conditions that are not accessible experimentally. This progress has greatly improved our understanding of the mechanism of amyloid aggregation and its relationship with neurodegenerative diseases. To date, the majority of single molecule studies on amyloid aggregation have been performed in vitro, but the simplified experimental conditions in vitro may not fully represent the more complicated cellular context. Due to the difficulty in achieving fluorescence labeling as well as improving the signal to noise ratio and fluorescence stability in vivo, it remains challenging to apply single molecule detection to living cells. However, a promising approach is the introduction of dye labels into living cells with the recent applications of microinjection or electroporation [47][48]. Meanwhile, advanced illumination techniques such as light-sheet or single-plane illumination microscopy that enable 3D sectioning with highly reduced background and limited phototoxicity provide opportunities for in vivo single molecule measurements of protein dynamics [49]. These and anticipated further development of new techniques will no doubt facilitate in vivo studies of the assembly of amyloid proteins at the single molecule level, therefore providing guidance on potential therapies for neurodegenerative diseases in the future.

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