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Review

A flash in the pan: Dissecting dynamic amyloid intermediates using fluorescence

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ABSTRACT

Several widespread and severe degenerative diseases are characterized by the deposition of amyloid protein aggregates in affected tissues. While there is great interest in the complete description of the aggregation pathway of the proteins involved, a molecular level understanding is hindered by the complexity of the self-assembly process. In particular, the early stages of aggregation, where dynamic, heterogeneous and often toxic intermediates are populated, are resistant to high-resolution structural characterization. Fluorescence spectroscopy is a powerful and versatile tool for such analysis. In this review, we survey its application to provide residue-specific information about amyloid intermediate states for three selected proteins: IAPP, α-synuclein, and tau.
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1. Introduction

The pathological self-assembly and deposition of proteins is a hallmark of a wide variety of devastating human disorders classified as protein misfolding or amyloid diseases (reviewed in [1–3]). These disorders are widespread and severe, encompassing 3 of the top 15 causes of death in the USA (Alzheimer's disease (AD), Type II Diabetes Mellitus (T2D) and Parkinson's disease (PD)) [4] as well as other major conditions including Chronic Traumatic Encephalopathy (CTE), Huntington's disease and Creutzfeldt-Jakob disease. There is therefore urgent and significant clinical interest in understanding the factors that trigger pathological self-assembly, and the development of pharmacological means by which this disease-related phenomenon may be arrested or mitigated. Initial efforts to understand the molecular mechanisms of amyloid diseases focused on characterization of fibrillar aggregates. Since then, however there has been growing interest in the structures formed along the aggregation pathway (Fig. 1a), investigating the hypothesis that these early structures are relevant to toxicity and thus are potential targets for therapeutic intervention.

The aggregation of amyloidogenic proteins generally display nucleation-dependent kinetics, wherein the conversion from monomer to aggregate displays a lag phase dependent on the accumulation of a nucleus – an on-pathway, high-energy intermediate that must be populated in order for subsequent self-assembly to progress. The lag phase is followed by a rapid, cooperative fibril growth phase until the reaction saturates upon depletion of monomer, and may be reduced in duration or eliminated by the addition of a small amount of 'seed' of preformed amyloid material (Fig. 1b) [5,6]. This behavior means that the conformation and population of specific, transiently-populated intermediates early in the self-assembly process play major roles in determining the nature and prevalence of subsequent toxic states. The dynamic and often heterogeneous nature of these transient intermediates makes them highly challenging targets for conventional structural biology approaches. Indeed, recent years have seen the development and application of a number of powerful biophysical approaches to the characterization of intermediates in the self-assembly process [7]. Significant insight has been gained into the thermodynamics driving aggregation as well as the secondary, tertiary and in some cases quarternary structures of molecular species relevant to pathological self-assembly [8–10].

Fluorescence spectroscopy has a number of advantages for the characterization of amyloidogenic proteins, including

Abbreviations: AD, Alzheimer's disease; ANS, 1-anilinonaphthalene-8-sulfonic acid; AS, α-synuclein; CMC, critical micellar concentration; CTE, chronic traumatic encephalopathy; EPR, electron paramagnetic; ET_{eff}, energy transfer efficiency; FRET, Förster resonance energy transfer; IAPP, islet amyloid polypeptide; IDP, intrinsically disordered protein; MTBR, microtubule-binding region; NFT, neurofibrillary tangle; NMR, nuclear magnetic resonance; PD, Parkinson's disease; PheCN, *p*-cyanophenylalanine; PRR, proline-rich region; SDS, sodium dodecyl sulfate; T2DM, type II diabetes mellitus; TCCD, two-color coincidence detection; ThT, thioflavin-T

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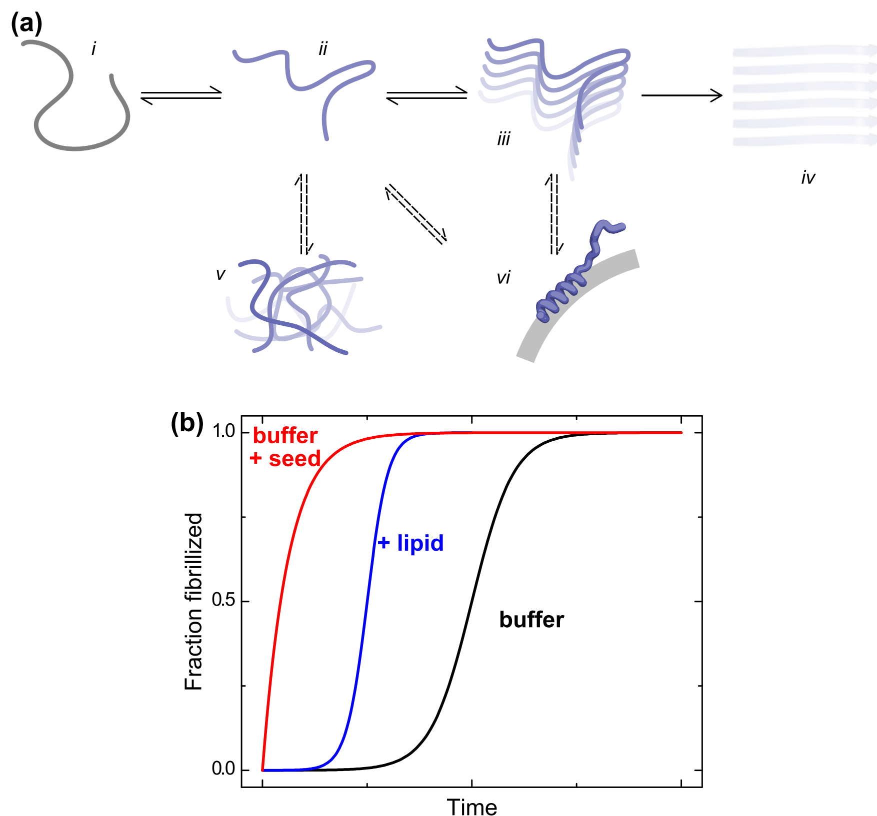

Fig. 1. (a) Conversion of soluble protein to amyloid aggregates. Proteins may convert from their native states or conformational ensembles (i) to an aggregation-prone form (ii), and then proceed via a population of on-pathway oligomers (iii) to amyloid fibers (iv). Proteins may also form off-pathway oligomers (v), or bind to membranes (vi) in modes which could inhibit or enhance fiber formation. Biologically relevant toxicity could result from states ii, iii, v or vi, while states i and iv are thought to be comparatively inert. (b) Schematic of aggregation kinetics displayed by amyloidogenic proteins. Sigmoidal fibrillization kinetics are observed in buffer (black) that can be accelerated, in many cases, by the addition of suitable lipid compositions (blue). The lag phase is bypassed upon the addition of a seed of preformed fibrillar material (red), demonstrating the nucleation-dependent nature of this process.

compatibility with low concentrations of protein (pM or nM in many cases) which inhibits rapid fiber formation, as well as enabling measurements under conditions (such as bound to membranes or even in cells) that are not easily accessible by other approaches. The major drawback is a lack of high resolution structural information relative to techniques such as NMR or EPR. However, in conjunction with molecular dynamics simulations and other computational modeling approaches, fluorescence methods can overcome resolution limitations and generate unique and valuable insights into the conformation and dynamics of amyloidogenic proteins in monomeric and oligomeric states [7,11,12]. In this review, we survey selected ensemble and single molecule fluorescence studies that demonstrate the level of residue-level structural information that can be obtained on intermediate species of interest.

Fluorescence approaches to the investigation of amyloidogenic proteins can be broadly divided into three classes. The first capitalizes on the phenomenon of Förster resonance energy transfer (FRET), wherein the efficiency of non-radiative energy transfer from a donor fluorophore to an acceptor reports on the distance between them. FRET measurements may be obtained in steady-state or time-resolved ensemble formats, or via single molecule FRET (smFRET; reviewed in [13–16]). Donor and acceptor fluorophores may be placed on the same molecule for intramolecular FRET, which allows for an assessment of the conformational ensemble and dynamics of the protein, or may be placed on different molecules for intermolecular FRET to interrogate oligomerization of the protein. The second class of measurement involves the site-specific attachment in the target protein chain of environment-sensitive fluorophores, so as to probe local environment and dynamics in aggregation-prone states or through the course of the aggregation reaction. The third class relies on environment-sensitive fluorescence from histological and diagnostic dyes that bind non-covalently to fibers and/or intermediates in the assembly process, such as Thioflavin-T (ThT) and -S (ThS), Congo Red, Nile Red, and 1-anilinonaphthalene-8-sulfonic acid (ANS) [17–19]. While there has been recent progress in defining the structural modes of interaction of these probes with amyloid and non-amyloid states [20–23], and these methods have generated fundamental insights into our understanding of pathological self-assembly, structural information from environment-sensitive dyes is generally low-resolution and coarse-grained relative to site-specific approaches. The use of these dyes also raises the prospect of false positives [20] and negatives [24] with respect to the existence of amyloid structure. In this review, we therefore focus on the first two classes of measurement described above, and survey selected ensemble and single molecule fluorescence studies that demonstrate the level of residue-level structural information that can be obtained on intermediate species of interest.

We have selected three amyloidogenic proteins for further discussion: islet amyloid polypeptide (IAPP; also called amylin), α-synuclein (AS) and tau. IAPP, AS and tau are implicated in the pathologies of T2D, PD and AD respectively, and share intriguing commonalities in terms of toxic mechanism, the nucleated kinetics of self-assembly, and the importance of electrostatics and templated self-assembly to the aggregation process. Moreover, they are all examples of intrinsically disordered proteins (IDPs): in solution, under physiological conditions, they each sample an ensemble of diverse, extended conformations rather than folding

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