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Novel aminobenzanthrone dyes for amyloid fibril detection

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ABSTRACT

A series of novel fluorescent aminobenzanthrone dyes have been tested for their ability to identify and characterize the oligomeric and fibrillar aggregates of lysozyme. The parameters of the dye binding to native, oligomeric and fibrillar protein have been calculated from the results of fluorimetric titration. Furthermore, several additional quantities reflecting the preference of the probe to either pre-fibrillar or fibrillar protein aggregates, have been evaluated. Based on the comparative analysis of the recovered parameters, AM4 was recommended for selective detection of protein pre-fibrillar assemblies, while the dyes AM1, AM2, AM3 were selected as the most prospective amyloid tracers.

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1. Introduction

A great number of so-called conformational diseases, including neurological disorders (Parkinson's, Alzheimer's and Huntington's diseases), type II diabetes, spongiform encephalopathies, thyroid carcinoma, systemic amyloidosis, etc., are strongly associated with the deposition in tissues of highly ordered pathogenic protein aggregates, called amyloid fibrils. Amyloidogenesis refers to an *in vivo* process in which one of the human amyloidogenic proteins abnormally self-associates into a fibril 60–100 E in width and of a variable length. Amyloid fibrils share a characteristic cross-β-sheet structure with β-strands orienting perpendicular to long axis of the fibril and β-sheets propagating in its direction. The driving forces for amyloid assembly *in vivo* are thought to involve heat shock, oxidative stress or gene mutations, while *in vitro*, the conditions favoring fibril formation include lowering pH, elevating temperature, adding organic solvents or denaturants, etc.

Given the crucial role of protein fibrillar aggregates in the development of debilitating diseases, correct identification and accurate characterization of amyloid assemblies are of utmost importance. The process of fiber formation is being studied with a vast majority of powerful techniques including transmission electron and atomic force microscopy [1,2], nuclear magnetic and electron paramagnetic resonance [3,4], circular dichroism (CD) [2,5], X-ray diffraction analysis [6], fluorescence spectroscopy [7], etc. Of these, the method based on the measurement of Thioflavin T (ThT) fluorescence is one of the gold standards for protein fibril detection [8]. Though being widespread, this assay suffers from several drawbacks: (i) high affinity of ThT for native proteins and bacteria; (ii) slow binding kinetics; (iii) sensitivity to amino acid sequence, pH and ionic strength of solution [9,10]. In view of this, a wide range of novel fluorophores, such as, for instance, T-49 [11], T-284 [12], DCVJ [8], ANS, TNS [13], stilbene derivatives [14], CRANAD-2 [15] have been developed for both *in vivo* and *in vitro* amyloid detection. Ideally, a prospective amyloid marker should fulfill the following requirements: (i) high affinity for protein aggregates ($\sim 1\ \mu M^{-1}$), (ii) high quantum yield, extinction coefficient ($\sim 10^4\ M^{-1}\ cm^{-1}$), Stokes shift and binding stoichiometry, (iii) low background fluorescence, (iv) photo- and chemical stability, (v) nontoxicity for living cells [16]. Despite the broad selection of available fluorophores, fibril tracers that fully meet the above challenges have to be found. Our previous work revealed one prospective fluorescent probe, benzanthrone derivative ABM, sensitive to the presence of amyloid aggregates [17]. Cumulative data from the binding, resonance energy transfer and red edge excitation shift (REES) studies allowed us to give comprehensive characteristic of lysozyme (Lz) fibrillar aggregates. Inspired by such unrivalled potential of ABM in identifying the amyloid assemblies, in the present work we evaluated the specificity of a range of novel aminobenzanthrones, referred to here as A4, A6, A8, AM1, AM2, AM3 and AM4, to the protein oligomeric and fibrillar aggregates. More specifically, our goals were: (i) to identify the fluorophores possessing the highest sensitivity to lysozyme pre-fibrillar and fibrillar aggregates; (ii) to quantitatively characterize the dye binding to native, oligomeric and fibrillar lysozyme; and (iii) to analyze the properties of putative binding sites for aminobenzanthrones in amyloid fibrils.

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