Spectral properties and factors determining high quantum yield of thioflavin T incorporated in amyloid fibrils

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Abstract. Benzthiazole dye thioflavin T (ThT) is widely used for studying amyloid fibrils structure and formation. Nevertheless, till now there is no common opinion concerning molecular mechanisms of ThT binding to amyloid fibrils and the reasons of dramatic increase of its fluorescence quantum yield on incorporation into amyloid fibrils. Our data prove that ThT molecules incorporate in amyloid fibrils in monomeric form and there is no ground to suppose the formation of ThT dimers, eximers, or micelles. It was shown that the increase of quantum yield of ThT incorporated in amyloid fibrils is caused by restriction of benzthiazole and aminobenzene rings torsion fluctuations about each other.

Keywords: Amyloid fibrils, thioflavin T, quantum yield, fluorescence, absorption, dimers, eximers, micelles

1. Introduction

Amyloid fibrils are enriched β -sheet a polymeric forms of proteins with the strands perpendicular to the long axis of the fibril [2,3]. A number of human diseases, including several neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, transmissible spongiform encephalopathies, etc., are characterised by deposition of proteins in the form of regular amyloid fibrils [1,6,12].

Usually, amyloid fibrils structure and formation are studied via the use of specific dyes such as Congo red and thioflavin T (ThT). ThT interaction with amyloid fibrils is highly specific, as this dye does not interact with proteins in native, unfolded and partially folded monomeric forms, oligomers or amorphous aggregates [7,11]. Despite the fact that the number of papers describing the dye application for the analysis of amyloid fibrils is rapidly growing the molecular mechanism of specific dye binding to amyloid fibrils and the reasons of dramatic changes in its fluorescence properties on complexes formation is poorly understood. Furthermore, there are a number of inconsistencies in description of the spectral properties of this dye.

In some papers strong long-wavelength shift of fluorescence excitation spectra of ThT accompanying dye incorporation into amyloid fibrils was observed. It was taken as a ground to suppose that fluorescent properties and the increase of fluorescence quantum yield of the dye incorporated in amyloid fibrils are caused by dimer, eximer or micell forms of ThT [4,5,8,9]. However, nobody paid attention to the fact

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that being excited at the long-wavelength absorption band, ThT in water emits light at the same spectral area as ThT incorporated in amyloid fibrils, whereas short-wavelength fluorescence excitation spectra of ThT in water and alcohol solutions do not coincide with the position of the long-wavelength absorption band of dye.

The main goal of the current study was an attempt to make a choice between the existing models of ThT interaction with amyloid fibrils and explain the increase of fluorescence quantum yield accompanying the incorporation of this dye into amyloid fibrils.

2. Materials and methods

2.1. Materials

We used samples of ThT (Sigma-Aldrich and AnaSpec, CA, USA), ATTO (ATTO-TEC, Germany), glycerol (Merk, Germany), hydrochloric and acetic acid, NaCl and insulin (Sigma, USA) without further purification. Viscosity of the water–glycerol mixtures was determined refractometric by refractometer Abbe (LOMO, Russia). Insulin amyloid fibrils were produced by the incubation of insulin in 20% acetic acid solution in the presence of 100 mM NaCl (pH 2.0) at 37°C at constant stirring.

2.2. Spectral measurements

Absorption spectra were recorded by ESP-3T (Hitachi, Japan) spectrophotometer. Fluorescence was measured by spectrofluorometer with the stationary and impulse excitation described elsewhere [10]. The fluorescent dye ATTO-425 has spectral characteristics similar to spectral characteristics ThT. We use this dye as a reference when fluorescence quantum yield of ThT was determined. ThT and ATTO-425 fluorescence was excited at 435 nm and registered at 480 nm.

3. Results and discussion

Absorption spectrum and molar extinction coefficient of ThT in a water solution do not depend on concentration of dye in a range from 10^{-5} to 10^{-3} M (Fig. 1A). Therefore there is no ground to suppose the formation of ThT dimers. Measurement lifetime of the dye in water–glycerol mixtures allowed to conclude that in a water solution it equals to 0.001 ns. It means that there is no ground to suppose the formation of ThT excimers. Dependence of intensity of ThT fluorescence on concentration of dye does not give grounds to assume formation ThT micelles (Fig. 1B).

The presence of the methyl group at nitrogen atom in benzthiazole ring makes nonplanar conformation of ThT energetically profitable and decreases barrier of intramolecular rotation of benzthiazole and aminobenzene rings relative to each other. Therefore in water solutions ThT represents dynamic ensemble of molecules with π -conjugated bonds of the benzthiazole and aminobenzene rings disturbed in various degree and the part of molecules would have the angle between the planes of the rings close to 90°. In this case the isolated fragments of ThT should have shorter wavelength positions of the absorption and fluorescence spectra in comparison with those of the whole ThT molecule. It explains the existence of short wavelength bands of ThT fluorescence and fluorescence excitation spectra in solvents with low viscosity, e.g. in aqueous solutions.

Fig. 1. Photophysical properties of thioflavin T. (A) Absorption spectra of thioflavin T (ThT) in water at 5 different concentration of the dye from 7.5×10^{-6} to 3.7×10^{-3} M. (B) Dependence of ThT fluorescence intensity upon the dye concentration ($\lambda_{\text{ex}} = 435$ nm, $\lambda_{\text{em}} = 480$ nm). (C) Absorption spectra of equal concentrations of ThT in water solution (curve 1) and ThT incorporated in insulin amyloid fibrils (curve 2). (D) Variation of ThT fluorescence quantum yield with the change of solvent viscosity and temperature.

Absorption spectrum of ThT in the presence of amyloid fibrils represents superposition of the absorption spectrum of free dye molecule and that incorporated in the amyloid fibrils. It was determined that ThT incorporation in amyloid fibrils is accompanied by strong long-wavelength shift of the absorption spectrum (from 413 to 450 nm) and increase in molar extinction coefficient in the spectrum maximum (Fig. 1C).

The short-wavelength position absorption spectrum of ThT in water solution is explained by the fact that the ground state is stabilized by the orientational interactions of the polar solvent dipoles with the positively charged ThT fragments, whereas the configuration of the solvation shell of the ThT molecule in the excited Franck–Condon state is likely far from being equilibrium. The increase of molar extinction coefficient of ThT on incorporation in amyloid fibrils can be explained by the increase of the conjugation of π -electron system of the benzothiazole and aminobenzene rings because incorporated molecules are more planar.

Fluorescence quantum yield of ThT recorded in a wide range of solvent temperatures and viscosity confirmed the assumption that the increase of quantum yield of ThT incorporated in amyloid fibrils is caused by restriction of benzothiazole and aminobenzene rings torsion fluctuations about each other (Fig. 1D).

All experimental data prove the model according to which ThT incorporates in amyloid fibrils in monomer form. The binding occurs in “channels” that run along the long axis of amyloid fibril.

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