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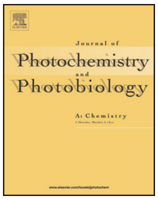

Ultrafast torsional dynamics of Thioflavin-T in an anionic cyclodextrin cavity

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Using sub-picosecond fluorescence upconversion spectroscopy, the effect of supramolecular confinement on the ultrafast excited state torsional dynamics of an amyloid fibril sensing dye, Thioflavin-T (ThT), is investigated in a novel cyclodextrin derivative, Sulphobutylether β-cyclodextrin (SBE-β-CD). The encapsulation of ThT inside the nanocavity of SBE-β-CD results in a significant increase in the emission intensity and the excited state lifetime of ThT when compared with native β-CD. Detailed analysis of the time-resolved emission spectra (TRES) shows that the time dependent changes in the integrated area, mean frequency and the width of the emission spectra are appreciably slower as compared with bulk water and native β-CD. These features suggest a significant confinement effect on the ultrafast torsional dynamics of ThT. The ionic strength of the medium significantly affects the complexation of ThT with SBE-β-CD, which indicates that the interaction between the host and the guest is predominantly electrostatic in nature. The results further suggest that the hydrophobic interaction in SBE-β-CD, although minor in contribution in the present case, is comparatively stronger than the native β-CD.

1. Introduction

Thioflavin T (ThT), a benzothiazole-based cationic dye, is used as an extrinsic fluorescence sensor to monitor and estimate the formation of amyloid fibrils, which are fibrous polypeptide aggregates comprising of cross-β structure. These amyloid fibrils are encountered in several debilitating conditions such as Alzheimer's disease and Parkinson's diseases [1–4]. The basis for the employment of ThT, in amyloid fibril sensing, lies in dramatic enhancement of its fluorescence intensity in fibrillar media as compared to bulk water, and protein in its native form [5–8]. ThT is very weakly emissive in bulk water, and in recent investigations it has been established that, upon electronic excitation in solution, a large amplitude non-radiative torsional relaxation around central C—C bond of ThT takes place on a barrier-less potential energy surface [9–12]. This torsional relaxation leads to the rapid conversion of initially populated strongly emissive locally excited state (LE) state to a very weakly emissive twisted intramolecular charge transfer (TICT) state, which quenches its emission in water. It is believed that this very efficient non-radiative torsional relaxation in ThT is strongly inhibited on association with amyloid fibrils leading to a drastic fluorescence enhancement [9,13–15]. Owing to the operation of such a large amplitude torsional motion in the excited state of ThT, the rate of this excited state reaction is also very sensitive to the viscosity of the medium, making ThT a very suitable candidate for the viscosity sensor. The emission quantum yield of ThT has been shown to vary linearly with the viscosity of the medium [10,16]. Because of its extreme sensitivity to the surrounding viscosity, ThT is also classified as ultrafast molecular rotor, which are an emerging class of sensitive reporters for the micro-viscosity of different chemical and biological environments [17–21]. Because of its extreme sensitivity to the surrounding environment, ThT has been recently shown to detect the small structural changes during premelting in the natural DNA [22], and is also shown to detect weak hydrophobic interaction between DNA and ionic liquids [23].

Amyloid fibrils, which consist of a generic cross-β structure, bears several grooves or channels made up of hydrophobic, aromatic and charged amino acid residues [24]. They represent very complex biological media offering a wide range of local non-covalent interactions to the binding ligands such as charge-transfer, electrostatic, hydrogen bonding, and hydrophobic interactions. Therefore, it is very relevant to study the ultrafast torsional relaxation dynamics of ThT in relatively simpler

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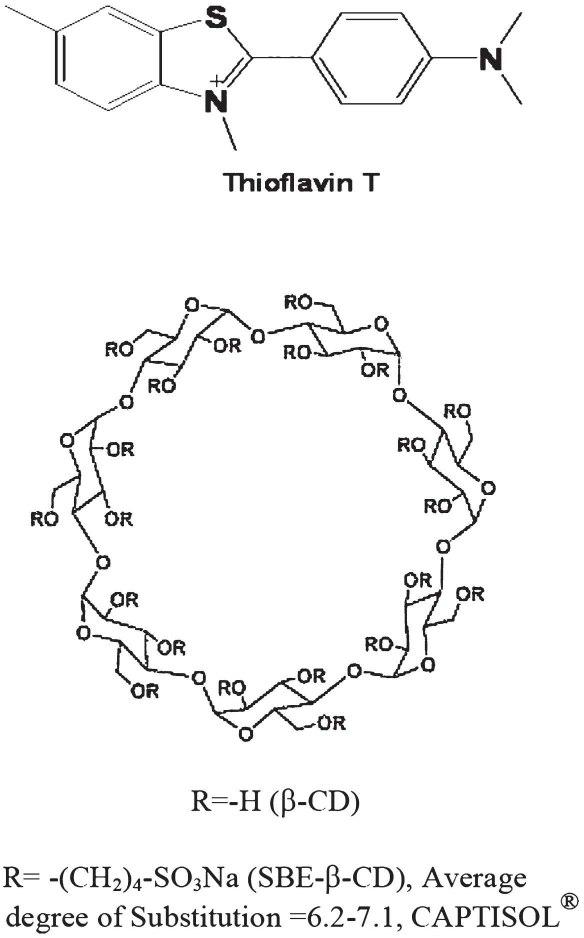
bio-mimetic confined environments, especially the ones which offers the possibility of multiple non-covalent interactions, to understand how each of these interaction individually affect the excited state dynamics of ThT. Knowledge of the ultrafast molecular dynamics of ThT on the excited state potential energy surface in such bio-mimetic confined environments will help in understanding the mechanism responsible for their amyloid fibril sensing ability as well as sensing other bio-molecules like DNA.

Sulphobutylether β-cyclodextrin (Captisol or SBE-β-CD, Scheme 1), which is a polyanionic β-cyclodextrin derivative with a sodium sulfonate salt separated from the native β-cyclodextrin cavity by a butyl ether spacer group, represents an important example of a bio-mimetic confined media. Natural cyclodextrins (CDs) are macrocyclic molecules comprising of elementary glucopyranose units, and the number of these glucopyranose units determines the size of their hydrophobic cavity [25,26]. The sulfonate group in SBE-β-CD makes the exterior of these cavities extremely hydrophilic whereas the presence of butyl ether group extends the hydrophobic cavity of parent β-CD, and offers stronger hydrophobic interaction towards the guest molecules [27,28]. They further display better toxicity profile and improved water solubility as compared to native β-CD [29]. Although there are some literature reports that explore the structural information on the host-guest complexes of the SBE-β-CD with some potential drug molecules [27,29,30], the information on the dynamical behavior of guest molecules in this potential lipophilic nanocavity, especially in the ultrafast time regime, is rather scarce.



Scheme 1. Molecular structure of Thioflavin T, β-cyclodextrin and sulfobutylether-β-cyclodextrin.

In the present paper, we examine the effect of confinement of the sulphobutyl SBE-β-CD nanocavity on the ultrafast torsional relaxation dynamics of ThT, using femtosecond resolved fluorescence upconversion spectroscopy which is uniquely suited to reveal the information in the ultrafast time regime. We have performed detailed analysis of the various features of the time-resolved emission spectra which includes mean frequency, width (FWHM) of the emission spectra and the integrated area under the emission spectra, and the results are compared with that reported in water and native β-CD. The effect of the ionic strength of the medium is also explored to analyze the contribution of electrostatic interaction in the complexation of ThT with SBE-β-CD.

2. Experimental

Thioflavin T (ThT) was obtained from Sigma as the chloride salt of the dye and was re-crystallized twice from methanol. The purity of the re-crystallized ThT was checked through NMR spectra. β-CD was obtained from TCI Mark, Tokyo and was used as received. Sulfobutylether-β-cyclodextrin (CAPTISOL®, average degree of sulfobutyl substitution: seven; average MW 2162) was kindly supplied by CyDex Pharmaceutical (La Jolla, California, USA). Sodium chloride was obtained from the Sigma–Aldrich. All samples were prepared using nanopure water (conductivity less than 0.1 μS cm^{-1}) obtained from a Millipore Milli-Q system. All measurements were performed using freshly prepared solution of the dye in SBE-β-CD.

Steady-state fluorescence measurements were carried out in a Hitachi spectrofluorimeter, model F-4500. The measured spectra, I (λ), were in wavelength domain and were converted to frequency domain, $I(\overline{\nu})$, by using the following equation [31].

$$I(\overline{\nu}) = \lambda^2 I(\lambda) \tag{1}$$

Time-resolved fluorescence measurements were carried out using a femtosecond fluorescence upconversion instrument (FOG 100, CDP Inc., Russia) which has been described earlier [32]. Briefly samples were excited using a second harmonic laser pulse (410 nm, 50 fs, 88 MHz) after frequency doubling of the fundamental output (820 nm) of a Ti-sapphire oscillator. The remaining fundamental laser beam was used as gate beam. The fluorescence signal collected from the sample was upconverted by overlapping with the gate beam into a BBO crystal after passing through a delay rail. The upconverted signal was allowed to pass through a bandpass filter to cut off excitation and gate beams and was then dispersed in a double monochromator. The instrument response function (IRF) was independently measured through the cross correlation of the fundamental and the excitation laser pulse. The IRF was found to have a Gaussian intensity profile with FWHM of 220 fs. All the fluorescence lifetime measurements were done at magic angle polarization, with respect to the horizontal excitation pulse, to remove any contributions from rotational reorientation on the decay traces. The reproducibility of the measurements was checked by measuring each decay trace 2–3 times. Samples were taken in a rotating cell of path length 0.4 mm to avoid the photo-degradation of the sample.

For spectrum reconstruction, fluorescence transients were recorded at 10 nm intervals across the steady-state emission spectrum. All these fluorescence transients were fitted with a tri-exponential function using the iterative convolution method. Time-resolved emission spectra (TRES) were reconstructed following the method proposed by Maroncelli and Fleming [33]. Each reconstructed spectrum was fitted using the lognormal function of the following form [33].