Journal of Applied Spectroscopy, Vol. 81, No. 2, May, 2014 (Russian Original Vol. 81, No. 2, March–April, 2014)

PHOTOPHYSICAL PROPERTIES OF *trans*-2-[4-(DIMETHYLAMINO)STYRYL]-3-ETHYL-1,3-BENZOTHIAZOLIUM PERCHLORATE, A NEW STRUCTURAL ANALOG OF THIOFLAVIN *T*

A. V. Lavysh,[a*] A. I. Sulatskaya,[b] A. A. Lugovskii,[c] E. S. Voropay,[c] I. M. Kuznetsova,[b,d] K. K. Turoverov,[b,d] and A. A. Maskevich[a]

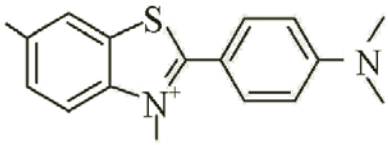
UDC 535.371:577.3

Spectral properties of a newly synthesized thioflavin T (ThT) derivative, trans-2-[4-(dimethylamino)styryl]-3-ethyl-1,3-benzothiazolium perchlorate (DMASEBT) with absorption and fluorescence spectra shifted to longer wavelengths (than ThT), were studied. Quantum-chemical calculations established that DMASEBT is planar in the ground state. The energy minimum of the excited molecule corresponded to a twisted conformation (TICT-state) with a 90° angle between the planar fragments. Charge in the molecule redistributed and a non-fluorescing TICT-state was formed if the fragments rotated. Fluorescence occurred from the non-equilibrium excited state (LE-state). It was shown that limited torsional rotation of the molecular fragments and; therefore, a decreased probability of transitioning into the non-fluorescing TICT-state, were responsible for the significantly increased quantum yield and fluorescence lifetime of DMASEBT upon increasing the solvent viscosity and incorporating it into amyloid fibrils.

Keywords: *thioflavin T, trans-2-[4-(dimethylamino)styryl]-3-ethyl-1,3-benzothiazolium perchlorate, fluorescence, molecular rotors, amyloid fibrils, intramolecular charge-transfer state.*

Introduction. Disruption of protein folding produces incorrectly folded states and stabilizes them through aggregation. This forms ordered insoluble structures known as amyloid fibrils (AF). It is currently thought that the development of diseases such as neurodegenerative Alzheimer′s and Parkinson′s, prion diseases, etc. is related to the formation and accumulation of AF in various human organs and tissues [1–3]. The study of AF structure has considerable practical value for medicine in the search for ways to prevent their formation and to solve the fundamental problem of protein structural organization and folding.

An effective method for detecting AF *in vivo* and *in vitro* is based on recording fluorescence of the benzothiazole dye thioflavin T (ThT) [4–7]:

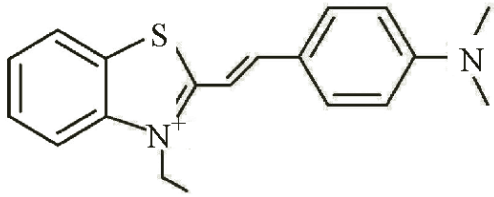


This is due to the fact that this dye in aqueous solution has an extremely low fluorescence quantum yield. Its fluorescence quantum yield increases by almost three orders of magnitude compared with the free dye upon interaction with proteins in the AF state that are enriched in the β-folded structure. Natural ThT or its analogs with absorption and fluorescence spectra that are shifted to longer wavelengths are useful in order to decrease light scattering by fibrils *in vitro* and to diminish absorption and fluorescence of tissues *in vivo*. The creation of such ThT analogs is exceedingly important to testing for AF in living cells and tissues.

*To whom correspondence should be addressed.

[a]Yanka Kupala State University, 22 Ozheshko Str., Grodno, 230023, Belarus; e-mail: andrewlavysh@mail.ru; [b]Institute of Cytology, Russian Academy of Sciences, St. Petersburg; e-mail: kkt@incras.ru; [c]Belarusian State University, Minsk; e-mail: voropay@bsu.by; [d]St. Petersburg State Polytechnical University, Russia; e-mail: imk@incras.ru. Translated from Zhurnal Prikladnoi Spektroskopii, Vol. 81, No. 2, pp. 209–218, March–April, 2014. Original article submitted November 4, 2013.

Herein the spectral properties of newly synthesized ThT derivative *trans*-2-[4-(dimethylamino)styryl]-3-ethyl-1,3-benzothiazolium perchlorate (DMASEBT) are examined:



The absorption and fluorescence spectra of DMASEBT have longer wavelengths than those of ThT. The ability to use the new dye to detect and study AF was demonstrated.

Experimental. The benzothiazole dye DMASEBT was synthesized in the Department of Laser Physics and Spectroscopy, Belarusian State University. Like ThT, DMASEBT is a cation at neutral pH values. A characteristic difference of DMASEBT and ThT is the longer bridge joining the benzene and benzothiazole rings.

Aqueous glycerol solutions (AGS) were prepared by mixing H_2O and 99% glycerol (Sigma-Aldrich, USA) in given ratios. The glycerol content in AGS was determined using the refractive index, which was measured using an Abbe refractometer (LOMO, Russia). AF were prepared by incubating insulin (Sigma, USA) in HOAc (20%) containing NaCl (100 mM) (pH 2.0) at 37°C with constant stirring for 24 h. The solution protein concentration was 2 mg/mL.

Absorption spectra of DMASEBT solutions were recorded on a Specord 200 spectrophotometer (Carl Zeiss, Germany); steady-state solution fluorescence spectra, on an SM2203 spectrofluorimeter (Solar, Belarus). The emission lifetime was measured on a pulsed spectrofluorometer [8] using time-correlated single-photon counting [9]. The fluorescence quantum yield (Φ) of DMASEBT was determined by the comparative method of Williams et al. [10]. The standard for measuring the DMASEBT quantum yield was rhodamine 6G in EtOH, for which Φ = 0.98 [11].

Quantum-chemical calculations of DMASEBT ground and excited states were performed using the PC-GAMESS 7.1.G program (Firefly). The molecular ground state was optimized using a restricted Hartree–Fock (RHF) method in basis set 3-21G. The excited state was calculated using a time-dependent density-functional theory (TDDFT) method with the three-parameter Becke–Lee–Yang–Parr composite exchange-correlated functional (B3LYP).

Results and Discussion. *Quantum-chemical calculations of the DMASEBT structure and ground- and excited-state energies.* The DMASEBT molecule was divided into two fragments. These were I, the benzothiazole ring with an ethyl group (C_2H_5), and II, the benzene ring with the dimethylamino group [$N(CH_3)_2$]. Two torsion angles that affected most strongly the molecular geometry were selected for optimizing the DMASEBT S_0 ground state structure. These were the angle ψ between the planes formed by atoms 1-2-3 and 2-3-4 and the angle φ between the planes formed by atoms 3-4-5 and 4-5-6 (Fig. 1a). Angles φ = 0° and ψ = 0° corresponded to a planar molecular geometry. The optimization was carried out with fixed angles φ and ψ while the other parameters were varied. The ground-state energy E_{S_0} of the conformers as a function of torsion angles (φ, ψ) was obtained by varying the angles from 0 to 360° in steps of 10° (Fig. 1b).

Quantum-chemical calculations showed an extremely high energy barrier of ~$1.5 \cdot 10^5$ cm^{-1} for rotation around the C=C bond in the bridge between fragments I and II. Therefore, this rotation was not considered further for the conformational analysis. Rotation of the dimethylamino group relative to the benzene ring was also not considered because such rotation was hindered by a high energy barrier of ~2800 cm^{-1} according to previous quantum-chemical calculations for ThT [12]. An analysis of the potential-energy surface showed that the energy minimum in the ground state corresponded to torsion angles φ = 0° and ψ = 0°.

Thus, the conformational analysis indicated that DMASEBT in the ground state was planar. The energy minimum of the ThT ground state corresponded to an angle of φ ≈ 37° between its two fragments [12]. This was explained by steric interactions of the benzothiazole methyl (CH_3) with the H atoms of the benzene ring. We supposed that the DMASEBT benzothiazole ethyl (C_2H_5) did not interact sterically with the benzene H atoms because of the long ethylene bridge. This made the structure of DMASEBT in the ground state planar.

The energies of the ground (S_0) and Franck–Condon excited (S_1^*) states as functions of φ and ψ (Fig. 1c and d) showed that the energy minimum of the excited molecule corresponded to φ = 90° (for ψ = 0°) and ψ = 90° (for φ = 0°). However, the energy minimum of ~10,700 cm^{-1} for φ = 90° was much less than for ψ = 90° (~17,000 cm^{-1}). Moreover, a barrier of ~1500 cm^{-1} occurred at ψ ≈ 50° for rotation of the benzothiazole ring that increased ψ (Fig. 1c). It could be assumed that rotation of the benzothiazole ring was improbable because of the energy barrier and also because this rotation did not convert the molecule into the lowest energy state.