CASCADE ENERGY TRANSFER BETWEEN BENZOTHIAZOLE, BENZANTHRONE AND SQUARAINE DYES IN BETA-STRUCTURED PROTEIN AGGREGATES

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Benzanthrone dyes, a well known class of fluorescent compounds with emission in a wide spectral region, have found numerous applications in different areas as laser dyes, disperse dyes for textiles and polymers, daylight fluorescent pigments, fluorescent probes for biomedical technologies, etc. Our previous studies provided evidence for the applicability of benzanthrones to detecting beta-structured protein aggregates, amyloid fibrils, that are thought to be involved in the development of more than 40 human disorders [1]. In the present work we evaluated the possibility of using this class of dyes as mediators in the cascade Förster resonance energy transfer (FRET) occurring on the amyloid fibril scaffold. To this end, we used one of the amyloid-sensitive benzanthrones, referred to here as A6, in combination with the classical amyloid marker, benzothiazole dye Thioflavin T and two squaraine dyes, SQ4 and SQ1, to form the energy transfer cascade ThT→A6→SQ4→SQ1. This system, containing ThT as a primary donor, A6 and SQ4 as jumper dyes and SQ1 as a final acceptor, was tested for its ability to differentiate between the fibrillar (InsF) and control or non-fibrillized (InsN) forms of insulin.

At the first step of the study the energy transfer efficiency was calculated from the quenching of the donor fluorescence at varying acceptor concentrations both in fibrillar and control proteins. The absence of the energy transfer from ThT to A6 was found for non-fibrillized protein (Table 1), while in the presence of InsF the FRET efficiency for this donor-acceptor pair reached the value ~ 80 %. Despite the compatible energy transfer efficiencies in the donor-acceptor pairs A6-SQ4 and SQ4-SQ1, the resulting fluorescence intensity of the final acceptor was ~ 5 times lower in InsN compared to InsF. That's why the distinction between InsN and InsF at the first step of the cascade FRET plays an essential role in the whole energy transfer process. The FRET parameters, such as the donor quantum yields (Q_d), overlap integrals (J), Förster radii (R_0) and donor-acceptor distances (r) were calculated for the fibrillar insulin. The R_0 value was maximal for A6 – SQ4 pair due to high values of the quantum yield of A6 and extinction coefficient of SQ4, along with significant overlap between A6 fluorescence and SQ4 absorption spectra. The variation of the average inter-chromophore distances r from 2.3 nm to 4.9 nm suggests that the employed dyes reside in the different fibril grooves. To summarize, our findings indicate that the proposed approach, based on the multi-step FRET in the four-chromophore system, can be useful for the development of sensitive fluorescence techniques for amyloid fibril detection in vivo.

Table 1. The FRET parameters

Donor – acceptor pair	E_{InsN}, %	E_{InsF}, %	Q_d	J, $M^{-1}cm^{-1}nm^4$	R_0, nm	r, nm
ThT – A6	-	80.3	0.022	$3.19 \cdot 10^{14}$	2.9	2.3
A6 – SQ4	76.6	73.7	0.128	$1.87 \cdot 10^{16}$	5.8	4.9
SQ4 – SQ1	78.9	71.3	0.014	$1.52 \cdot 10^{16}$	3.9	3.3

[1] Vus K., Trusova V., Gorbenko G., Sood R., Kirilova E., Kirilov G., Kalnina I., Kinnunen P. (2014) Fluorescence investigation of interactions between novel benzanthrone dyes and lysozyme amyloid fibrils. *J. Fluoresc.*, 24, 493–504.