CASCADE ENERGY TRANSFER IN INSULIN AMYLOID FIBRILS DOPED BY THIOFLAVIN T, BENZANTHRONE AND SQUARINE DYES

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The three-step Förster resonance energy transfer (FRET) within the cascade of four dyes, including the classical amyloid marker Thioflavin T as a primary donor, two jumper dyes, benzanthrone ABM and squaraine SQ4, and terminal acceptor SQ1, was tested as a possible tool for detection and characterization of insulin amyloid fibrils. The results obtained confirm the occurrence of highly efficient multistep FRET (msFRET) in the chromophore ensemble in the presence of insulin fibrils formed at elevated temperature under pH 2 (InsF1) or pH 7.4, 0.15 M NaCl (InsF2), while negligible FRET efficiencies were obtained for the control unfibrillized protein, suggesting the specificity of msFRET to cross-β-sheet architecture characteristic of amyloid fibrils. Specifically, the efficiencies of FRET for the donor-acceptor pairs ThT-ABM, ABM-SQ4 and SQ4-SQ1 at maximum acceptor concentrations (~0.4 µM – 1.6 µM) were estimated to be 86%/94%, 48%/34% and 66%/32%, respectively, in the presence of InsF1/InsF2. The most significant differences between InsF1/InsF2 and the control protein were observed for the donor-acceptor pair ThT-ABM, suggesting that ABM is the key mediator in the whole process of msFRET. Assuming the isotropic rotation of the fluorophores, the average donor-acceptor distances were estimated in the presence of InsF1, yielding the values 1.3 nm, 5.3 nm, and 3.9 nm for the ThT-ABM, ABM-SQ4 and SQ4-SQ1 pairs, respectively. The obtained distances are indicative of different fibril binding sites for the chromophores in the insulin fibrils, although due to their high specificity to the fibrillar structure, the dyes are most likely to localize in the surface grooves of β-sheets running along the main axis of amyloid fibril. Remarkably, the differences in the insulin amyloid morphology can be clearly distinguished using msFRET. As evidenced from TEM, InsF2 were thinner, shorter and contained amorphous aggregates, as compared to InsF1. Thus, different amyloid formation pathways under neutral and acidic pH resulted in the changes in the dye affinity for to the fibril binding sites, and, as a consequence, in the distinct msFRET efficiencies, especially for the pair SQ4-SQ1. The ability of ThT to serve as an efficient amplifier for the two near-infrared dyes, SQ4 and SQ1, with the benzanthrone fluorophore ABM as a jumper dye, allows detection of fibrillar insulin in the optical window of the biological samples, with the Stokes shift of the four-chromophore being ca. 240 nm. The proposed msFRET-based approach can be employed not only for insulin amyloid detection but also for distinguishing between different amyloid fibril morphologies and gaining further insights into the mechanisms involved in the development of the injection-localized insulin amyloidosis.

KEYWORDS: ABM, cascade resonance energy transfer, insulin amyloid fibrils, quantum yield, Thioflavin T.

Förster resonance energy transfer (FRET) is known as a physical process by which an excited state fluorophore (donor) non-radiatively transfers energy to another fluorophore via distance-dependent long-range dipole-dipole interactions [1]. Due to this feature, FRET technique is proven to be highly informative in the structural characterization of a wide variety of biological macromolecules and their assemblies [1-4]. The efficiency of FRET depends on the distance between the chromophores employed as energy donor and acceptor, orientation of the donor and acceptor transition dipoles, and the donor quantum yield [1,5]. Numerous studies indicate that the maximum FRET efficiency can be achieved via multiple interacting FRET pathways rather than independent channels, by which the energy travels from an initial donor chromophore through the intermediate donors/acceptors onto a final acceptor chromophore [6,7]. Therefore, during the past decades the multistep FRET (msFRET), manifesting itself in the energy transfer within multiple chromophore systems through cascade route, attracts increasing attention [8,9]. To exemplify, the msFRET was applied for protein labeling [10], simultaneous enzyme detection in multicomponent biological samples [9], genotyping of single nucleotide polymorphism [11], DNA sequencing [12], estimating the stoichiometry of protein complexes [13], determination of the tumor necrosis factor [14] and analysis of multicomponent interactions in living cells [15], screening of multiple enzyme inhibitors [7], developing the light harvesting systems and molecular photonic wires [6,7,16,17]. The vast majority of the above implementations of msFRET are based on the application of DNA as a framework for arraying multiple fluorophores at precise positions and controlling the inter-chromophore distances in such a way that the energy transfer occurs on the nanoscale [6,7,11,12,17].

Recently we have found that amyloid fibrils can also serve as a molecular scaffold for controlled positioning of a multitude of chromophores communicating via the two-step FRET [18,19]. Moreover, we have demonstrated that the technique based on the msFRET can be effectively used for the identification and characterization of insulin amyloid fibrils along with classical Thioflavin T (ThT) assay, thus allowing the increase of amyloid detection sensitivity [19,20].

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For further investigation of the ability of amyloid fibrils to act as a molecular framework for arraying multiple fluorophores, herein we directed our efforts to the identification of a set of fluorophores capable of reinforcing the amyloid-sensing potential of the classical amyloid marker ThT. More specifically, the aim of the present study was to examine the applicability of the chromophore system, containing Thioflavin T, benzanthrone dye ABM, and two squaraine compounds, SQ1 and SQ4, to detecting and characterizing the insulin amyloid fibrils of different morphology.

EXPERIMENTAL SECTION

Materials

Bovine insulin, dimethyl sulfoxide (DMSO), Tris, Thioflavin T (ThT) were purchased from Sigma. Benzanthrone dye ABM [21], and squaraine dyes, SQ1, SQ4 [19], were synthesized in the Daugavpils University and University of Sofia, respectively. All other reagents were used without further purification.

Preparation of working solutions

The insulin stock solutions 10 mg/ml (InsF1) or 0.3 mg/ml (InsF2) were prepared in 10 mM glycine buffer (pH 2.0) or in 10 mM Tris-HCl buffer (0.15 M NaCl), respectively. The reaction of the protein (stock solutions) fibrillation was conducted at 37 °C for 5 days (InsF2) and for 10 hours, followed by incubation at 55 °C for 8 hours (InsF2) under constant agitation on the orbital shaker (108 and 143 r.p.m. for InsF1 and InsF2, respectively). The kinetics of amyloid formation was monitored using the Thioflavin T assay [22], revealing the dye fluorescence intensity increase at 480 nm ca. 142 and 591 times for InsF1 and InsF2, respectively (data not shown). Hereafter, the fibrillar protein and its non-fibrillized counterpart (the insulin solution in glycine or Tris-HCl buffers that was not subjected to heating and agitation) are denoted as InsF1/InsF2 and InsN1, respectively, where indexes 1 and 2 correspond to glycine and TRIS buffers, respectively.

The dyes stock solutions were prepared in DMSO (SQ1 and SQ4) and ethanol (ABM), while ThT was dissolved in 10 mM Tris buffer (pH 7.4). The fluorimetric measurements were carried out in 10 mM Tris-HCl buffer (pH 7.4). Insulin fibril samples for transmission microscopy were contrasted by 1.5% (w/v) phosphotungstic acid, dried and viewed with the EM-125 electron microscope (Selmi, Ukraine), as described previously [19].

Spectroscopic measurements

The absorption spectra of the examined dyes were recorded with the spectrophotometer Shimadzu UV-2600 (Japan) at 25 ºC. The dye concentrations were determined spectrophotometrically using the extinction coefficients \( \varepsilon_{444}^{ABM} = 9.3 \times 10^3 \, \text{M}^{-1}\text{cm}^{-1} \), \( \varepsilon_{444}^{SQ1} = 2.3 \times 10^3 \, \text{M}^{-1}\text{cm}^{-1} \), \( \varepsilon_{444}^{SQ4} = 4.21 \times 10^4 \, \text{M}^{-1}\text{cm}^{-1} \) and \( \varepsilon_{412}^{ThT} = 3.6 \times 10^4 \, \text{M}^{-1}\text{cm}^{-1} \) for ABM, SQ1, SQ4 and ThT, respectively. Steady-state fluorescence spectra were recorded with RF-6000 spectrofluorimeter (Shimadzu, Japan). Fluorescence measurements were performed at 25 °C using 10 mm pathlength quartz cuvettes. Fluorescence spectra were recorded within the range 460–800 nm with the excitation wavelength 440 nm. The excitation and emission slit widths were set at 10 nm.

The efficiency of energy transfer was determined from the quenching of the donor fluorescence in the presence of acceptor using the theory described in our previous paper [19]. Briefly, the following formulas were used [1]:

\[
E = 1 - \frac{I_{\text{A}}}{I_{\text{D}}}
\]

Fig. 1. Transmission electron microscopy photographs of the insulin amyloid fibrils prepared in 10 mM glycine buffer (pH 2) (A) 10 mM Tris-HCl buffer (pH 7.4) (B).
where \( I_D \) and \( I_{DA} \) are fluorescence intensities of the free donor and in presence of the acceptor, respectively. The donor fluorescence recorded upon addition of the acceptor was corrected for inner filter effect [19]. The Förster radius was calculated using the Mathcad 15.0 software and the equations:

\[
R_0 = 979 \left( \kappa^2 n^2 Q_D J \right)^{1/6}, J = \int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^2 d\lambda / \int_0^\infty F_D(\lambda) d\lambda
\]

where \( J \) is the overlap integral; \( F_D(\lambda) \) is the donor fluorescence intensity, \( \varepsilon_A(\lambda) \) is the acceptor molar absorbance at the wavelength \( \lambda \), \( n \) is the refractive index of the medium; \( Q_D \) is the donor quantum yield; \( \kappa^2 \) is the orientation factor [19]. Finally, quantum yield of the dyes was estimated from the equation:

\[
Q_d = Q_s \left( \frac{1 - 10^{-6}}{1 - 10^{-4}} \right) \frac{S_d n_d^2}{S_s n_s^2}
\]

where \( Q_s \) is the quantum yield of the standard, \( A_d \) and \( A_s \) are the absorbances at the donor excitation wavelength, \( S_d \) and \( S_s \) are the areas under the fluorescence curve, \( n_d \) and \( n_s \) are the refractive indexes of the solutions in which fluorescence spectra of the donor and standard are measured, respectively.

**RESULTS AND DISCUSSION**

The three-step sequential FRET was observed in the system of four dyes including the ThT (donor), ABM (the main mediator), squaraine dyes SQ4 (acceptor for ABM) and SQ1 (acceptor for SQ4) in the presence of fibrillar insulin and control unfibrillized protein (Fig. 2). A sequential chromophore addition to InsF1 and to the control unfibrillized protein, starting from the terminal acceptor (SQ1) and ending by the primary donor (ThT), resulted in the following SQ1 fluorescence intensity increases at 680 nm: SQ1+SQ4 (~2), +ABM (~20), +ThT (~100) for InsF1 and SQ1+SQ4 (~1.2), +ABM (~14), +ThT (~15) for the control protein (Fig. 3A, B). Notably, increase in the fluorescence intensity of the terminal acceptor SQ1 (~100 for InsF1) in the presence of ABM in the four-chromophore system was higher than that in the case of 4-dimethylaminochalcone (DMC) [19], suggesting that the examined benzanthrone dye is better enhancer of SQ1 fluorescence in the msFRET occurring in the insulin amyloid fibrils. As illustrated in Fig. 3C, titration of the fibril-bound ThT with ABM is accompanied by the decrease in ThT fluorescence intensity at 480 nm, allowing the calculation of FRET efficiency. The sample containing InsF1, ThT and ABM was then titrated with SQ4, followed by the titration with SQ1 (data not shown), and FRET efficiencies within the pairs ABM-SQ4 and SQ4-SQ1 were determined. Similar approach was employed to determine the msFRET efficiencies for the donor-acceptor pairs bound to InsF2.

![Fig. 2. Scheme of a three-step sequential energy transfer between 4 chromophores.](image)

Interestingly, addition the dyes in the following order: SQ1, SQ4, ABM (0.3 µM), ABM (1.6 µM) to the InsF2-ThT system, resulted in the increase of SQ1 fluorescence at 680 nm by ~4, ~8, ~18, ~75 times, respectively (Fig. 3D). These data indicate that ABM acts as a jumper dye in the FRET between ThT and SQ4, since the squaraine fluorescence intensity increases with increasing the ABM concentration (Fig. 3D). Furthermore, final increase in SQ1 fluorescence at 680 nm was about 100 (18) times for InsF1 (InsF2) at the SQ1, SQ4 and ABM concentrations, 0.46, 0.25, and 0.3 µM, respectively. The differences observed between InsF1 and InsF2 suggest the sensitivity of msFRET to amyloid fibril morphology (Fig. 3A,D). The donor quantum yields \( (Q_d) \) presented in Table 1 were calculated using eq. (3). Notably, the ThT quantum yield was increased two orders of magnitude upon the dye binding to fibrillar insulin, that is in a good agreement with our previous data [19].

The FRET efficiencies \( (E) \) evaluated for the donor-acceptor pairs ThT-ABM, ABM-SQ4 and SQ4-SQ1 at the maximum acceptor concentrations (InsF1: 0.8 µM, 0.4 µM and 1.2 µM; InsF2: 1.6, 0.3 and 0.4 µM for ABM, SQ4 and SQ1, respectively) are given in Table 2. Similar to our previous study with DMC [19], the energy transfer efficiencies for all donor-acceptor pairs in the cascade were higher in the presence of fibrillar insulin, as compared to the control unfibrillized protein, suggesting the specificity of msFRET for amyloid state of polypeptide chain.
Fig. 3. Changes in the emission spectra of the squaraines (SQ1 and SQ4) upon addition of donors (ThT and ABM) in the presence of the fibrillar insulin InsF1 (A) and control protein (B). Fluorescence spectra of fibril-bound ThT upon titration with the main mediator ABM (C) or SQ1, SQ4, ABM (D). The protein and ThT concentrations were 5.6 and 3.6 µM (A–C), 1.8 and 0.16 µM (D), respectively. SQ1 and SQ4 concentrations were 0.46 and 0.25 µM, respectively. ABM concentration was 0.3 µM (A,B), and was varied from 0.2 to 0.8 µM (C) and from 0.3 to 1.6 µM (D). The samples were incubated for 3–16 minutes after addition of each aliquot of the dye stock solutions to the InsF2-ThT mixture (D).

Notably, the benzanthrone dye ABM is the key mediator in a whole msFRET process, because the energy transfer efficiency for the pair ThT-ABM was the highest (Table 2). ABM appeared to be a better mediator than DMC, because only ~60 % FRET efficiency was observed for ThT-DMC pair in the presence of insulin amyloid fibrils [19].

Notably, FRET efficiency for the pair Q4-SQ1 was 2-fold lower in InsF2, as compared to that in InsF1 (Table 2). Furthermore, only 4% FRET efficiency was observed for this donor-acceptor pair immediately after the addition of SQ1 to the ThT-ABM-SQ4-InsF2 system, and it reached the value of 32% only after 19 hours of sample incubation at room temperature (Table 2), probably because of steric restrictions for the binding of bulky SQ1 molecule to InsF2. The distinct FRET efficiencies in the ensemble of four dyes in the presence of InsF1 and InsF2 can be explained by variations in fibril morphology resulting from different pH, concentration, and ionic strengths [26]. As seen in Fig. 1A, the mature fibrils of 1.0±0.2 µm in length and 30±6 nm in diameter were formed under acidic pH in the absence of NaCl [27]. Instead, shorter (0.9±0.7 µm), thinner (24±6 nm) and blurred aggregates were observed upon insulin incubation at physiological pH (7.4) and ionic strength 150 mM (Fig. 1B). In fact, InsF2 fibrils seem to be immature, e.g., broken fibrils or protofilaments, resulting in the different appearance of InsF1 and InsF2 structures (Fig. 1) [28]. Thus, a more pronounced loss of insulin tertiary structure induced by acidic pH provides fast and complete maturation (InsF1, Fig. 1A), while some amount of amorphous aggregates are formed at pH 7.4 presumably due to screening of the electrostatic repulsion between the protein molecules (InsF2, data not shown). The latter is in good agreement with the results of Yoshihara and co-workers, who reported common amyloid structural properties of insulin fibrils formed at pH 1.6, 0.1 M NaCl, such as high beta-sheet content and exposed C-terminal and N-terminal parts of the B-chain [28]. In turn, the aggregates prepared at pH 7.4, 0.14 M NaCl were featured by the presence of unordered structures and exposed only the C-terminal part of the B-chain. Remarkably, both electrostatic and hydrophobic intermolecular
interactions govern insulin amyloid fibril assembly, and thus, at low pH insulin molecules exist as monomers, which may form amyloid nuclei.

Table 1
Donor quantum yields in the presence of fibrillar insulin (InsF1)

| Donor | $Q_i$ | Standard |
|-------|-------|----------|
| ThT   | 0.011 | ThT in buffer ($Q_s=10^{-4}$) [23] |
| ABM   | 0.10  | DSP-6 in ethanol ($Q_s=0.05$) [24] |
| SQ4   | 0.039 | Nile Blue in water ($Q_s=0.01$) [25] |

In turn, both pH 7.4 and 0.15 M NaCl reduce electrostatic repulsion, enabling amorphous aggregates formation, and, furthermore, it is the insulin dimer, which most likely forms amyloid nuclei in this case [26, 28]. Notably, addition of NaCl resulted in decrease in the lag time of insulin growth at pH 1.6 [26]. Next, 15-times greater lag time and 12-fold slower insulin fibril growth rate were observed in 0.1 M NaCl at pH 7.4, as compared to the corresponding values at pH 1.6, 0.1 M NaCl [26]. Furthermore, insulin amyloid aggregates were not formed at pH 7.4 without stirring, suggesting a slower nucleation than at pH 1.6 (presumably due to slow structural transformations of the insulin dimer).

Table 2
FRET efficiencies in the system containing ThT, ABM, SQ4, SQ1, fibrillar (InsF1/InsF2) or control (InsN1) insulin at the maximum acceptor concentration

| Donor-acceptor pair | $E_{\text{fibril}}$, % | $E_{\text{InsN}}$, % | $E_{\text{InsF2}}$, % |
|---------------------|-----------------------|---------------------|----------------------|
| ThT-ABM             | 86                    | 12                  | 94                   |
| ABM-SQ4             | 48                    | 25                  | 34                   |
| SQ4-SQ1             | 66                    | 4                   | 32                   |

It should also be emphasized that ThT affinity for insulin amyloid fibrils formed at pH 1.6 was about 2-3 times smaller than for the protein aggregates prepared at pH 7.5, although the binding stoichiometry in both cases was reported to be ~0.1 [29]. This fact may result in about 4-fold greater fluorescence increase of ThT in the presence of InsF2, as compared to that in InsF1 (591 and 142 for InsF2 and InsF1, respectively), and, as a consequence, to different FRET efficiencies (Table 2). Interestingly, a very high FRET efficiency was recorded in InsF2 for the ThT-ABM pair, although its value for the pair SQ4-SQ1 was close to that for the control unfibrillized insulin, suggesting low affinity of SQ1 for insulin fibrils prepared at pH 7.4. The observed effect can be explained by the low accessibility of the InsF2 binding sites for SQ1 whose molecule is the longest among the four employed chromophores (SQ1 length is ~2.1 nm [19]). Thus, the InsF1 and InsF2 species can be clearly distinguished using msFRET within the above four-chromophore cascade, specifically, through estimating the FRET efficiency for the SQ4-SQ1 donor-acceptor pair. Furthermore, two types of binding sites were observed for ThT in the insulin fibrils formed at pH 7.4 [29], presumably accounting for a slightly greater FRET efficiency for the ThT-ABM pair in InsF2 as compared to that for InsF1 (Table 2). The above considerations indicate that the examined four-chromophore system can be employed not only for insulin amyloid detection, but also for distinguishing between different amyloid fibril morphologies.

The overlap integral values ($J$) derived by numerical integration, the Förster radii and the donor-acceptor distances (calculated using the FRET efficiency values at the maximum acceptor concentration, 0.8 µM, 0.4 µM and 1.2 µM for ABM, SQ4 and SQ1, respectively) are presented in Table 3.

Table 3
FRET parameters obtained under assumption of isotropic rotation of the fluorophores in the presence of InsF1

| System   | $J$, M$^{-1}$cm$^{-1}$nm$^2$ | $R_0$, nm | $r$, nm |
|----------|-----------------------------|-----------|--------|
| ThT-ABM  | 2.12·10$^{14}$              | 1.8       | 1.3    |
| ABM-SQ4  | 1.27·10$^{16}$              | 5.2       | 5.3    |
| SQ4-SQ1  | 1.20·10$^{16}$              | 4.4       | 3.9    |

The pairs ABM-SQ4 and SQ4-SQ1 showed the greatest $J$ values due to small shifts (~22 nm and ~14 nm, respectively) between ABM(SQ4) fluorescence and SQ4(SQ1) absorption maxima, and high extinction coefficients of SQ4(SQ1). The largest value of the Förster radius (Table 3) was obtained for the pair ABM-SQ4 due to the greatest value of SQ4 extinction coefficient. The recovered distances are suggestive of the different binding sites for the chromophores within fibril structure, although due to their high specificity to the fibrillar assemblies, the dyes are most likely to localize in the surface grooves of the β-sheets running along the main fibril axis. Furthermore, the ABM and DMC seem to reside in the different fibril grooves due to two-fold lower ThT-ABM distance (Table 3), as compared to that for the pair ThT-DMC (~2.4 nm) [19]. As a consequence, ABM is localized further from SQ4, while the $r$ value for the DMC-SQ4 pair was 4.5 nm [19]. Notably, similar distances between SQ4 and SQ1 were obtained in the two studies,
InsF2 binding sites for the dye. The estimated donor-acceptor distances for the pairs ThT-ABM, ABM-SQ4 and SQ4-InsF2, as compared to that of InsF1, can be due to: i) the greater affinity of ThT for InsF2, and ii) the higher rigidity of the various types of insulin aggregates. For instance, about 6-fold greater increase in ThT emission in the presence of morphology of the fibrillar species InsF1 and InsF2, suggesting the applicability of msFRET for distinguishing between the various types of insulin aggregates. For instance, about 6-fold greater increase in ThT emission in the presence of InsF2, as compared to that of InsF1, can be due to: i) the greater affinity of ThT for InsF2, and ii) the higher rigidity of InsF2 binding sites for the dye. The estimated donor-acceptor distances for the pairs ThT-ABM, ABM-SQ4 and SQ4-SQ1 in the presence of InsF1, assuming the isotropic rotation of the fluorophores, fall in range 1.3 nm – 5.3 nm, suggesting that the dyes occupy different sites on the amyloid surface, most likely represented by the β-sheet grooves running parallel to the main fibril axis. The obtained results can be useful for the development of sensitive fluorescence techniques for amyloid fibril detection in vivo, based on the cascade FRET within the systems containing classical amyloid marker Thioflavin T, a near-infrared fluorescent dye and effective enhancers of its fluorescence.

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высокие значения эффективности многоэтапного ИРПЭ в данном ансамбле хромофоров в присутствии амилоидных фибрилл инсулина, сформированных при повышенных температурах и pH 2 (InsF1) или pH 7.4, 0.15 М NaCl (InsF2), в то время как незначительный ИРПЭ наблюдался в нефибриллизированном белке (контроль), что свидетельствует о специфичности зондов каскада к β-складчатой архитектуре амилоидных фибрилл. В частности, эффективность ИРПЭ для донорно-акцепторных пар ThT-ABM, ABM-SQ4 и SQ4-SQ1 при максимальной концентрации акцептора (~ 0.4 µM – 1.6 µM) составила 86%/94%, 48%/34% и 66%/8%, соответственно, в присутствии InsF1/InsF2. Наиболее существенные отличия между InsF1/InsF2 и контрольным белком обнаружены для донорно-акцепторной пары ThT-ABM, что свидетельствует о ключевой роли ABM как мидиатора в процессе многоэтапного ИРПЭ. При оценке расстояний между донором и акцептором в фибриллярных агрегатах InsF1, при условии изотропного вращения зондов получены значения 1.3 нм, 5.3 нм и 3.9 нм для пар ThT-ABM, ABM-SQ4 и SQ4-SQ1, соответственно. Эти результаты свидетельствуют о разных сайтах связывания фибрилл для зондов каскада, хотя благодаря их высокой специфичности к фибриллярным структурам, красители должны локализоваться в поверхностных желобках β-листов, которые простираться вдоль оси амилоидной фибриллы. Следует отметить, что различия в морфологии амилоидных фибрилл можно четко охарактеризовать при помощи многоэтапного ИРПЭ. В частности, согласно данным электронной микроскопии, фибриллы InsF2 были более тонкими, короткими и содержали аморфные агрегаты, в отличие от InsF1. Очевидно, разные пути формирования амилоидных агрегатов при нейтральном и кислых значениях pH, обусловливают разную аффинность зондов к сайтам связывания фибрилл, и, следовательно, разные значения эффективности многоэтапного ИРПЭ, особенно для пары SQ4-SQ1. Способность ThT служить эффективным усилителем для SQ4 и SQ1, которые флуоресцируют в ближней инфракрасной области, при использовании бензантронового флуорофора ABM в качестве медиатора, позволяет детектировать амилоидные агрегаты инсулина в оптическом окне биологических образцов благодаря стоксовому сдвигу четыреххромофорной системы около 240 нм. Предложенный подход, который базируется на многоэтапном ИРПЭ, можно использовать не только для детектирования амилоидных фибрилл инсулина, но и для дифференцирования морфологии фибриллярных агрегатов и выяснения механизмов развития инъекционно-локализированного инсулинового амилоидоза.

КЛЮЧЕВЫЕ СЛОВА: ABM, каскадный индуктивно-резонансный перенос энергии, амилоидные фибриллы инсулина, квантовый выход, тиофлавин T.