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MODULATING EFFECTS OF POLYPHENOLS ON THE INTERACTIONS BETWEEN FUNCTIONAL PROTEINS AND AMYLOID FIBRILS

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The present study was focused on the investigation of the binary and ternary systems containing the functional proteins, polyphenols and amyloid fibrils using the differential absorption spectroscopy, intrinsic protein fluorescence and fluorescent probe techniques. The group of polyphenols (PF) included the representatives of four different classes: flavonoid quercetin (QR), phenolic acid (salicylic acid, SA), curcuminoid curcumin (CR) and tannin gallic acid (GA). The functionally important proteins were represented by hemoglobin (Hb), cytochrome c (Ct) and serum albumin (BSA). The amyloid fibrils were prepared from the N-terminal (1-83) fragment of apolipoprotein A-I with amyloidogenic mutation G26R (ApoA-IF). It was demonstrated that the spectral markers such as the position and intensity of the bands in differential absorption spectra, fluorescence intensity and emission maxima of the intrinsic (Trp) and extrinsic (TDV) fluorophores can be regarded as sensitive indicators of the protein structural modifications induced by amyloid fibrils and polyphenols. The observed changes in the Trp and TDV fluorescence were interpreted in terms of several processes: i) PF-induced conformational changes of the protein molecules accompanied by the alterations in microenvironment of Trp residues; ii) competition between PF and TDV for the protein binding sites; iii) non-resonance quenching of Trp or TDV fluorescence by PF; iv) resonance quenching (Forster resonance energy transfer) between Trp or TDV and PF. Taken together, the results obtained suggest that polyphenolic compounds can modulate the interactions between functional proteins and amyloid fibrils and can be considered as perspective agents for reducing the amyloid toxicity.
Key words: *Amyloid fibrils; Functional proteins; Polyphenols; Differential absorption spectroscopy; Fluorescence*
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Amyloid fibrils, the ordered aggregates with a core β-sheet structure, are currently associated with a variety of human diseases, including neurodegenerative disorders, systemic amyloidosis, type-II diabetes, cancer, etc. [1-3]. Among the proposed mechanisms of amyloid cytotoxicity are disruption of plasma and intracellular cell membranes [4-7], suppression of proteasomal degradation [7], mitochondrial dysfunction [8] and generation of reactive oxygen species [9]. It was also demonstrated that amyloid fibrils can form complexes with endogenous proteins, thereby affecting their structural and functional properties. For instance, Aβ peptide are capable of interacting with human serum albumin [10], while islet amyloid polypeptide, β-amyloid and insulin amyloid fibrils directly interact with the receptor protein NLRP3 resulting in NLRP3 inflammasome activation and pyroptotic cell death [11-13]. In view of this, the aim of the present study was to explore the interactions between functionally important proteins differing in their structure and physicochemical properties such as serum albumin, hemoglobin and cytochrome c. with the amyloid fibrils from N-terminal fragment of apolipoprotein A-I and a series of polyphenols including quercetin (QR), curcumin (CR), gallic acid (GA) and salicylic acid (SA). The choice of the endogenous proteins for experimental study was governed by their availability and significant physiological roles. Albumin, the major protein in human plasma, is responsible for maintaining the osmotic pressure, transport of various small molecules, regulation of the immune response, etc. [14]. Hemoglobin accounts for transport of oxygen and carbon dioxide, modulation of erythrocyte metabolism, heat transduction via oxygenation-deoxygenation reactions [15], while cytochrome c functions as a component of the electron transport chain in the inner mitochondrial membrane and displays pro-apoptotic activity [16]. Polyphenols (PF), which are abundant in various fruits and vegetables, have garnered attention for their potential to modulate different types of biomolecular interactions [17]. These compounds can interact with both amyloid fibrils and functional proteins, but the implications of these interactions are not yet fully understood.

MATERIALS AND METHODS

The bovine serum albumin (BSA), horse hemoglobin (Hb), cytochrome c from bovine heart (Ct) and polyphenols were purchased from Sigma. The phosphonium dye TDV was kindly provided by Prof. Todor Deligeorgiev (Faculty of Chemistry, University of Sofia, Bulgaria). The N-terminal (1-83) fragment of apolipoprotein A-I with amyloidogenic mutation G26R was kindly provided by Professor Hiroyuki Saito (Kyoto Pharmaceutical University, Japan). All other reagents were of analytical grade and used without further purification.

The fibrillization of N-terminal (1-83) fragment of apolipoprotein A-I with mutations G26R, W50F and W72F was conducted at 37 °C with constant agitation on an orbital shaker after the protein dialysis from 6M guanidine hydrochloride

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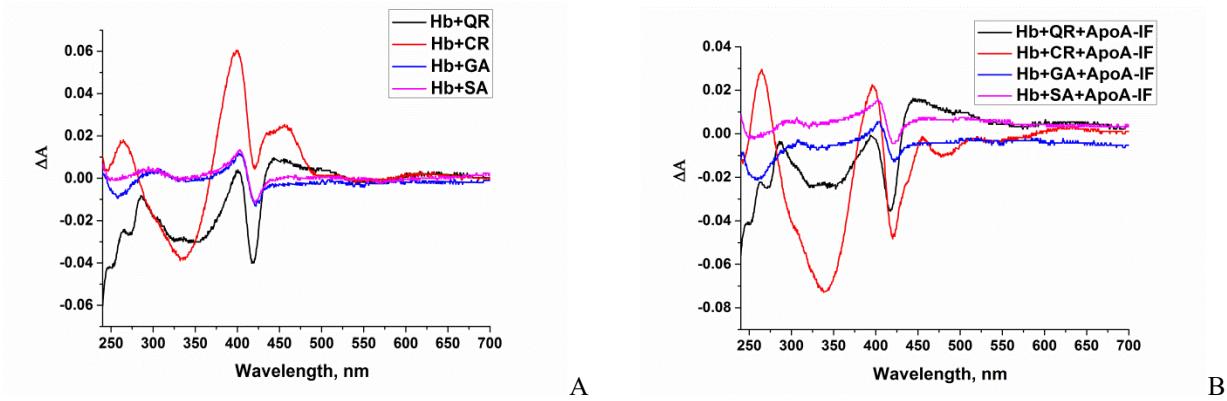
solution into 10mM Tris-HCl buffer, 150 mM NaCl, 0.01% NaN3, pH 7.4. The fibril growth was monitored through measuring the intensity of Thioflavin T fluorescence at excitation and emission wavelengths of 440 and 484 nm, respectively. Hereafter, the fibrillar form N-terminal fragment of apolipoprotein A-I is referred to as ApoA-IF. The stock solutions of polyphenols, *viz.* quercetin, curcumin, salicylic and gallic acids, were prepared in dimethylsulfoxide (DMSO) in the concentration 620 µM. The absorption measurements were performed in 10 mM Tris-HCl buffer (pH 7.4) with a Shimadzu UV-Visible spectrophotometer UV-2600 (Shimadzu, Japan) using the 10 mm path-length quartz cuvettes. The protein concentrations were determined spectrophotometrically using the following extinction coefficients $\varepsilon_{406}^{Hb} = 1.415 \cdot 10^5$ $M^{-1}cm^{-1}$, $\varepsilon_{406}^{Ct} = 1.05 \cdot 10^5$ $M^{-1}cm^{-1}$, $\varepsilon_{280}^{BSA} = 4.25 \cdot 10^4$ M-1cm-1 for hemoglobin (per heme), cytochrome c and albumin, respectively. The fluorescence measurements were carried out in 10 mM Tris-HCl buffer (pH 7.4) with a Shimadzu RF-6000 spectrofluorimeter (Shimadzu, Japan) using the 10 mm path-length quartz cuvettes. The protein fluorescence spectra were recorded from 300 to 500 nm with the excitation wavelength 280 nm, while TDV emission spectra were measured from 490 to 700 nm with the excitation wavelength 470 nm. The excitation and emission slit widths were set at 10 nm.

RESULTS AND DISCUSSION

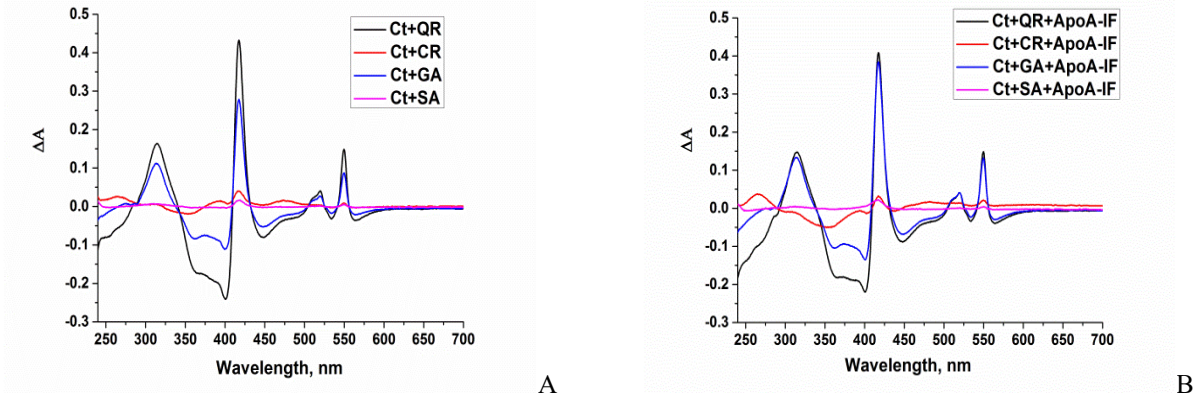
The first series of experiments was aimed at exploring the influence of polyphenols on the protein absorbance in the absence in presence of amyloid fibrils. Shown in Fig. 1 are the differential absorption spectra of the binary (Hb + PF) and ternary (Hb + PF + ApoA-IF) mixtures of Hb with PF and ApoA-IF, calculated as ((Hb+PF)-Hb-PF, red line) and ((Hb+PF+ApoA-IF)-(Hb+ApoA-IF)-PF, black line). The effects produced by ApoA-IF in the systems Hb+QR included slight intensity decrease of the peaks ~ 340 nm and ~ 418 nm, accompanied by the hypsochromic shift of the peak around 400 nm. At the same time, in the systems containing GA and SA, the addition of ApoA-IF led to reduction in the intensity of PF-induced peaks observed at ~340, 400 nm (GA) and ~340, 425 nm (SA). Notably, the most pronounced effects of amyloid fibrils were observed in the system Hb+CR+ApoA-IF where the peaks at ~340, 400, 420 nm undergo greater changes compared to the other polyphenols. Overall, the comparison of the differential absorption spectra indicates that the binding of all examined polyphenols to hemoglobin is followed by appearance of the peaks around 340, 400 and 420 nm. These peaks turned out to be sensitive to Hb-fibril complexation, suggesting that both PF and fibrillar ApoA-I modify the protein structural state trough affecting the intramolecular interactions of the heme group.



A B

Figure 1. The differential absorption spectra of Hb in the systems Hb + PF and Hb + PF + ApoA-IF. Hb concentration was 1.4 µM, polyphenol concentration was 27 µM, ApoA-IF concentration was 5.3 µM.

Shown in Fig. 2 are the differential absorption spectra of Ct calculated as ((Ct+PF)-Ct-PF, red line) and ((Ct+PF+ApoA-IF)-(Ct+ApoA-IF)-PF, black line).



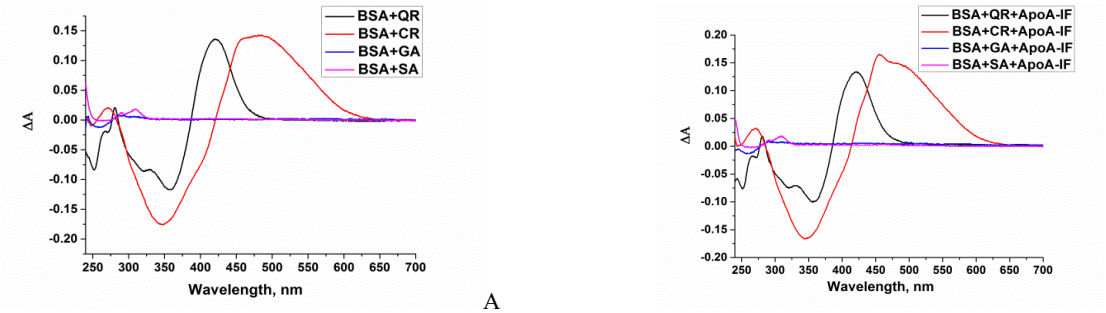
A B

Figure 2. The differential absorption spectra of Ct in the systems Ct + PF and Ct + PF + ApoA-IF. Ct concentration was 9.8 µM, polyphenol concentration was 27 µM, ApoA-IF concentration was 5.3 µM

As can be seen, Ct-QR binding is followed by the emergence of the intense peaks at 314 nm, 417 nm, 520 nm and 550 nm. The appearance of the peaks at 520 and 550 nm instead of the broad band centered at ~ 530 nm indicates that quercetin causes the transition of cytochrome c from the ferric to ferrous form. The oxidation state of heme group in Ct is known to change between + 3 (ferric form) and + 2 (ferrous form), thereby accounting for Ct ability to transfer electrons in the inner mitochondrial membrane [18]. Our findings indicate that quercetin can serve as an effective reducing agent for cytochrome c. Among the other investigated polyphenol's similar ability was revealed for GA, whereas CR and SA did not display any reducing ability (Fig. 2, A).

It appeared that in the system Ct+QR ApoA-IF does not significantly perturb the structure of cytochrome c (Fig. 2, B). In the presence of GA ApoA-I fibrils produced the decrease of the peaks at 314 nm, 417 nm, 520 nm and 550 nm, with the magnitude of this effect being greatest at 417 nm peak. The spectral changes caused by ApoA-IF in the system Ct+CR turned out to be rather pronounced, especially in the differential peaks at ~350 nm and ~400 nm. Thus, the influence of QR and GA on the structural state of cytochrome c is much stronger than that of CR and SA, whereas the effects of amyloid fibrils manifest themselves most strongly in the CR-containing systems.

The formation of BSA-PF complexes resulted in the appearance of characteristic differential peaks centered around 280 nm, 360 nm, 425 nm (QR), 350 nm, 480 nm (CR), 300 nm (GA), 310 nm (SA) (Fig. 3).

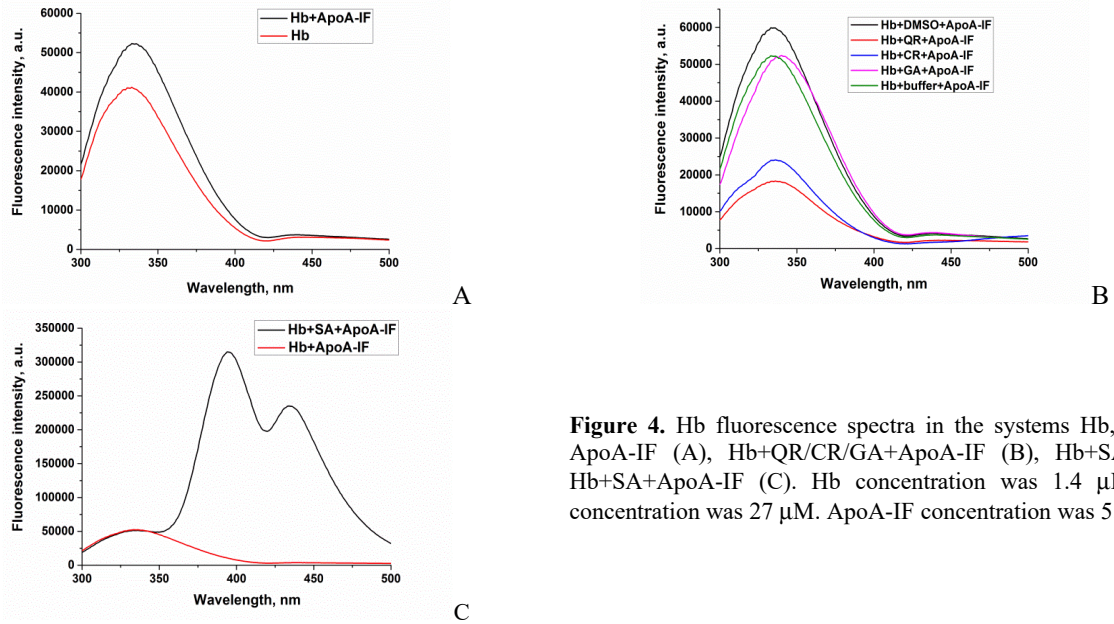


A B

Figure 3. The differential absorption spectra of BSA in the systems BSA + PF and BSA + PF + ApoA-IF. BSA concentration was 15.4 μM, polyphenol concentration was 27 μM, ApoA-IF concentration was 5.3 μM.

In contrast to Hb and Ct, the differential absorption spectra of BSA showed very weak changes on the addition of ApoA-IF. Taken together, the absorption measurements provided evidence for the ability of polyphenols to modify the structural and physicochemical properties of the examined proteins, thereby affecting their responsiveness to amyloid fibrils.

The second series of experiments was aimed at obtaining information on the interactions between functional proteins, polyphenols and amyloid fibrils using the methods of intrinsic protein fluorescence and fluorescent probes. As illustrated in Fig. 4, A, Hb fluorescence spectrum has a maximum at 333 nm. Hb is known to contain different types of intrinsic fluorophores, such as tryptophan, tyrosine and phenylalanine.



A B C

Figure 4. Hb fluorescence spectra in the systems Hb, Hb + ApoA-IF (A), Hb+QR/CR/GA+ApoA-IF (B), Hb+SA and Hb+SA+ApoA-IF (C). Hb concentration was 1.4 μM, PF concentration was 27 μM. ApoA-IF concentration was 5.3 μM.

Among the six tryptophan residues, two αβ dimers of Hb molecule have three Trp residues, α-Trp14, β-Trp15 and β-Trp37. Likewise, there are five tyrosine residues in each αβ dimer, viz. α-Tyr24, α-Tyr42, α-Tyr140, β-Tyr34 and β-Tyr144. It was found that Hb fluorescence originates predominantly from the β-Trp37 residue residing at the α1β2 interface [19]. The addition of amyloid fibrils from ApoA-I was followed by the red shift of the emission maximum by ~ 3 nm and increase of fluorescence intensity (Fig. 4, A).

The complexation of Hb with polyphenols prior to addition of ApoA-IF resulted in the following changes in Hb fluorescence spectra: i) significant decrease of fluorescence intensity in the presence of QR and CR without any marked shift of emission maximum; ii) a red shift of emission maximum by ~ 5 nm in the presence of GA, without the changes in fluorescence intensity At the same time, in the SA-containing system Hb emission spectrum was superimposed with the strong fluorescence signal from SA (Fig. 4).

As shown in Fig. 5, in the absence of PF ApoA-IF gives rise to fluorescence increase of a phosphonium probe TDV and blue shift of its emission maximum by ~3 nm.

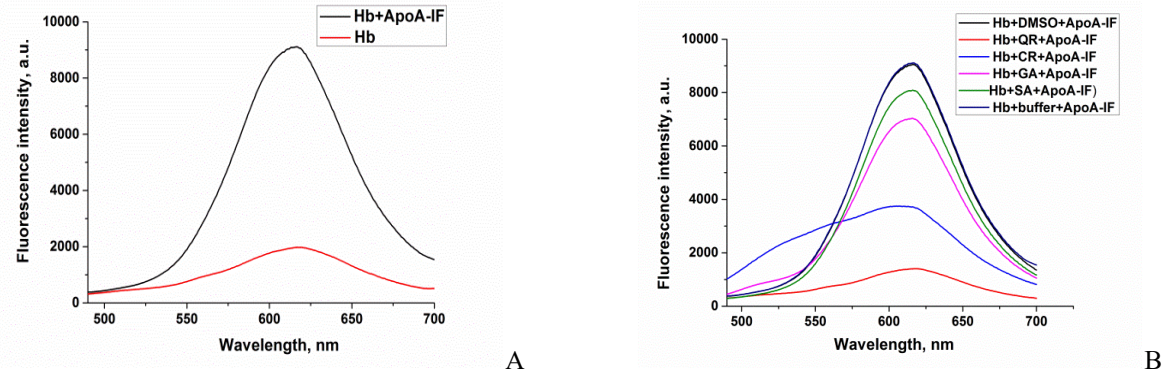


Figure 5. TDV fluorescence spectra in the systems Hb and Hb + ApoA-IF (A) and Hb + DMSO/PF +ApoA-IF (B). Hb concentration was 1.4 µM, TDV concentration was 0.4 µM, PF concentration was 27 µM, ApoA-IF concentration was 5.3 µM.

The analysis of the TDV fluorescence spectra in the systems Hb+PF+ApoA-IF revealed that: i) the maximum of TDV fluorescence (~616 nm) remains virtually unchanged in the presence of GA and SA, while fluorescence intensity shows a decrease by about 22% and 12%, respectively; ii) QR and CR provoke a decrease in TDV fluorescence by 84% and 59%, respectively; iii) CR produces a blue shift in emission maximum ~10 nm. The results obtained indicate that polyphenols are capable of modulating the effects of amyloid fibrils on the structural state of hemoglobin molecule. The observed decrease in the fluorescence of intrinsic hemoglobin fluorophore Trp37 and extrinsic fluorophore TDV can be interpreted in terms of several processes which require further investigation: i) PF-induced conformational changes of Hb accompanied by the alterations in microenvironment of Trp37; ii) competitive binding of PF and TDV to Hb; iii) non-resonance quenching of Trp or TDV fluorescence by PF; iv) resonance quenching (Forster resonance energy transfer) between Trp or TDV and PF.

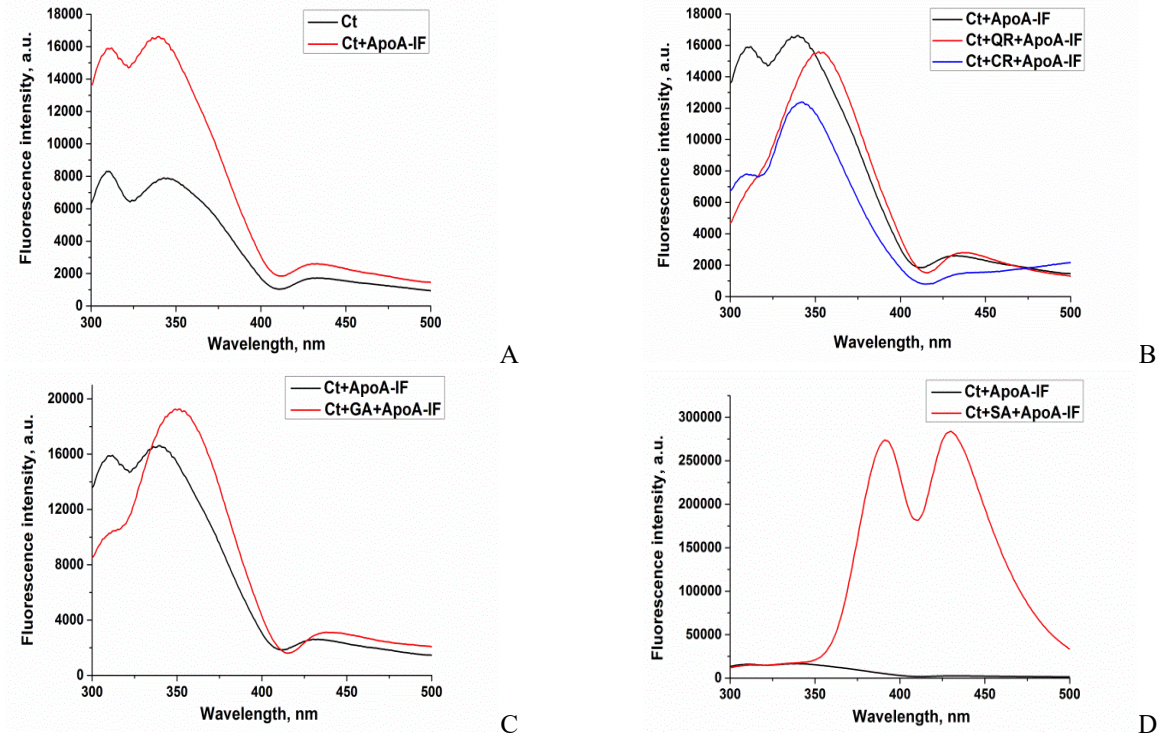


Figure 6. Ct fluorescence spectra in the systems Ct + ApoA-IF (A), Ct+PF+ApoA-IF (B-D). Ct concentration was 9.8 µM, PF concentration was 27 µM. ApoA-IF concentration was 5.3 µM.

Shown in Fig. 6, are Ct fluorescence spectrum measured in the absence and presence of polyphenols and fibrillar ApoA-I. Cytochrome c has a single tryptophan residue at position 59 whose fluorescence is significantly quenched by the heme group of the protein. The Ct emission spectrum has a maximum at 342 nm and is distorted by the Raman scattering

peak centered around 309 nm. In the system Ct+ ApoA-IF the emission maximum shifted by ~2 nm towards shorter wavelengths, to ~340 nm, whereas the fluorescence intensity showed about 2-fold increase (Fig. 6, A).

The changes in Ct fluorescence spectra upon the formation of Ct-PF complexes involved: i) slight decrease of fluorescence intensity and a bathochromic shift of emission maximum by ~10 nm in the presence of QR (Fig. 6, B; ii) fluorescence decrease by 25% without any shift of emission maximum in the presence of CR (Fig. 6, B; iii) fluorescence increase by 16% and a red shift of emission maximum by ~10 nm upon addition of GA (Fig. 6, C). Similar to Hb, fluorescence of SA was significantly stronger than that of Ct, thereby masking the effects of this polyphenol on Ct structure (Fig. 6, D).

The spectral behavior of TDV in Ct+ApoA_IF mixture was similar to that in the mixture Hb + ApoA-IF – the addition of amyloid fibrils resulted in the fluorescence increase and blue shift of TDV emission maximum by ~6 nm. However, in the Ct-containing systems the fibril-induced rise of TDV fluorescence was about twofold smaller compared to Hb (Figs. 7, A, 5, A). In the systems Ct+PF+ApoA-IF the maximum of TDV emission (~615 nm) did not undergo any marked change for QR, GA and SA, while in the presence of CR a blue shift (~3 nm) of emission maximum was observed. The fluorescence intensity decreased by ~25% for QR, and showed only slight alterations for CR, GA and SA (Fig. 7, B, C).

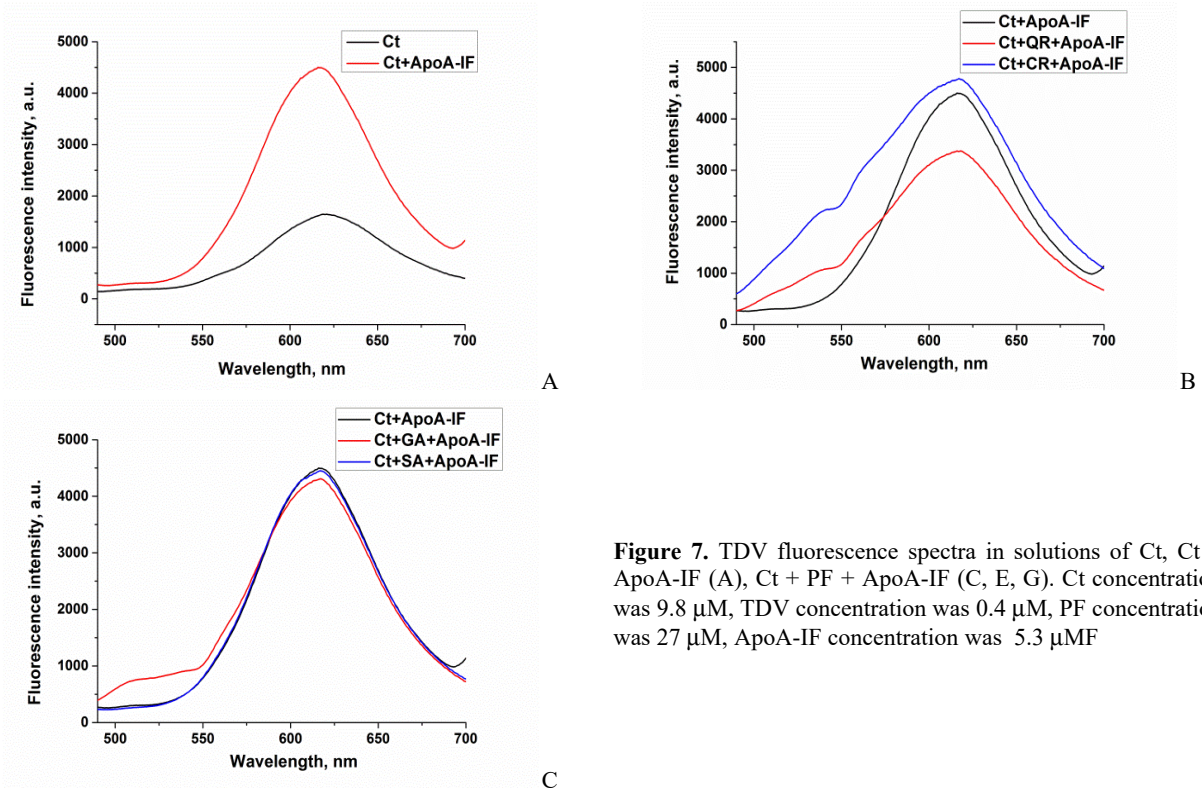


Figure 7. TDV fluorescence spectra in solutions of Ct, Ct + ApoA-IF (A), Ct + PF + ApoA-IF (C, E, G). Ct concentration was 9.8 μM, TDV concentration was 0.4 μM, PF concentration was 27 μM, ApoA-IF concentration was 5.3 μMF

In a separate series of experiments, we measured the intrinsic fluorescence of BSA upon varying concentrations of polyphenols, QR/CR – from 0 to 3.9 μM, GA/SA – from 0 to 39 μM. The molecule of BSA consists of three homologous domains (I, II, III) divided into nine loops (L1-L9) by 17 disulfide bonds. The intrinsic fluorescence of BSA originates from two tryptophan residues: Trp-134 from the first domain and Trp-212 from the second domain. Of these, Trp-212 resides within a hydrophobic binding pocket, while Trp-134 is located on the surface of the molecule [20]. As shown in Fig. 8, all examined polyphenols are capable of quenching the BSA fluorescence, with the magnitude of this effect being the greatest for quercetin.

To describe the BSA-PF complexation quantitatively, the decrease in fluorescence intensity observed in the presence of polyphenols (ΔF), was assumed to be proportional to the concentration of bound dye (B) defined in terms of the Langmuir adsorption model:

$$\Delta F_i = F_0 - F_{Z_i} = aB = 0.5a\left[Z_i + nP + K_a^{-1} - \sqrt{\left(Z_i + nP + K_a^{-1}\right)^2 - 4Z_i nP} \right] \quad (1)$$

where F_0, F_{Z_i} are the protein fluorescence intensities in the absence and presence of polyphenol, respectively; a is the coefficient of proportionality between the fluorescence changes and concentration of bound polyphenol; P and Z are the concentrations of the protein and polyphenol, respectively; K_a is the association constant; n is the binding stoichiometry (in mole of PF per mole of protein).

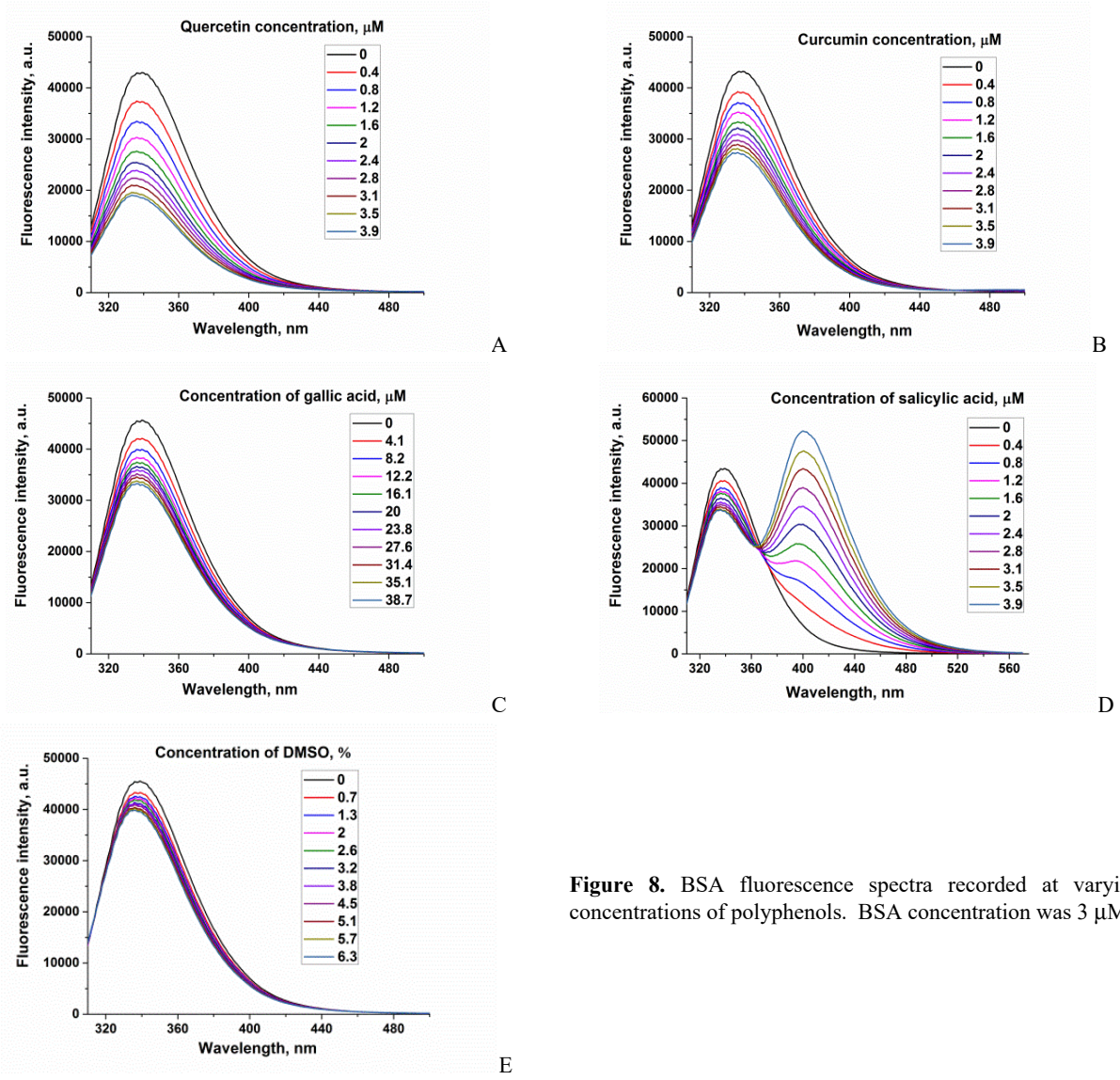


Figure 8. BSA fluorescence spectra recorded at varying concentrations of polyphenols. BSA concentration was 3 μM.

To determine the quantitative parameters of the BSA-PF complexation (K_a, n, a) the $\Delta F_i^{theor}(Z_i)$ values calculated from the Eq. (1), were fitted to the experimental ΔF values ($\Delta F_i^{\exp}(Z_i)$) through minimization of the function:

$$f = \frac{1}{N}\sum_{i=1}^{N}\left(\Delta F_i^{theor} - \Delta F_i^{\exp}\right) \quad (2)$$

where N is the number of the experimental points ($N = 10$).

Table 1. Quantitative parameters of polyphenol binding to BSA

Polyphenol	K_a, M^{-1}	n	a, M^{-1}
Quercetin	$3.3\times10^6 \pm 4.3\times10^5$	1.02 ± 0.2	$1.1\times10^{10} \pm 1.7\times10^9$
Curcumin	$3.2\times10^6 \pm 4.1\times10^5$	1.01 ± 0.3	$7.7\times10^9 \pm 8.2\times10^8$
Gallic acid	$1.7\times10^5 \pm 3.3\times10^4$	1.7 ± 0.3	$2.7\times10^9 \pm 3.7\times10^8$
Salicylic acid	$2.7\times10^6 \pm 3.6\times10^5$	0.78 ± 0.4	$5.6\times10^9 \pm 4.6\times10^8$

As seen in Table 1, quercetin, curcumin and salicylic acid have comparable high affinities for BSA, while the affinity of gallic acid was found to be ~20-fold lower. In contrast to Hb and Ct, fibrillar ApoA-I did not cause any marked changes of BSA fluorescence in the control sample (when DMSO was added instead of PF, (Fig. 9, E)), while in the presence of polyphenols amyloid fibrils induced the increase of BSA fluorescence (Fig. 9, A-D). One possible explanation for this effect is the competition between ApoA-IF and PF for the protein binding sites and lowering the extent of the BSA fluorescence quenching by polyphenols.

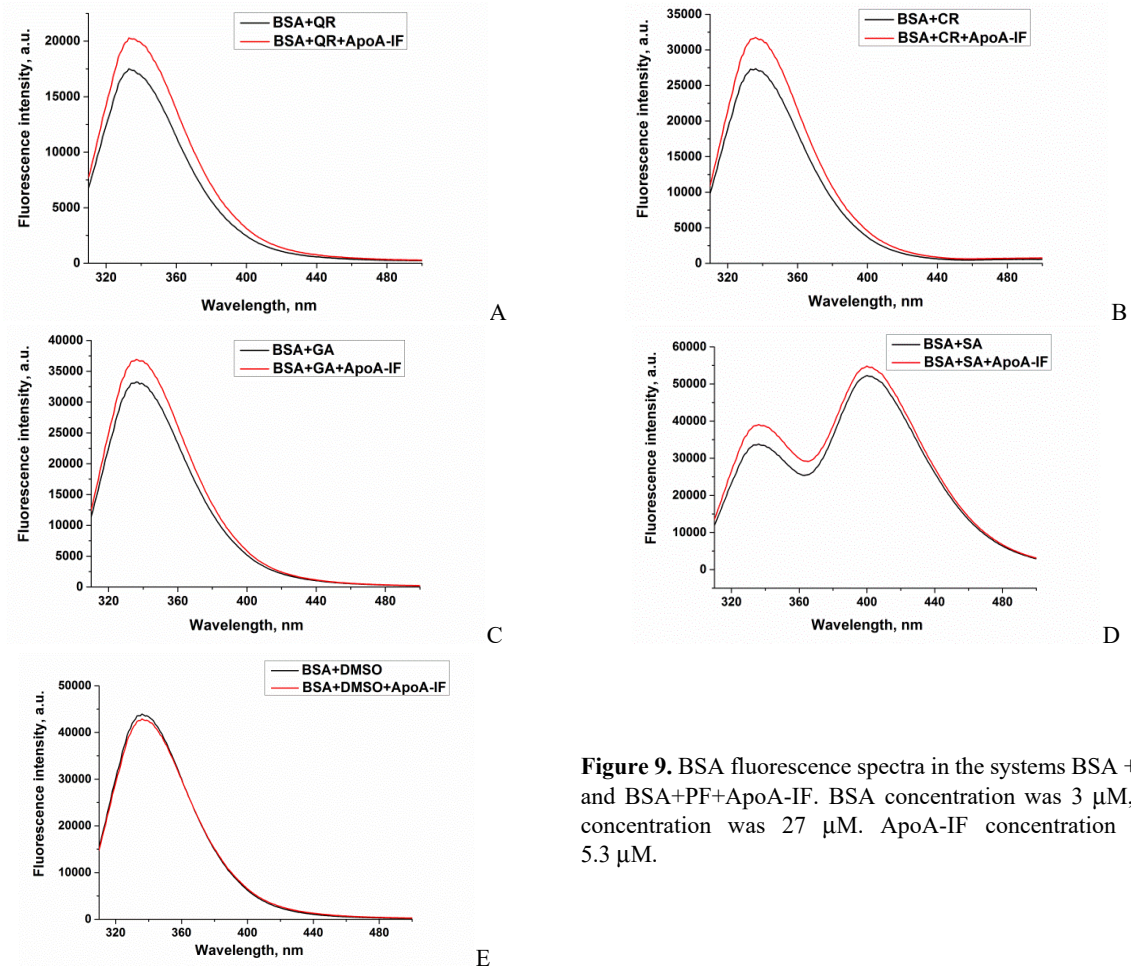


Figure 9. BSA fluorescence spectra in the systems BSA + PF and BSA+PF+ApoA-IF. BSA concentration was 3 μM, PF concentration was 27 μM. ApoA-IF concentration was 5.3 μM.

The measurements of TDV fluorescence in the BSA-containing systems revealed the effects differing from those observed for Hb and Ct. As shown in Fig. 10, A, B, amyloid fibrils brought about significant red shift of the TDV emission maximum (from 592 nm to 604 nm) without marked changes of fluorescence intensity.

Quercetin induced further shift of TDV emission maximum towards 606 nm and slight decrease of fluorescence intensity, while in the presence of curcumin emission maximum of TDV was close to that in BSA solution (593 nm), but fluorescence intensity showed about 5-fold increase (Fig. 10, B). In the systems with gallic and salicylic acids the longest wavelength emission maximum of TDV (~609 nm) and insignificant decrease of fluorescence intensity were observed.

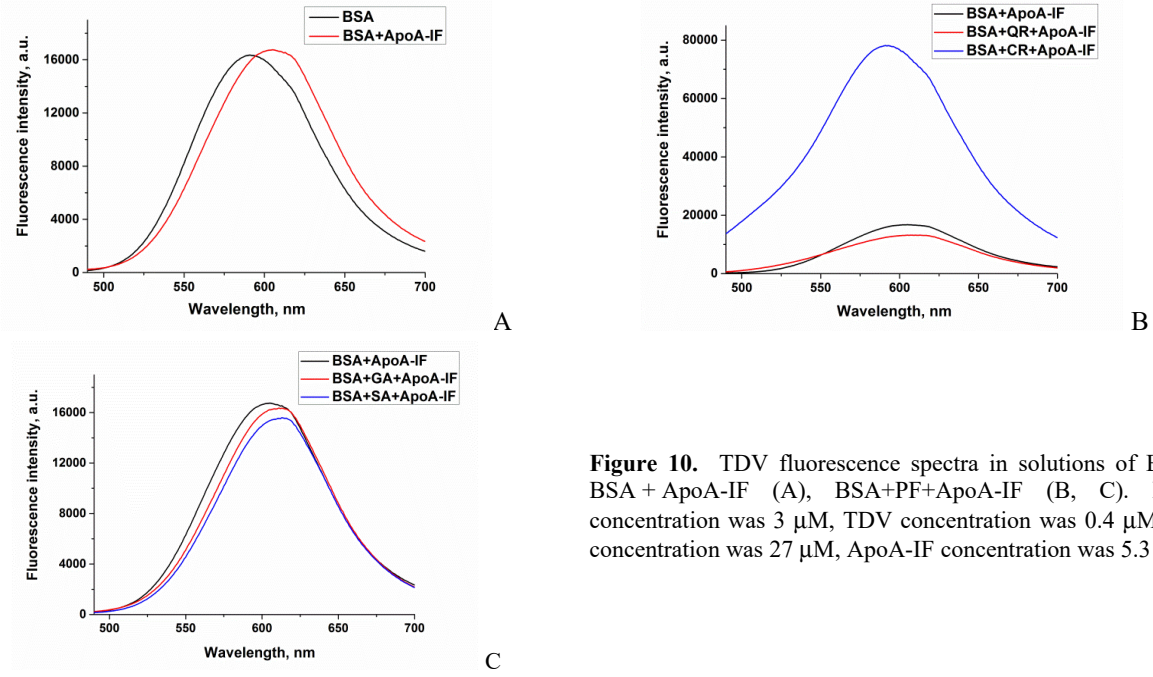


Figure 10. TDV fluorescence spectra in solutions of BSA, BSA + ApoA-IF (A), BSA+PF+ApoA-IF (B, C). BSA concentration was 3 μM, TDV concentration was 0.4 μM, PF concentration was 27 μM, ApoA-IF concentration was 5.3 μM

CONCLUSIONS

In summary, the present study provided new information on the interactions of quercetin, curcumin, gallic and salicylic acids with functionally important proteins such as hemoglobin, cytochrome c and serum albumin, and modulating effects of polyphenols on the binding of amyloid fibrils from N-terminal fragment of apolipoprotein A-I to the proteins. Using several spectroscopic techniques including the differential absorption spectroscopy, intrinsic protein fluorescence and fluorescent probes, the spectral markers of polyphenol modulating abilities have been identified. The analysis of the differential absorption spectra revealed that: i) in the systems Hb+PF+ApoA-IF the peaks around 340, 400 and 420 nm are sensitive to Hb-fibril complexation; ii) quercetin gives rise to the transition of cytochrome c from the ferric to ferrous form as judged from the appearance of the peaks at 520 and 550 nm; iii) the effects of amyloid fibrils are the strongest in the system Ct+CR+ApoA-IF; iv) the formation of BSA-PF complexes resulted in the appearance of characteristic differential peaks depending on PF structure, while amyloid fibrils ApoA-IF did not affect the BSA absorbance. The measurements of the intrinsic protein fluorescence showed that: i) amyloid fibrils bring about the fluorescence increase of Hb and Ct, accompanied by the bathochromic (Hb) or hypsochromic (Ct) shifts of emission maximum; ii) QR and CR produce significant decrease of Hb fluorescence intensity, while the main effect of GA involves a bathochromic shift of Hb emission maximum; iii) the most pronounced changes in Ct fluorescence spectra (bathochromic shift of emission maximum by ~10 nm) were induced by QR and GA; iv) the quantitative interpretation of the data on BSA fluorescence quenching by PF gives the following order for the affinities of polyphenols for BSA: QR ≥ CR > SA >> GA. The spectral alterations of a phosphomium fluorescent probe TDV involved; i) fluorescence increase and hypsochromic shift of emission maximum on the binding of ApoA-IF to Hb and Ct; ii) significant decrease in TDV fluorescence in the systems cotaining Hb and QR/CR; iii) a hypsochromic shift in TDV emission maximum (10 nm for Hb + CR and 3 nm for Ct + CR); iv) considerable (12 nm) bathochromic shift of TDV emission maximum upon BSA complexation of BSA with ApoA-IF, that was enhanced in the presence of QR, GA and SA. Overall, the above findings show that the spectral parameters such as the position and intensity of the bands in differential absorption spectra, the intensity and emission maxima of the intrinsic (Trp) and extrinsic (TDV) fluorophores can serve as sensitive indicators of the protein structural changes produced by amyloid fibrils and polyphenols.

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МОДУЛЮЮЧИЙ ВПЛИВ ПОЛІФЕНОЛІВ НА ВЗАЄМОДІЇ МІЖ ФУНКЦІОНАЛЬНИМИ БІЛКАМИ ТА АМІЛОЇДНИМИ ФІБРИЛАМИ

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В даній роботі з використанням методів диференційної абсорбційної спектроскопії, власної флуоресценції білків та флуоресцентних зондів були досліджені подвійні та потрійні системи, що містили функціональні білки, поліфеноли та амілоїдні фібрили. Група поліфенолів включала представників чотирьох різних класів: флавоноїд кверцетин (QR), фенольну кислоту (саліцилова кислота (SA)), куркуміноїд куркумін (CR) і таннін галова кислоту (GA). Функціонально важливі білки були представлені гемоглобіном (Hb), цитохромом с (Ct) та сироватковим альбуміном (BSA). Амілоїдні фібрили були отримані із N-термінального (1-83) фрагменту аполіпопротеїну A-I з амілоїдогенною мутацією G26R (ApoA-IF). Було продемонстровано, що спектральні маркери, такі як положення та інтенсивність смуг в диференційних спектрах поглинання, інтенсивність флуоресценції та максимуми емісії внутрішніх (Trp) та зовнішніх (TDV) флуорофорів можуть слугувати чутливими індикаторами структурних модифікацій білків під впливом амілоїдних фібрил та поліфенолів. Спостережувані зміни флуоресценції Trp та TDV були інтерпретовані в рамках наступних процесів: i) PF-індукованих конформаційних змін білкових молекул, що супроводжуються змінами мікрооточення залишків Trp; ii) конкуренцією між PF та TDV за білкові сайти зв'язування; iii) нерезонансне гасіння флуоресценції Trp та TDV поліфенолами; iv) резонансне гасіння (Фьорстерівський резонансний перенос енергії) міжTrp чи TDV та PF. В цілому, отримані результати дозволяють припустити, що поліфенольні сполуки здатні модулювати взаємодії між функціональними білками та амілоїдними фібрилами, та можуть розглядатись як перспективні агенти для зниження токсичності амілоїдів.

Ключові слова: *амілоїдні фібрили; функціональні білки; поліфеноли; диференційна абсорбційна спектроскопія; флуоресценція*