Protocols

Interaction of α -synuclein with *Rhus typhina* tannin – Implication for Parkinson's disease

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ABSTRACT

The etiology of Parkinson's disease (PD) relates to α -synuclein, a small protein with the ability to aggregate and form Lewy bodies. One of its prevention strategies is inhibition of α -synuclein oligomerization. We have investigated the interaction of α -synuclein and human serum albumin with 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose (a tannin isolated from the plant *Rhus typhina*). Using fluorescence spectroscopy method we found that this tannin interacts strongly with α -synuclein forming complexes. Circular dichroism analysis showed a time-dependent inhibition of α -synuclein aggregation in the presence of the tannin. On the other hand, 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose had a much stronger interaction with human serum albumin than α -synuclein. The calculated binding constant for tannin-protein interaction was considerably higher for albumin than α -synuclein. This tannin interacted with albumin through a "sphere of action" mechanism. The results lead to the conclusion that 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose is a potent preventive compound against Parkinson's disease. However, this tannin interacts very strongly with human serum albumin, significantly reducing the bioavailability of this compound.

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by symptoms of tremor, bradykinesia and rigidity of muscles [1,2]. Irrespective of etiology of the disease, PD is connected with the appearance of cytoplasmic and neuritic fibrillar inclusions, known as Lewy bodies (LB), in surviving dopaminergic neurons, and Lewy neuritis as well as their death, mainly in the substantia nigra pars compacta [2]. Aggregation of the presynaptic protein, α -synuclein, is assumed to underlie PD pathogenesis as its oligomeric forms are found in the brains of patients. α -synuclein (α -syn.) is a small protein of 140 amino acids, divided into 3 major domains: amphipathic N-terminal region, NAC (non-amyloidogenic component) domain and C-terminal region [3]. The physiological role of α -syn. is not completely understood. Its localization in neuronal synaptic terminal and ability to interact with membranes indicates α -synuclein involvement in neurotransmission.

It is known that this small protein is involved in a synaptic plasticity, and regulation of dopamine release and re-uptake [4]. α -syn. can also influence on the concentration of dopamine by regulating the activity of tyrosine hydroxylase, which is involved in dopamine synthesis [5]. Despite its important physiological role, α -syn. also has neurotoxic effects due to its ability to aggregate. Structural changes of α -syn. due to several missense mutation or increase of its concentration results in overexpression, leading to the formation different oligomers [1–3]. Aggregates of α -syn. may be secreted and captured by other cells. It is assumed that fibrillar forms of α -syn. serve as places for aggregation of soluble monomeric protein [6]. The mechanisms of neurotoxicity of the aggregated forms of α -syn. remain the subject of debate as they are not fully understood. Oligomeric α -syn. can probably form a pore-like structure, cause overload of Ca^{2+} and increase the formation of ROS, which in turn triggers cell death [7].

Fibrils of α -syn. are structural components not only of Lewy bodies in Parkinson's disease, but are found in other disorders; for example, aggregation has been observed in dementia with Lewy bodies, multiple systems atrophy and the Lewy body variant of Alzheimer's disease, all of which that are known collectively as α -synucleinopathies [6]. Inhibition of polypeptide aggregation is

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one of strategies to prevent and treat neuropathologies including PD [8] and other amyloid-associated neurological syndromes [9].

Small polyphenol molecules like flavonoids bind to β -amyloid and prevent amyloid fibril formation or induced disaggregation of preformed α -syn. oligomers [9,10]. Caruana et al. [8] found *in vivo* that such polyphenols as myricetin, baicalein, EGCG (epigallocatechin gallate), scutellarein and black tea extract can be inhibitors of α -syn. oligomerization. Another polyphenol – curcumin – modulates α -syn. aggregation, decreasing the population of toxic oligomeric intermediates [11].

Despite investigations of some interested groups of polyphenols as potential agents in neuroprotection, the tannins (except tannic acid) have not generally been investigated as anti-aggregation factors against α -syn. oligomerization. Tannins, plant polyphenols, are divided into 2 groups, hydrolysable and condensed (proanthocyanidin) [12,13]. They exhibit a wide spectrum of biological activity, acting as antioxidants, antimicrobials, antitumor and antimutagenic drugs [14–16].

The strong biological activity of tannins is the result of their interplay with a variety of biomolecules, proteins in especially [17–22]. Protein-tannin interactions can change protein structure and functionality, and modify their capacity to form aggregates. For example, Fujiwara et al. [23] showed that PGG (1,2,3,4,6-penta-O-galloyl- β -D-glucopyranose) – a hydrolysable tannin isolated from *Paeonia suffruticosa* – inhibits formation of A β _{1–42} fibrils in the Alzheimer SK-N-SH cell line model. From *in vivo* experiments, the same authors found that PGG strongly reduces A β _{1–40} and A β _{1–42} formation in Tg2576 mice hemibrain.

We have investigated the interaction between 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose (RTmc, the main compound of *Rhus typhina* plant extract) with α -syn. to verify whether it is a potential inhibitor of α -syn. aggregation. RTmc was also chosen due to its strong antioxidant activity [24–26], because it can act against free radicals stress – the second main reason of neurodegenerative disorders [27]. We also experimented with HSA, which is the most abundant protein in blood, with an important role as a transporter of different guest molecules [28,29]. Thus the investigation of RTmc interaction with HSA is essential for estimating how albumin can affect the bioavailability of tannin in biological systems.

2. Materials and methods

2.1. Materials

Fatty acid-free HSA and α -syn. were purchased from Sigma-Aldrich (St. Louis, MO, USA). The main compound following water-acetone extraction of *Rhus typhina* leaves is 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose, (RTmc, Fig. 1), which was isolated at the Institute of Bioorganic Chemistry Academy of Science of Uzbekistan by the method of Islambekov [26]. All other compounds were of analytical grade. Distilled or filtered water (conductivity $\sim 0.1 \mu\text{S}$) was used to prepare all the solutions.

2.2. Fluorescence spectroscopy

Fluorescence analysis was used to study the interaction between 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose and proteins. Experiments were done using excitation wavelengths of $\lambda_{\text{exc.}} = 280 \text{ nm}$ and $\lambda_{\text{exc.}} = 295 \text{ nm}$ for α -syn. and albumin, respectively. The emission spectrum range was 290–425 nm. Scanning speed was set at 40 nm/sec. Excitation and emissions slits were set at 7 nm and 5 nm for α -syn., and both at 2.5 nm for HSA. Measurements were made in quartz cuvettes at 37 °C with a Perkin-Elmer LS-55B spectrofluorimeter (Perkin Elmer, UK). The fluorescence

sources were the aromatic tyrosine in α -syn., and tryptophan residues in HSA. The solutions were prepared before experimentation. Proteins were dissolved in phosphate buffer saline ($C = 10 \text{ mM}$, $\text{pH} = 7.4$) and used for experiments at final concentrations of 8 and 4 μM for α -syn. and albumin, respectively. A stock solution of tannin was prepared in water. Because used compound slightly absorb UV light the values of fluorescence intensity received from experiments were corrected (using below equation) to avoid inner filter effect [30,31]:

$$F_{\text{cor.}} = F_{\text{obs.}} \times 10^{(A_{\text{exc.}} + A_{\text{em.}})/2}$$

where: $F_{\text{cor.}}$ and $F_{\text{obs.}}$ are the corrected and observed fluorescence, $A_{\text{exc.}}$ and $A_{\text{em.}}$ are the absorbance of *Rhus typhina* main compound at the excitation and emission wavelength.

2.3. Circular dichroism (CD)

Circular dichroism studies were applied to investigate the influence of 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose on the secondary structure of synuclein and albumin.

The CD spectra for α -syn. and HSA in the presence of RTmc were registered using Jasco J-815 CD spectrometer (Oklahoma, Japan). Concentration-dependent measurements were carried out in a 10 mM Na-phosphate buffer, $\text{pH} = 7.4$.

CD spectra were recorded in the range of 260 and 190 nm using 0.5 cm path-length Helma quartz cell. For analysis, 2 μM concentration of α -syn. and 1 μM concentration of HSA were taken. Spectra show the average of minimum three independent scans.

2.4. Statistical analysis

All experiments were repeated at least 4 times independently. Values are presented as mean $\pm$ SD. Calculations and graphs were made with Microsoft Excel software. Data were verified using Grubb's test with standard α (0.05) to eventually detect outlying values. Statistical significance (results were compared to control) was estimated using one sample *t*-Test, using GraphPad software QuickCalcs: (<http://www.graphpad.com/quickcalcs/>).

3. Results and discussion

Fluorescence analysis is widely used in studies of protein-guest molecule interactions. This technique provides data on the basis of which some interaction parameters can be calculated, including the Stern-Volmer constant (K_{sv}), the binding constant (K_{b}), the quenching constant (k_{q}) and the number of binding sites (n). The type of protein-ligand interaction can also be determined based on fluorescence measurements. Changes in the fluorescence of tyrosines and tryptophan residues were monitored.

3.1. Fluorescence analysis of RTmc – α -syn. interaction

α – synuclein possesses 4 tyrosines (Tyr, Y). A single Tyr is localized in the N-terminal domain at position 39 (Y-39) and 3 are placed at the end of C-terminal domain in positions 125, 133 and 136 [32]. The interaction between α -syn. and RTmc shows that the adding tannin results in a concentration-dependent and statistically significance (with the $p < 0.0004$) decrease in Tyr residue fluorescence indicating that tannin does indeed interact with α -syn. At the lowest RTmc concentration (0.25 μM), fluorescence decreased by 15.8% compared to the control, whereas it was reduced to 70.5% of the initial value at the highest tannin level (4 μM). A strong decrease of Tyr fluorescence suggests that RTmc preferentially interacts with the C-terminal domain of α -syn., which is due to RTmc-synuclein complexation (static quenching mechanism) or collisional encoun-

Fig 1. Structure of 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl-β-D-glucose (RTmc, C₅₅H₄₀O₃₄).

ters between the 2 compounds (a dynamic quenching mechanism). To determine which mechanism is involved in this interaction, the Stern-Volmer equation (Eq. (1)) [33] describing the fluorescence quenching process was used.

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

where: K_{SV} – Stern-Volmer constant, F_0 –fluorescence in the absence of quenching molecules, F – fluorescence in the presence of quencher, $[Q]$ – quenchers concentration.

Our data were set out in a Stern-Volmer plot (Fig. 2A) as a basis for the calculation of K_{SV} . The plot of α -syn. quenching is not a straight line, but has a downward deviation. These results indicate the unequal accessibility of the quencher to more than one fluorophore [34]. Therefore, double Stern-Volmer lines were drawn and 2 Stern-Volmer constants calculated: $K_{SV1} = 542.5 \pm 61.3 \text{ mM}^{-1}$ and $K_{SV2} = 32.1 \pm 14.5 \text{ mM}^{-1}$. As the value of K_{SV1} is much higher compared with K_{SV2} , we supposed that RTmc interacts with specific α -syn. regions. Much higher K_{SV1} may be the result of predominant tannin binding to the C-terminal domain (containing 3 Y residues), followed by weak connection to the N-terminal region (one Y).

Our hypothesis is supported by the biological role of the C-terminal region as a ligand-binding domain [3]. Furthermore, the C-terminal region of α -syn. is rich in proline (at P-108, 117, 120, 128 and 138) that are close to Y residues (Y-125, 133 and 136) [32] and which are known to determine the extent of protein–tannin binding [35,36]. To identify the type of interaction between the α -syn. and tannin, we calculated the quenching constant (k_q) using Eq. (2) [37].

$$k_q = K_{SV}/\tau_0 \quad (2)$$

where τ_0 (5×10^{-9} sec) is the average of fluorescence lifetime of molecules in the excited state.

Both calculated k_q values ($k_{q1} = 10.85 \times 10^{13} \text{ M}^{-1}\text{s}^{-1}$ and $k_{q2} = 0.64 \times 10^{13} \text{ M}^{-1}\text{s}^{-1}$) are higher than the maximum scattering collision quenching constant ($2 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$), indicating the formation of complexes between RTmc and α -syn. (a static quenching mechanism).

Because α -syn. has 4 Y, it was interesting to analyze what of part of fluorophores were accessible (f_i) to the quencher. Therefore, we used modified Stern-Volmer equation (Eq. (3)) [38].

$$F_0/(F_0 - F) = 1/f_i + 1/(f_i \times K_{SVi}) \times 1/[Q] \quad (3)$$

where: F_0 –fluorescence in the absence of quenching molecules, F – fluorescence in the presence of quencher, $[Q]$ – quenchers concentration, K_{SVi} – Stern-Volmer constant for the i -th fluorescent species accessible to the quencher, f_i – fraction of total fluorescence that is accessible to the quencher.

Based on our data, a modified Stern-Volmer plot was obtained (Fig. 2B). Due to its linear character, the plot can be described by the function “ $y = ax + b$ ”, where “ b ” is defined as $1/f_i$ (see Eq. (3)). The calculated f_i was 30%, indicating that only about one-third of the Y fluorescence is available to RTmc compound. For further measurement of the physicochemical parameters of these interactions, the binding constant (K_b) and the number of binding sites (n) were calculated using the equation Eq. (4) [39].

$$\log[(F_0 - F)/F] = \log K_b + n \log [Q] \quad (4)$$

where: F_0 –fluorescence in the absence of quenching molecules, F – fluorescence in the presence of quencher, $[Q]$ – quenchers concentration, K_b – binding constant, n – number of binding sites

Our constants were: $K_b \approx 0.015 \times 10^3 \text{ M}^{-1}$ and $n = 0.3$. When calculated “ n ” is very close to 1, indicating only one high affinity binding site [29]. Received quite low value of “ n ” (Fig. 2C) suggests that α -syn., despite ligand binding domain, do not have the typical

Fig. 2. Stern-Volmer plot of fluorescence quenching of α -syn. tyrosine residues by RTmc (A), modified variant of Stern-Volmer plot (B), plot of $\log [(F_0-F)/F]$ vs. $\log [Q]$ for RTmc interaction with α -Synuclein (C).

binding site for guest molecules what explain obtained small value of binding constant.

3.2. Effect of RTmc on α -syn. aggregation – CD analysis

The fluorescence results clearly demonstrated that RTmc has an affinity for α -syn., with fluorescence quenching occurs by static mechanism. Therefore, circular dichroism (CD) analysis was used to estimate whether RTmc can inhibit α -syn. aggregation.

Fig. 3A shows the time-dependence of the CD spectra of the pure α -syn. The control value of CD [mdeg] maximum, taken for non-incubated α -syn., was -8.69 . After 8 and 48 h incubation at 37°C the CD [mdeg] signal decreased by $\sim 23\%$ and 34% , respectively, compared to the control, indicating aggregation of α -syn. Similar results for pure α -syn. were observed by Milowska et al. [40,41] and Gosh et al. [42]. Both groups observed time-dependent α -syn. secondary structure changes, leading to the appearance a β -structure – the first step in α -syn. aggregation.

Adding RTmc to sample decreased α -syn. aggregation depending on the incubation time (Fig. 3C and D). RTmc was added at 0.25 and $1 \mu\text{M}$ to keep the same RTmc – synuclein molar ratio ($1:8$ and $1:2$) as in fluorescence analysis. RTmc in non-incubated samples of α -syn. (Fig. 3B) changed slightly the CD spectrum. After prolonged incubation (8 and 24 h at 37°C), RTmc the inhibitory effects on α -syn. aggregation were stronger. For the 8 h incubation (Fig. 3C), α -syn. changed in structure by $\sim 6.4\%$ and 7.3% , respectively, whereas at 24 h they were 10.7% and 15% for $0.25 \mu\text{M}$ and $1 \mu\text{M}$ RTmc, respectively (Fig. 3D).

Fluorescence and CD studies of RTmc-synuclein interaction demonstrated that RTmc (3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose) interacts with α -syn. and inhibits its oligomerization. From our data, we propose the simplified mechanism (Fig. 4) of α -syn. aggregation (pathway 1) and its inhibition by RTmc (pathway 2).

Our results are in good accordance with other works. Caruana et al. [8] showed that some types of polyphenol compounds can inhibit α -syn. oligomerization and cause their disaggregation. Most activity was seen with tannic acid at an $\text{IC}_{50} = 61 \text{ nM}$, whereas for smaller polyphenol molecules such as EGCG and baicalein, the values were $0.79 \mu\text{M}$ and $2.03 \mu\text{M}$, respectively. Another polyphenol – curcumin – could also bind to α -syn. oligomers and fibrils, reducing their toxicity in cell culture [11]. It should be emphasized that RTmc can inhibit α -syn. aggregation not only directly by formation of RTmc-synuclein complexes, but it may also act indirectly by preventing the phosphorylation and some post-translational modifications of α -syn. *In vitro* studies describe phosphorylation of serine-129 and its glycosylation (a post-translational modification), and also nitration of 3 Y located in C-terminal (Y-125, 133 and 136) promote α -syn oligomerization [32]. We suggest that RTmc, by interaction with α -syn. – probably by binding with the proline-rich C-terminal domain – leads to changes in protein conformation that hinder its phosphorylation, glycosylation and nitration.

3.3. Interaction of RTmc with HSA

Bioavailability of tannins can be diminishing via their interaction with other proteins in organism especially with this one, which are in the highest concentration and play an important role as transporters for different compounds. Protein, which can potentially very strong reduce bioavailability of tannins is human serum albumin – the main blood protein, acting as carrier for different compounds like fatty acids, drugs or metal ions [43]. HSA forms complexes with tannins, the strength of which is largely determined by the structure of the tannins [21,35], making it important for us to investigate the interaction between RTmc and albumin (Fig. 5).

Tannin (with statistically significance $p < 0.0005$) decreased Trp-214 fluorescence of HSA in a concentration-dependent manner. At the highest RTmc concentration ($4 \mu\text{M}$), fluorescence was reduced by $\sim 59\%$ compared to the control (taken as 100%). For RTmc interacting with α -syn., fluorescence decreased by 29.5% at the same tannin concentration. From these results, it can be concluded that RTmc has a stronger affinity for HSA than α -syn. The Stern-Volmer plot (Fig. 5A) shows an upward deviation, probably as a result of both static and the dynamic or “sphere of action” fluorescence quenching mechanism [21]. Therefore, we redrew the modified Stern-Volmer graph (Fig. 5B) as previously described [21], which gave a constant of $K_{SV} = 222.8 \pm 13.2 \text{ mM}^{-1}$. The resulting linear relationship indicates that the RTmc-albumin interaction takes place by complex formation, with fluorescence quenching being due to the “sphere of action” mechanism.

Based on the plot in Fig. 5C, the binding constant “ K_b ” and the number of binding sites “ n ” for RTmc in albumin were calculated as $K_b = 3.22 \times 10^6 \text{ M}^{-1}$ and $n = 1.2$, respectively. The “ n ” value shows

Fig. 3. CD analysis of α -syn. aggregation without (A) and with RTmc (B–D).

Fig. 4. Simplified mechanism of α -syn. aggregation (pathway 1) and inhibition of α -syn. aggregation by RTmc (pathway 2).

that HSA has more than one independent class of binding sites for RTmc. The calculated binding constant (K_b) of RTmc is much higher for albumin than α -syn., proving a stronger interaction with HSA. This significant difference in binding constants results probably is related to protein structure and function. Alpha-synuclein has only one domain responsible for ligand binding (the C-terminal region) [3], whereas HSA has 2 major binding sites: site I and site II [29], and is one of the main transport proteins.

CD analysis was done to verify whether the RTmc-HSA interaction induces changes in albumin secondary structure (Fig. 6).

The measurements show that tannin, at the RTmc/HSA molar ratios used here, do not change albumin's secondary structure. Similar results were obtained previously for bihexahydroxydiphenyl-trigalloylglucose (BDTG) isolated from *Geranium sanguineum* and 1-O-galloyl-4,6-hexahydroxydiphenyl- β -D-glucose (OG β DG) from *Oenothera gigas* [21]. The slightly distorted intensity of the CD [mdeg] signal, but without any changes in the secondary

Fig. 5. Stern-Volmer plot of Trp-214 fluorescence quenching in HSA (A); modified Stern-Volmer plot of Trp-214 fluorescence quenching in HSA (B); Plot of $\log[(F_0 - F)/F]$ vs. $\log [Q]$ for RTmc interaction with HSA (C).

Fig. 6. CD spectra of pure HSA and after added RTmc.

structure, supports our hypothesis that the “sphere of action” mechanism is responsible for RTmc-HSA interaction. Therefore, it can be concluded that interaction between albumin and RTmc is of a superficial nature.

4. Conclusions

Studies on interaction between *Rhus typhina* main compound (3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl- β -D-glucose) with α -syn. and HSA show that the tannin we have used strongly interacts with both proteins. RTmc decreases α -syn. tyrosine fluorescence as the result of complexation with this protein. Furthermore, this tannin inhibits α -syn. aggregation. On the other hand, a very strong interaction with HSA can significantly reduce RTmc bioavailability to α -syn. Despite these promising results, further research focused on RTmc as a potential protector against the development of Parkinson's disease is needed.

Conflict of interest

The authors declare no conflict of interest.

References

- [1] K.E. Paleologou, G.B. Irvine, O.M.A. El-Agnaf, *Biochem. Soc. Trans.* 33 (2005) 1106.
- [2] W. Xin, S. Emadi, S. Williams, Q. Liu, P. Schulz, P. He, N.B. Alam, J. Wu, M. R. Sierks, *Biomolecules* 5 (2015) 1634.
- [3] S. Moussaud, D.R. Jones, E.L. Moussaud-Lamodière, M. Delenclos, O.A. Ross, P.J. McLean, *Mol. Neurodegener.* 9 (43) (2014) 1.
- [4] G. Di Rosa, D. Puzzo, A. Sant'Angelo, F. Trinchese, O. Arancio, *Histol. Histopathol.* 18 (2003) 1257.
- [5] R.G. Perez, J.C. Waymire, E. Lin, J.J. Liu, F. Guo, M.J. Zigmond, *J. Neurosci.* 22 (8) (2002) 3090.
- [6] E.A. Waxman, B.I. Giasson, *J. Neurochem.* 113 (2010) 374.
- [7] P.R. Angelova, M.H.R. Ludtmann, M.H. Horrocks, A. Negoda, N. Cremades, D. Klenerman, C.M. Dobson, N.W. Wood, E.V. Pavlov, S. Gandhi, A.Y. Abramov, *J. Cell Sci.* 129 (2016) 1792.
- [8] M. Caruana, T. Högen, J. Levin, A. Hillmer, A. Giese, N. Vassallo, *FEBS Lett.* 585 (2011) 1113.
- [9] Y. Porat, A. Abramowitz, E. Gazit, *Chem. Biol. Drug Des.* 67 (1) (2006) 27.
- [10] M.E. Herva, S. Zibae, G. Fraser, R.A. Barker, M. Goedert, M.G. Spillantini, *J. Biol. Chem.* 17 (2014) 11897.
- [11] P.K. Singh, V. Kotia, D. Ghosh, G.M. Mohite, A. Kumar, S.K. Maji, *ACS Chem. Neurosci.* 4 (2013) 393.
- [12] S.M. Mavlyanov, S.Y. Islambekov, A.I. Ismailov, D.N. Dalimov, N.G. Abdulladzhanova, *Chem. Nat. Compd.* 37 (2001) 1.
- [13] V. Kolečkar, K. Kubikova, Z. Rehakova, K. Kuca, D. Jun, L. Jahodar, L. Opletal, *Mini Rev. Med. Chem.* 8 (2008) 436.
- [14] J. Serrano, R. Puupponen-Pimia, A. Dauer, A.-M. Aura, F. Saura-Calixto, *Mol. Nutr. Food Res.* 53 (2009) S310.
- [15] T. Okuda, H. Ito, *Molecules* 16 (2011) 2191.
- [16] A. Smeriglio, D. Barreca, E. Bellocco, D. Trombetta, Br. J. Pharmacol. (September) (2016), <http://dx.doi.org/10.1111/bph.13630>.
- [17] E. Haslam, *J. Nat. Prod.* 59 (1996) 205.
- [18] M. Labieniec, T. Gabryelak, *J. Photochem. Photobiol. B* 82 (2006) 72.
- [19] C. Le Bourvellec, C.M. Renard, *Crit. Rev. Food Sci. Nutr.* 52 (2012) 213.
- [20] M. Li, A.E. Hagerman, *Curr. Drug Metab.* 14 (2013) 432.
- [21] S. Sekowski, M. Ionov, M. Kaszuba, S. Mavlyanov, M. Bryszewska, M. Zamaraeva, *Colloids Surf. B: Biointerfaces* 123 (2014) 623–628.
- [22] S. Sekowski, M. Ionov, A. Dubis, S. Mavlyanov, M. Bryszewska, M. Zamaraeva, *J. Membr. Biol.* 249 (2016) 171.
- [23] H. Fujiwara, M. Tabuchi, T. Yamaguchi, K. Iwasaki, K. Furukawa, K. Sekiguchi, Y. Ikarashi, Y. Kudo, M. Higuchi, T.C. Saido, S. Maeda, A. Takashima, M. Hara, N. Yaegashi, Y. Kase, H. Arai, *J. Neurochem.* 109 (2009) 1648.
- [24] E. Olchowik, A. Sciepek, S. Mavlyanov, N. Abdullajanova, M. Zamaraeva, *Biomed. Prev. Nutr.* 2 (2011) 99.
- [25] E. Olchowik, K. Lotkowski, S. Mavlyanov, N. Abdullajanova, M. Ionov, M. Bryszewska, M. Zamaraeva, *Cell. Mol. Biol. Lett.* 17 (2012) 333.
- [26] E. Olchowik-Grabarek, S. Mavlyanov, N. Abdullajanova, R. Gieniusz, M. Zamaraeva, *Appl. Biochem. Biotechnol.* 181 (2017) 495.
- [27] E. Niedzielska, I. Smaga, M. Gawlik, A. Moniczewski, P. Stankowicz, J. Pera, M. Filip, *Mol. Neurobiol.* 53 (2016) 4094.
- [28] U. Anand, S. Mukherje, *Biochim. Biophys. Acta* 1830 (2013) 5394.
- [29] A. Xing, B. Jia, X. Li, Z. Zhang, G. Gu, G. Wang, *J. Fluorine Chem.* 153 (2013) 107.
- [30] M. Li, A.E. Hagerman, *Free Radic. Res.* 49 (8) (2015) 946.
- [31] J.A. Molina-Bolívar, C. Carnero Ruiz, F. Galisteo-González, M. Medina-O'Donnell, A. Parra, *J. Lumin.* 178 (2016) 259.

- [32] A. Recchia, P. Debetto, A. Negro, D. Guidolin, S.D. Skaper, P. Giusti, *FASEB* 18 (2004) 617.
- [33] J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd ed., Kluwer Academic/Plenum Publishers, New York, 1999, 698.
- [34] V.M. da Silva Fragoso, C.P. de Morais Coura, L.Y. Hoppe, M.A. Gomes Soares, D. Silva, C.M. Cortez, *Int. J. Mol. Sci.* 17 (2016) 59.
- [35] S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouysegou, *Angew. Chem. Int. Ed.* 50 (2011) 586.
- [36] M.G. Ferruzzi, N. Bordenave, B.R. Hamaker, *Physiol. Behav.* 107 (2012) 591.
- [37] J.R. Lakowicz, G. Weber, *Biochemistry* 12 (1973) 4171.
- [38] K. Owczarek, O. Nowacka, B. Klajnert, J. Kujawa, M. Bryszewska, *Neuroendocrinol. Lett.* 34 (6) (2013) 573.
- [39] S. Naveenraj, S. Anandan, A. Kathiravan, R. Renganathan, M. Ashokkumar, *J. Pharm. Biomed. Anal.* 53 (2010) 804.
- [40] K. Milowska, M. Malachowska, T. Gabryelak, *Int. J. Biol. Macromol.* 48 (2011) 742.
- [41] K. Milowska, T. Gabryelak, M. Bryszewska, A.-M. Caminade, J.-P. Majoral, *Int. J. Biol. Macromol.* 50 (2012) 1138.
- [42] D. Ghosh, P.K. Singh, S. Sahay, N.N. Jha, R.S. Jacob, S. Sen, A. Kumar, R. Riek, S.K. Maji, *Sci. Rep.* 5 (2015) 9228, <http://dx.doi.org/10.1038/srep09228>.
- [43] A. Varshney, P. Sen, E. Ahmad, M. Rehan, N. Subbarao, R.H. Khan, *Chirality* 22 (2010) 77.