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Polyphenols and Alzheimer's Disease: Tau/polyphenol interactions investigated by NMR and molecular modelling

Marie Guéroux[a], Michel Laguerre[b], Magali Szlosek-Pinaud[a], Eric Fouquet[a] and Isabelle Pianet[a,*]
[a]*University of Bordeaux, CNRS, Institut des Sciences Moléculaires, Talence, France*
[b]*University of Bordeaux, CNRS, Chimie et Biologie des Membranes et Nanonobjets, Pessac, France*

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Abstract. Tau that belongs to the family of microtubule-associated proteins is the major constituent of intraneuronal fibrillar lesions described in Alzheimer's Disease (AD). Its sequence homology with salivary proline-rich proteins (sPRP), known to fix tannins, combined with some epidemiological evidences that a moderate consumption of polyphenols presents benefits in AD, suggests that tannins can bind Tau protein and, perhaps, could inhibit or modulate the cascade of events leading to AD symptoms. To study the affinity of tannins towards the Tau proline rich domain P2, we have first synthesized a peptide representative of this domain, determined its 3D-structure and its affinity towards different procyanidins (epicatechin-EC, epicatechin gallate-ECG, epigallocatechin gallate-EGCG and procyanidin B3 and B2) by using both NMR, molecular modelling and dynamic techniques. We have found that the Tau peptide is able to fix the different tested tannins in two distinct domains with an affinity in the mM range.

Keywords: Alzheimer, Tau, procyanidins, interactions, NMR, molecular modelling

1. Introduction

Alzheimer Disease (AD) is a progressive neurodegenerative disorder and major public health concern within an aging population. This pathology, leading to a progressive and irreversible loss of memory and cognitive functions, is characterized by the presence of β amyloid plaques and neurofibrillary tangles (NFT) in the brain. NFT are mainly composed of the microtubule-associated Tau protein in an abnormal hyperphosphorylated state. NFT are first present in the hippocampic region (involved in memory management), and appear in the associative cortical regions, and across the cerebral cortex. This chain of reactions leads to the death of a large number of neurons and, consequently to the appearance of clinical signs [1]. The involvement of Tau in AD is commonly established, notably, its abnormal phosphorylation leads to its polymerization into NFT and to the subsequent microtubules disorganization [2–4]. Phosphorylation of Tau takes place on serine/threonine residues principally located in the basic proline-rich domains P2 of the protein [3]. The similarities between P2 and the amino acid sequence of basic Proline-Rich proteins of saliva (sPRP) (richness in proline and glycine residues, presence of basic amino acids such as glutamine or arginine) suggest that these domains are potent targets able to fix polyphenols [5, 6] and that tannins (polyphenols) could inhibit Tau aggregation [7]. For this purpose, we synthesized a peptide of 20 residues length, issued from the P2 domain of Tau, corresponding to the G201-P220 segment (Scheme 1,

*Corresponding author: Isabelle Pianet, University of Bordeaux, CNRS, Institut des Sciences Moléculaires UMR5255, Talence, France. E-mail: i.pianet@ism.u-bordeaux1.fr.

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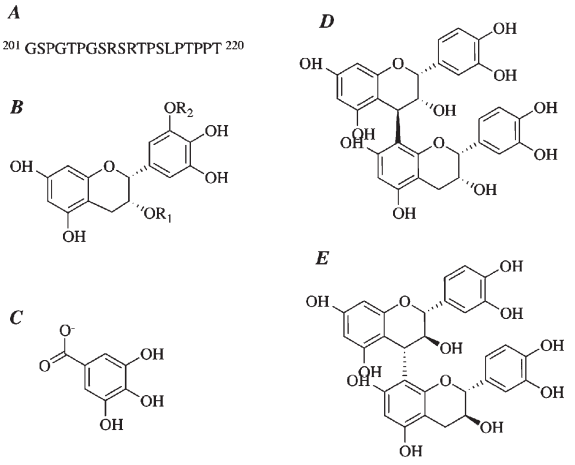

Scheme 1. Chemical structures of peptide P2 (A), Epicatechin (B, R1 = R2 = H), Epicatechin gallate ECG (B, R1 = C, R2 = H), Epigallocatechin gallate EGCG (B, R1 = C, R2 = OH), procyanidin B2 (D) and procyanidin B3 (E).

A) containing four serines and four threonines, some of which being known to be specifically phosphorylated in the disease [8]. Then we have monitored the interactions between five different tannins of the procyanidins family (Scheme 1, B to D) and the peptide by NMR and molecular dynamics. Monitoring the peptide proton chemical shift induced by adding the different procyanidins, gives rise to some physicochemical parameters of interest: the stoichiometry of the complex formed, the interaction sites (n) and the dissociation constant of the complex formation (Kd). The obtained results evidence the specific interaction between four of the five tested tannins in two distinct domains of the Tau peptide with different affinities depending on the tannin structure.

2. Materials and methods

2.1. Synthesis

Synthesis of Tau peptide (^{201}GSPGTPGSRSRT PSLPTPPT^{220}) was performed by using a solid phase strategy (Merrifield 1963) with a FastMoc chemistry on an Applied Biosystem synthesiser 431 A. ECG, B3 and B2 dimers were synthesised by using an approach previously developed [9]. EGCG and epicatechin were purchased from Sigma-Aldrich.

2.2. Samples preparation

For the structure determination of the peptide, a 4 mM solution was prepared in a H_2O/D_2O: 90/10 mixture or with only D_2O (to facilitate the proline assignments) with 10 mM of KH_2PO_4 buffered at pH 7. For titration experiments, tannins are progressively added to a 0.5 or 1 mM peptide solution in a KH_2PO_4 buffer, pH 6.2, dissolved in H_2O/D_2O 90:10 to scan a tannin concentration ranging from 0 to 5–10 mM. In order to keep constant the peptide concentration, tannins are added under a lyophilised form.

2.3. NMR spectroscopy

All NMR spectra were recorded at 293 K on a Bruker Avance III 600 spectrometer equipped with a 5 mm gradient inverse broadband probe with ^{2}H lock. Proton and carbone chemical shifts are given with respect to TSP (Tetramethyl Silyl Propionate). For the total assignment of the peptide NMR resonances and the constraints file built for molecular modelling purpose, the usual 2D NMR spectra were recorded: TOCSY, HSQC and NOESY. Data processing and analysis were performed using the Topspin software version 2.1.

For titration experiments, chemical shift variations of some protons of the peptide were analyzed as a function of procyanidin concentration using the equation previously described for a multisite model [10]:

$$\Delta\delta_i = {}^1\!/_2\,\Delta\delta_{max}\,[(1 + K_d/n[P_0] + [T_i]/n[P_0]) - \{(1 + K_d/n[P_0] + [T_i]/n[P_0])^2 - 4[T_i]/n[P_0]\}^{1/2}] \quad (1)$$

where $\Delta\delta_i$, is the chemical shift variation (ppm), $\Delta\delta_{max}$ the chemical shift difference between the chemical shift of the peptide alone and saturated with tannins, K_d the dissociation constant expressed in M, $[T_i]$, the total concentration of polyphenol able to fix the peptide (in M) by taking into account their self-association [11], $[P_0]$, the total concentration of peptide (in M), and n the number of polyphenol binding sites. K_d, n and $\Delta\delta_{max}$ were calculated using a least-squares-fitting routine within the software program Microsoft EXCEL.

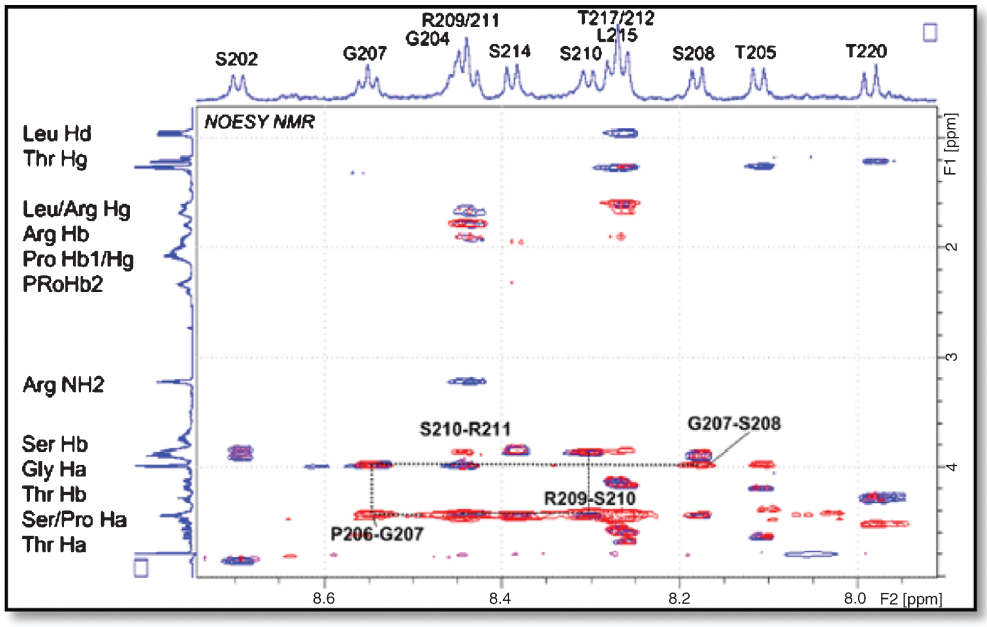

Fig. 1. NMR TOCSY (in blue) and NOESY (in red) spectra of Tau peptide.

2.4. Molecular and dynamic modelling

Minimization and molecular dynamic runs were performed under NMR constraints (NOE and ^{3}J). The following distance constraints were chosen according to the NOE correlation intensities: strong, 2.2 ± 0.4 Å; medium, 3.5 ± 0.9 Å; and weak, 5.0 ± 0.5 Å. These distance constraints are chosen in order to ensure three contiguous classes of distances without overlap but also without any "forbidden" intervals. Conformational minima were found using the modified AMBER* (1991 parameters) force field as implemented and completed in the MacroModel program and dynamic run calculations were accomplished using GROMACS version 4.5 and the GROMOS96 force field (G43a1).

3. Results

3.1. Tau peptide 3D structure

The total NMR resonances (^{1}H, ^{13}C and ^{15}N) assignment of all residues of the peptide was achieved using the sequential assignment strategy developed by Wüthrich [12]. The TOCSY spectrum gives access to the identification of the amino acid type thanks to its specific spin system (Fig. 1, blue): for example, S202 have its NH resonance as a doublet at 8.68 ppm correlated with its Hβ at 4.80 ppm and α around 3.8 ppm. The NOESY spectrum gives rise to the sequential assignment thanks to the correlation pattern between the NH of an amino acid (i + 1) and the Hβ of the previous residue (i) (Fig. 1, red) : for example, the NH resonance of S208 at 8.17 ppm is correlated with the Hβ of G207 at 4.0 ppm. The constraints file, composed of 13 ^{3}J (NH-Hβ) and 18 NOEs, was used to calculate the 3D-structure preference that the peptide adopts: the peptide presents a non-structured and extended confor-

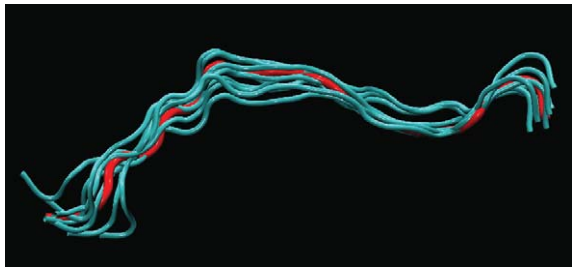

Fig. 2. The ten best conformations of Tau, the red bold trace representing the lowest energy conformer.

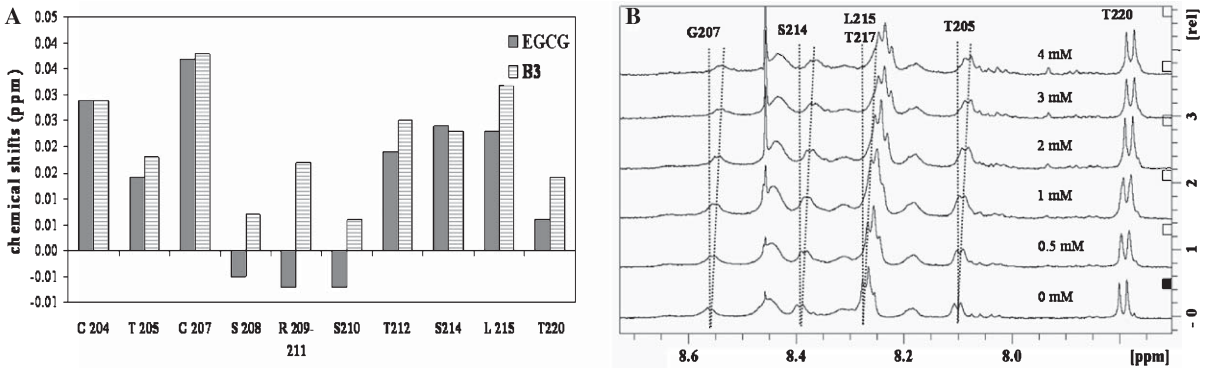

Fig. 3. NH Chemical shift variations of Tau peptide upon procyanidin additions. A. maximal amplitude observed at each residue (ppm). B. ^{1}H spectra recorded at 600 MHz for different ECG concentrations.

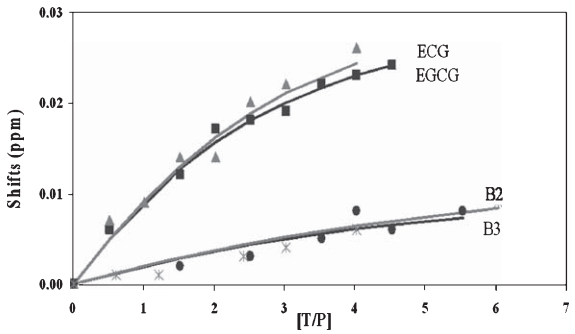

Fig. 4. Observed (symbol) and fitted (-) chemical shift differences ($\delta_{free} - \delta_{bound}$) of the NH resonance of T212 for the titration experiments with increasing concentration of ECG, EGCG, B2 or B3.

mation. Figure 2 displays the 10 best conformations obtained under NMR constraints.

3.2. Titration experiments

When procyanidins are progressively added to the peptide solution, significant chemical shift variations are observed, mainly in the NH region of the peptide (Fig. 3). Two regions are affected: around T205 and T212.

The chemical shift variations induced by the different tannins tested are displayed in Fig. 4 (NH of T212), and showed saturation curves characteristic of a specific binding. In fact, all the titration experiments were performed in a tannin concentration range avoiding their self-association [13, 11] and thus the consequential non-specific binding [14]. Experimental data points were fitted using Eq. [1] resulting from a simple host-guest model for which several sites (n) exhibit the same dissociation constant (Kd) towards the peptide [15]. The fitting was performed using Kd, n and $\Delta\delta_{max}$ as adjustable parameters, and the values obtained are reported on Table 1.

Table 1

Binding Data. Dissociation constant (Kd), and number of procyanidins binding sites (n) were obtained from the fit of experimental chemical shift variations using Eq. [1]. The different Kd ad n values obtained are averaged and reported ± SD. NM: not measurable

Procyanidins	Kd (mM)	N
Epicatechin	NM	NM
B3	4.0 ± 0.2	2.9 ± 0.2
B2	4.0 ± 0.2	2.9 ± 0.2
ECG	1.1 ± 0.4	2.4 ± 0.2
EGCG	0.9 ± 0.2	3.1 ± 0.2

It is noteworthy that, if epicatechin is not able to fix the peptide since no chemical shift variation is observed when it is added to the peptide solution, changes are observed for the two galloylated monomers ECG and EGCG or for the two dimers B2 and B3. Two set of compounds can be distinguished: contrary to the non-galloylated dimers B2 and B3, the galloylated procyanidins induce great chemical shift changes starting at 0.5 mM, and reach a plateau for 5 mM. Those differences are expressed in the Kd values calculated from the fitting of the experimental data points: the Kd for ECG or EGCG is close to 1 mM while it attains 4 mM for the two dimers. However, the number of fixation sites remains unchanged whatever the procyanidin considered and is close to three.

3.3. Molecular dynamics

Molecular Dynamic calculations were run on a system composed of one peptide and four procyanidins

ECG, EGCG, B2 or B3 in their preferred conformations, disposed randomly in a $(100\ Å)^3$ box full of water molecules. With such conditions, the *in silico* concentrations are about 7 mM for procyanidins and 1 mM for the peptide, and are close to the experimental values. All the calculations were run at least three times with different random seeds and, the most frequent scenario is described above.

At the end of the calculation corresponding to a 60 ns dynamic, in all the cases, two or even four procyanidins (whatever it is, *i.e.* EGCG, ECG, B2, B3) fix the peptide in the two distinct domains: around T205 and T212 (Fig. 5), confirming the data observed during the titration experiments. However, some discrepancies are observed from one procyanidin to another, notably in their ability to develop hydrogen bonds with the peptide. EGCG and, in a less extent, ECG appear to establish a high level of relatively stable hydrogen bonds with the peptide much more than the non-galloylated procyanidins B2 and B3 for which, at the most, one highly labile hydrogen bond is observed. These results can explain the affinity differences measured for the four procyanidins studied.

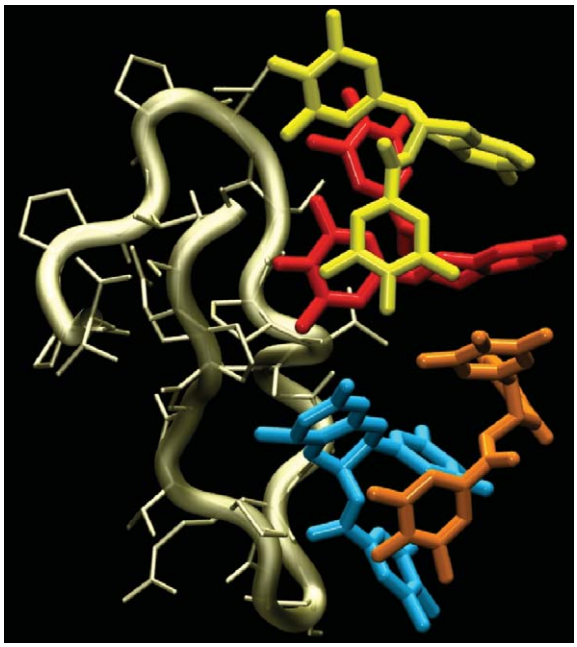

Fig. 5. Tau-EGCG complex.

4. Discussion

The aim of this work was to study the ability of some polyphenols belonging to the procyanidin family, to bind specifically the Proline-Rich region of Tau. The chosen peptide (G201-P220) arises from the P2 region of Tau, which is known to be the place of the multiple phosphorylations, that modulate both its physiological role of microtubule binding and its aggregation into NFT characterizing Alzheimer's diseased neurons. The compounds tested were chosen in order to scan the structural variations commonly observed for procyanidins, notably the degree of oligomerization, the galloylation or the stereochemistry. The objective was to evaluate the influence of the procyanidins structure upon their affinity for the peptide.

Both NMR and Molecular Modelling data converge to the same conclusions: except for the monomer epicatechin, all the tannins tested were able to fix specifically the peptide in two distinct regions (T205 and T212) by hydrogen bounds. In all cases, up to three procyanidins were able to bind the peptide but with different affinities depending on their structure. The galloylated procyanidins ECG and EGCG exhibit a higher affinity with respect to the non-galloylated procyanidins suggesting that the galloyl group can play a critical role in the interaction. However, and contrary to what was observed for saliva PRPs [14], the two dimers B2 and B3 have the same affinity, showing that the stereochemistry of the heterocyclic ring, meanwhile, does not influence the interaction strength.

Seed-derived polyphenols, such as procyanidins, are described to reduce Tau pathology in a mouse model of Tauopathy [16], probably by altering the ultrastructure of the NFT [6] or even by playing a key role in the phosphorylation process of Tau [18]: they appear to disrupt the filamentous structure of Paired Helical Filaments (PHF, intermediary Tau structure leading to the formation of NFT) and to interact with the P2 region [6]. Since this region is involved in the phosphorylation of Tau (notably S214 which phosphorylation is described to play a role in the aggregation process of Tau [8]), it could suggest that polyphenols could have a protective effect through the neutralization of this phosphoepitope [17]. Moreover, the correlation observed between a seed-derived polyphenols-enriched diet and the decrease in AD hallmarks (notably the inhibition of Tau aggregation) determined on mice model of AD lead to envisage that polyphenols could, at least, partially be able to pass through the hematoencephalic barrier [16].

Even if our present work contributes to understand the mode of action of these compounds at a molecular/atomic level, the low affinity measured for procyanidins towards P2 (in the mM range) has to be questioned in term of biological meanings. However, two facts have to be notified: first, this low affinity is surprising when we take into account the IC50 value measured to disrupt PHF (<μM range [6]). Nonetheless, in the present work, Kd were determined on a short peptide of 20 amino-acid residues and they are expected to be lower on the entire Tau protein (440 amino-acids) due to the ability of a longer peptide to fold and surround the procyanidins, thereby increasing the association by cooperative intra-molecular interactions [18, 19]. The second point to underline concerns the affinity of kinases towards their proline-rich motif target which is also described to be in the mM range [20], so that procyanidins and kinases could act as competitors towards this domain of Tau. All these preliminary results are undoubtedly encouraging in the search for new drugs susceptible to slow down or even to prevent the Alzheimer's Disease.

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References

[1] Dubois B, Feldman HH, Jacova C, Cummings JL, DeKosky ST, Barberger-gateau P, et al. Revising the definition of Alzheimer's disease: A new lexicon. Lancet Neurol. 2010;9:1118-27.

[2] Bulic B, Pickhardt M, Schmidt B, Mandelkow EM, Waldmann H, Mandelkow E. Development of tau aggregation inhibitors for Alzheimer's disease. Angew Chem Int Ed. 2009;48:1740-52.

[3] Sergeant N, Delacourte A, Buée L. Tau protein as a differential biomarker of tauopathies. Biochim Biophys Acta. 2005;1739:179-97.

[4] Buée L, Troquier L, Burnouf S, Belarbi K, Van der Jeugd A, Ahmed T, et al. From tau phosphorylation to tau aggregation: What about neuronal death. Biochem Soc T. 2010;38:967-72.

[5] Brunden KR, Ballatore C, Crowe A, Smith AB III, Lee VM-Y, Trojanowski JQ. Tau-directed drug discovery for Alzheimer's disease and related tauopathies: A focus on tau assembly inhibitors. Exp Neurol. 2010;223(2):304-10.

[6] Ksiezak-Reding H, Ho L, Santa-Maria I, Diaz-Ruiz C, Wang J, Pasinetti GM. Ultrastructural alterations of Alzheimer's disease paired helical filaments by grape seed-derived polyphenols. Neurobiol Aging. 2012;33:1427-39.

[7] Han LJ, Shi S, LZheng LF, Yang DJ, Yao TM, Ji LN. Flavonids inhibit heparin-induced aggregation of the third repeat of microtubule-binding domain of Alzheimer's Tau protein. B Chem Soc Jpn. 2010;83(8):911-22.

[8] Landrieu I, Lacosse L, Leroy A, Wieruszeski JM, Trivelli X, Sillen A, et al. NMR analysis of tau phosphorylation pattern. J Am Chem Soc. 2006;128:3575-83.

[9] Tarascou I, Barathieu K, Andre Y, Pianet I, Dufourc EJ, Fouquet E. An improved synthesis of procyanidin dimers: Regio- and stereocontrol of the interflavan bond. Eur J Org Chem. 2006;(23):5367-77.

[10] Charlton AJ, Baxter NJ, Lilley TH, Haslam E, McDonald CJ, Williamson MP. Tannin interactions with a full-length human salivary proline-rich protein display a stronger affinity than with single proline-rich repeats. FEBS Lett. 1996;382:289-92.

[11] Pianet I, André Y, Ducasse MA, Tarascou I, Lartigue JC, Pinaud N, et al. Modeling procyanidin self-association processes and understanding their micellar organization: A study by diffusion NMR and molecular mechanics. Langmuir. 2008;24(19):11027-35.

[12] Wüthrich K. The way to NMR structure of proteins. Nature Struct Biol. 2001;8:923-5.

[13] Fabre S, Pinaud N, Fouquet E, Pianet I. Colloidal behavior of wine galloylated tannins. CR Chimie. 2010;13(5):561-5.

[14] Cala O, Pinaud N, Simon C, Fouquet E, Laguerre M, Dufourc EJ, et al. NMR and molecular modeling of wine tannins binding to saliva proteins: Revisiting astringency from molecular and colloidal prospects. FASEB J. 2010;24(11):4281-90.

[15] Baxter NJ, Lilley TH, Haslam E, Williamson MP. Multiple interaction between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry. 1997;36:5566-77.

[16] Wang JZ, Santa-Maria I, Ho L, Ksiezak-Reding H, Ono K, Teplow DB, et al. Inhibition of tau aggregation by grape derived polyphenols in a mouse model of Alzheimer's disease. J Alzheimers Dis. 2010;22:653-61.

[17] Miyai S, Yamaguchi A, Iwasaki T, Shamsa F, Ohtsuki K. Biochemical Characterization of epigallocatechin-3-gallate as an effective stimulator for the phosphorylation of its binding proteins by glycogen synthase kinase-3b *in vitro*. Biol Pharm Bull. 2010;33(12):1932-7.

[18] Cala O, Dufourc EJ, Fouquet E, Manigand C, Laguerre M, Pianet I. The colloidal state of tannins impacts the nature of their interaction with proteins: The case of Salivary Proline-Rich Protein/procyanidins binding. Langmuir. 2012;28:17410-18.

[19] Searle MS, Williams DH. The cost of conformational order: Entropy changes in molecular associations. J Am Chem Soc. 1992;114:10690-7.

[20] Ball LJ, Khüne R, Schneider-Mergener J, Oschkinat H. Recognition of proline-rich motifs by protein-protein-interaction Domains. Angew Chem Int Ed. 2005;44:2852-69.