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How wine polyphenols can fight Alzheimer disease progression: towards a molecular explanation

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ABSTRACT

The present study provides clues at a molecular level towards an understanding of the mechanism of action of wine polyphenols on the protein Tau, whose aberrant phosphorylations are responsible for Alzheimer disease progression. Ten different flavan-3-ols that cover their natural structural diversity were tested with regard to their aptitude to fix a representative peptide of the proline-rich region of Tau (Gly 201-Thr 220) by using NMR and dynamic molecular modelling. The combination of these two techniques allows to locate the fixation sites: the fixation occurs in the peptide region where phosphorylations usually take place. The affinity was evaluated by titration curves and has been shown to depend on the presence of some procyanidin structural elements. Notably the presence of a galloyl moiety and the degree of polymerization are shown to increase the affinity. An ambivalence role of procyanidins has been evidenced: dynamic molecular data shows that they can play indifferently the role of hydrogen bond donor or acceptor.

1. Introduction

Alzheimer's Disease (AD) represents a major public health concern due to the general ageing of the population, especially in developed/ing countries. The two pathological hallmarks and therapeutic targets of Alzheimer's disease are senile plaques composed of β-amyloid peptides and neurofibrillary tangles (NFT) comprised of the microtubule-associated Tau protein. The involvement of Tau in AD, or even in others neurodegenerative pathologies, is now commonly established: the aggregation of Tau into filaments is the consequence of its hyper-phosphorylation,[1] and its polymerization into filaments would initiate a chain of events that cause the neuronal death in specific areas of the brain (notably enthorinal region, hippocampus and cortex). The phosphorylation/dephosphorylation of Tau regulates its ability to assemble tubulin in microtubules and implies that its pathological hyperphosphorylation induces the disruption of the neuronal cytoskeleton and may contribute to Tau fibrillization and neuronal death.[2,3]

Six Tau isoforms (Tau A to G), resulting from the alternative splicing of the mRNA issued from the single MAPT gene transcription and ranging from 352 to 441 amino acids, are found in the adult human brain.[4] They are composed of two main domains: the projection domain involved in the interaction with neural plasma membrane and cytoskeletal elements and the microtubule binding domain involved in the microtubule polymerization and stabilization.[5] A proline-rich motif (PR) is found in each domain and is described to be the recognition place of proline-directed kinases/phosphatases. Twenty-one phosphorylation sites are found in Tau, among them 10 are Ser/Thr-Pro motifs located in the two PR regions. The periodicity of prolines confers to these PR domains an extended left-handed polyproline II helix (PPII) and to the entire Tau protein, an unfolded structure. This PPII structure forms in itself a unique recognition motif between a protein substrate (like Tau) and a catalytic protein such as kinases,[6–8] prolyl *cis–trans* isomerase.[9] Beyond its key role in the structure of the PR domain, Proline is unique among the 20 common amino acids in having the side chain cyclised on to the backbone nitrogen atom.[10] This specificity is commonly described to induce the following consequences: (i) its backbone is restricted, (ii) the bulkiness of its N–CH_2 group (instead of a N–H) places restrictions on the conformation of the residue preceding proline, and (iii) proline is unable to act as a hydrogen bond donor due to the replacement of the amide proton by a CH_2 group, letting its exposed carboxyl group, which is more electron-rich than those of all other amino acids,[11] acting as

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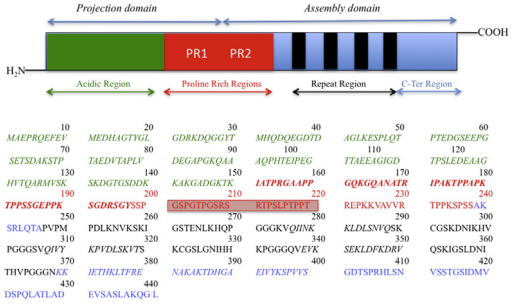
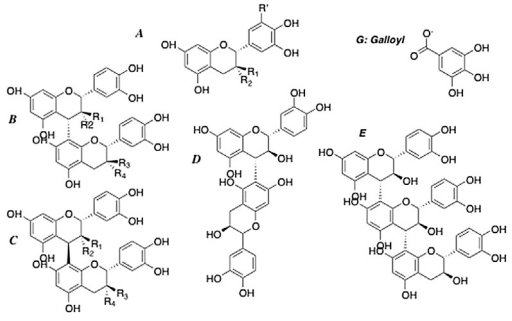
a hydrogen bond acceptor. All these properties could provide for proline-rich motifs a mechanism of highly discriminatory recognition without requiring high affinity (K_d typically in the μM to the mM range) and high cost in energy[12].

Proline-rich domains, thanks to their extended structure and the unusual property of proline, are potent targets able to fix a variety of compounds, especially polyphenols. The propensity of polyphenols to fix, even to precipitate, saliva proline-rich peptides (sPRP), phenomenon at the origin of the astringency sensation felt in the mouth when one drinks a beverage rich in tannins such as tea or red wine,[13] is well described[14–16]. Interestingly, the affinity that procyanidins exhibit towards sPRP is also in the mM range.[17,18]

In the present paper, we test the capacity of different flavan-3ols to fix Tau in its proline-rich regions for three main reasons: (1) Their protective role evidenced by epidemiological studies[19] or even on mouse models of Tauopathy;[20] (2) the sequence similarity between sPRP and Tau, and (3) the affinity of sPRP towards tannins, mainly of the flavan-3-ols family. The different purposes of this work are (i) to understand the action mode of polyphenols upon the Tau protein at a molecular level that could explain their beneficial effects upon the illness progression. In fact, two ways are conceivable: the location of polyphenols on the proline-rich domain of Tau that could prevent the kinases attack and the consequent pathologic Tau aggregation and/or cause the disaggregation of NFT; and (ii) to begin a Structure-Affinity Relationship by determining the chemical elements of procyanidins (degree of polymerization, stereochemistry of the heterocyclic ring, galloylation, see Scheme 2) capable to promote their affinity towards the proline-rich domain of Tau. If the molecular polymorphism of polyphenols of the flavan3-ols family represents an inexhaustible source of potent drugs, it also poses a risk of adverse health effects.[21]



Scheme 1. Schematic representation of the functional domains of Tau (top) and amino acid sequence of the Tau isoform F (bottom). The peptide representative of the Tau PR2 domain studied in the present work is framed in red.



Scheme 2. The different procyanidins used for the titration experiments: EC, Epicatechin (A, R_1=R′=H, R_2=OH); ECG, Epicatechin gallate (A, R_1=H, R′=OH, R_2=G); EGCG, Epigallogatechin gallate (A, R_1=H, R′=H, R_2=G); B3, Catechin 4α-8 Catechin (B, R_1/R_3=OH, R_2/R_4=H); B4, Catechin 4α-8 Epicatechin (B, R_1/R_4=OH, R_2/R_3=H); B3G, Catechin 4α-8 Catechin gallate (B, R_1/=OH, R_3=G, R_2/R_4=H); B2, Epicatechin 4β-8 Epicatechin (C, R_1/R_3=H, R_2/R_4=OH); B1, Epicatechin 4β-8 Catechin (C, R_2/R_3=OH, R_1/R_4=H) (C); B6, Catechin 4α-6 Catechin(D); C2, Catechin 4α-8 Catechin 4α-8 Catechin (E).

A few years ago, we have described a synthetic strategy allowing the synthesis of C4–C8 linked procyanidin with a total control of the stereochemistry at the interflavan link, the degree of polymerization,[22] and their 3-*O*-galloylation.[23] This strategy permits to produce a large panel of pure procyanidin reflecting their natural polymorphism and to assess their affinity towards a peptide representative of the Tau PR2 domain (^{201}G–^{220}T, Scheme 1). Beyond the monomers epicatechin, epigallocatechin, already commercially available, the four C4–C8 procyanidin dimers (B1 to B4), the C4–C6 dimers of catechin, the C4–C8 trimer of catechin and the B3 galloylated dimers (Scheme 2) were synthesized to test the influence of the stereochemistry, galloylation and oligomerization on affinity. The interactions were investigated by using NMR, CD and Molecular Modelling and Dynamics in order to get first insights of the specific binding of procyanidin on Tau at a molecular level by evidencing the chemical elements of procyanidin determining their affinity on Tau, by locating the amino acid target(s) upon which the fixation occurs, and by obtaining some physicochemical values. All these data provide answers that may explain the protective role of grape seed polyphenols upon the phosphorylation process of the Tau protein and give promising elements for exploring new drugs and/or therapeutic strategy to slow down the Alzheimer's disease evolution.

2. Results

2.1. Structure preferences of PR2

The peptide synthesis was performed using the Fmoc strategy, and was purified by HPLC. Purity was controlled by MALDI-TOF spectrometry (absence of truncated peptides from synthesis). Only one peak at 1951.7 Da (monoisotopic mass corresponding to M+H) was observed in the spectrum. The global yield of this synthesis reached 80%, corresponding to an amino acid grafting level up to 99% (S1). The 3D-structure of PR2 was determined in water, pH 7.0, using three complementary techniques: CD, NMR and molecular modelling.

Different CD spectra were recorded at different peptide concentrations (from 0.1 to 0.5 mM, Fig. 1). Spectra showed no significant modification, suggesting that neither conformational changes nor aggregation process occur when the peptide concentration increases in this concentration range. Best fit were obtained with a combination of a PII helix (12.5%) and a random coil contribution (70%), confirming that this part of Tau is not structured due to the high level of proline residue (6 prolines over 20 amino acids).

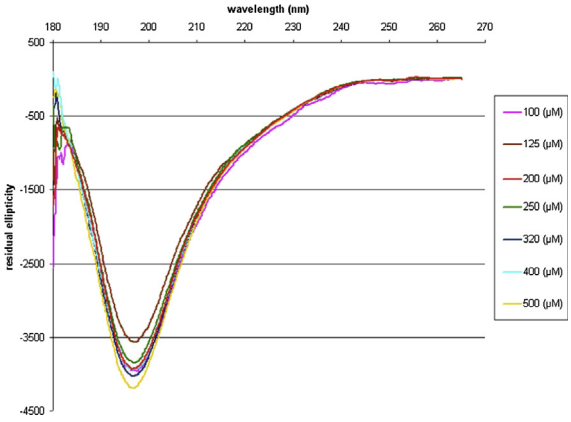


Fig. 1. CD spectra recorded at different concentrations in peptide (011–0.5 mM, pH, 7.0). CD spectra (190–250 nm) were recorded on a JASCO 720 polarimeter equipped with a 0.1 cm quartz cell, at 298 K.