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Binding site of different tannins on a human salivary proline-rich protein evidenced by dissociative photoionization tandem mass spectrometry

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ABSTRACT

The sensation of astringency is thought to originate from the interaction occurring between tannins and the salivary proline-rich proteins (PRPs). Astringency perception can be modified by the structure of tannins. Herein, we study the interactions occurring between the human salivary PRP, IB5, and three model tannins with different structure, epigallocatechin gallate and the procyanidin dimers B2 and B2 3′*O*-gallate, using the coupling of mass spectrometry and VUV-synchrotron radiation. The results obtained indicate that the structure of tannins, in particular the degree of polymerization and the galloylation, does not modify the binding site on IB5 involved in the interaction.

1. Introduction

Astringency is a major sensory attribute of wines and especially of red wines,[1] elicited primarily by tannins. It is usually accepted that tannin astringency results from their interaction with salivary proteins and subsequent aggregation and/or precipitation, causing a loss of the lubricating ability of saliva, although it may also involve adsorption of tannins on the oral mucosa.[2–4]

Tannins are ubiquitous in plants and believed to play a role in plant defense against pests and herbivores. Proanthocyanidins, i.e., oligomers and polymers of flavan-3-ols, also called condensed tannins, are the major tannins in foods and beverages, and particularly abundant in grapes and in red wine. They exhibit large structural diversity arising from the presence of several constitutive units and linkage positions, substitution, especially with galloyl groups, and varying degrees of polymerization. These structural features determine tannin properties, including astringency[5,6] and affinity for proteins and peptides that increase with chain length and galloylation[7–10] and are also impacted by tannin conformation.[11]

Among salivary proteins, proline-rich proteins (PRP) are particular prone to interact with tannins.[12] It has been suggested that secretion of PRPs, and especially of basic PRPs, whose only known function is tannin binding, is the first line of defense of herbivores against dietary tannins. Indeed, tannins inhibit digestive enzymes and impede assimilation of dietary proteins and other nutrients. Their binding by PRPs is believed to prevent these processes and could also reduce tannin consumption by triggering astringency. The high affinity of PRPs for tannins is associated to their structural characteristics. PRPs belong to intrinsically disordered proteins, and

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show random coil structure except for small polyproline helix segments. The most accepted model of PRP–tannin interactions describes three different stages as tannin concentration increases: (i) tannins, which are multidentate ligands, bind to several sites on the free protein, (ii) the stoichiometries of the complexes increase and tannins cross-link different protein molecules, (iii) the resulting multimeric aggregates grow until precipitation.

A human salivary PRP, referred to as IB5, and its interactions with tannins have been particularly investigated. Its sequence (Fig. 1A) presents tandem repeats of KPQGPP(P) and eight clusters of 2–5 proline residues. Experiments involving small angle X-ray scattering (SAXS),[13] circular dichroism (CD),[14,15] nuclear magnetic resonance (NMR),[16] and mass spectrometry (MS)[17] have shown that IB5 exhibits a random coil structure and undergoes disorder to order transition upon interaction with epigallocatechin gallate (EgCG), selected as a model tannin ligand.[14,15] Molecular modeling performed on a smaller truncated PRP, $IB9_{37}$, also indicated that structural rearrangement of the peptide occurs during the interaction.[18] Folding of the peptide chain around the tannin, as first proposed by Charlton et al.,[19] may explain the higher tannin affinity of $IB9_{37}$ and IB5 compared to a single proline-rich repeat.

precise binding sites of tannins could not be determined by NMR on full length PRP proteins because of the abundance of proline and repeated sequences. A new method using tandem mass spectrometry coupled to synchrotron radiation as an activation method has recently allowed unambiguous determination of demonstration of binding of the procyanidin dimer B2 3′OG (epicatechin-(4β-8)-epicatechin-3-*O*-gallate, Fig. 1) on the KPQGPPPPPQGG segment of IB5 sequence.[23] In the case of $IB7_{14}$ and $IB9_{37}$, the same three binding sites, corresponding to PP and GG clusters, were involved for all tannin dimers and trimers tested but the binding force was higher for tannins showing extended conformation and longer chain length.[18,11] Once three tannins are linked to the peptide, regardless of its chain length, non-specific cross-linking occurs.[18,11] Similarly, upon, binding with EgCG, the full length protein IB5 forms aggregates with a core structure containing proteins that have bound at least three EgCG molecules and a less dense corona with fewer bound tannins.[21]

Regarding these different observations, it appears important to know if, as for shorter $IB9_{37}$, the same binding site on IB5 is involved in the interaction with tannins having different structures. Therefore, in the present paper, we apply the recent introduced method,

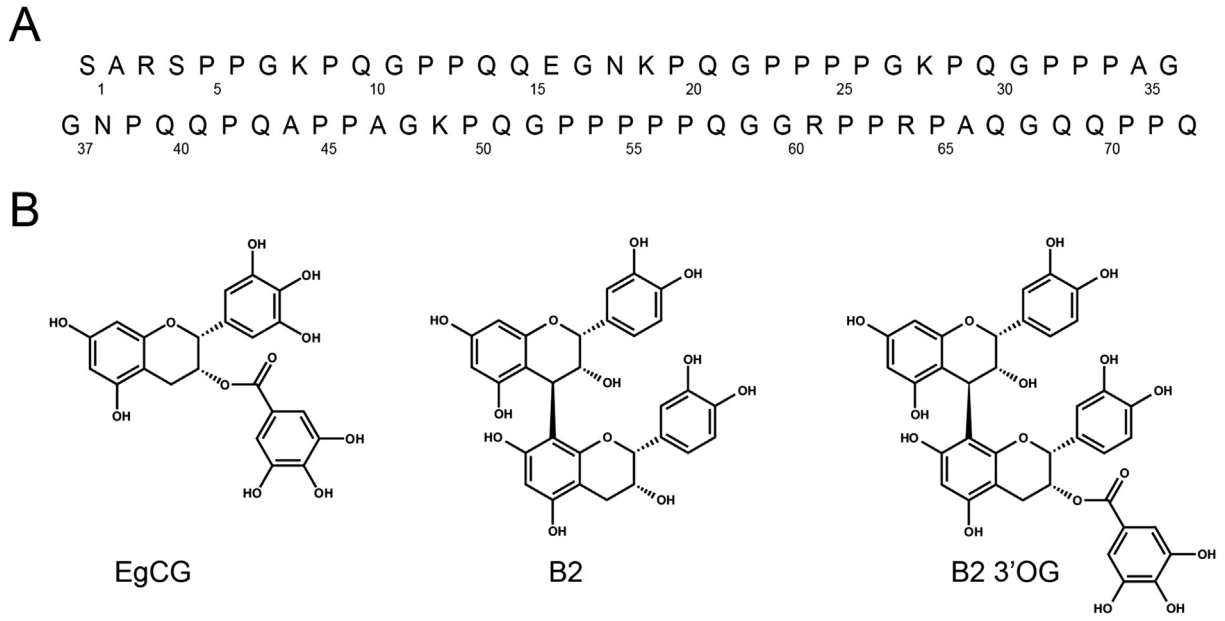

Fig. 1. (A) Sequence of the IB5 human salivary protein. (B) Molecular structure of the epigallocatechin gallate (EgCG), (epicatechin-4β-8)-epicatechin (B2) and epicatechin-(4β-8)-epicatechin-3-*O*-gallate (B2 3′OG).

NMR[20] and a multitechnique approach involving MS and SAXS[21] indicated that at least three EgCG molecules per protein or peptide are required to form PRP–EgCG aggregates. Comparison of different tannins showed that the stability of IB5·tannin complexes increases with the number of hydroxyl groups in the molecule, indicating the involvement of hydrogen bonds.[10] NMR studies performed on two proline-rich peptides ($IB7_{14}$ and $IB9_{37}$) also showed specific hydrophilic interaction at low tannin concentration.[18,11] Mass spectrometry has revealed that IB5·EgCG complexes are present in solution under a distribution of various stoichiometries and fragmentation experiments of complexes with stoichiometries from 1:1 to 1:9 suggested the presence of eight equivalent and independent binding sites on the protein, matching the number of proline clusters.[21] NMR data on complexes of tannins with IB5 or PRP peptides indicated the involvement of Pro and Gly residues in specific non-covalent interactions.[22,18,11,16] However, the coupling MS and vacuum-ultraviolet (VUV) radiation, to localize the binding sites of EgCG and B2 ((epicatechin-4β-8)-epicatechin, Fig. 1B.) on the full PRP, IB5, in 1:1 complexes, and to compare them to that of B2 3′OG.

2. Results and discussion

IB5 is a model of basic salivary PRP, which has been obtained by heterologous expression of the human gene PRB4S in the yeast *Pichia pastoris*.[24] The mass spectrum obtained by electrospraying the protein solution displayed a series of protonated peaks corresponding to four IB5 isoforms with charge states ranging from 5+ to 10+. IB5 isoforms differ from each other by few amino acids at their N-terminal end as previously observed.[23] The sequence of the main isoform is presented in Fig. 1A. Fig. 2A presents a close-up of the mass spectra of IB5 solution and IB5/tannin mixtures for the