Food Chemistry 253 (2018) 79–87

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Mechanisms of astringency: Structural alteration of the oral mucosal pellicle by dietary tannins and protective effect of *b*PRPs

Sarah Ployon[a], Martine Morzel[a], Christine Belloir[a], Aline Bonnotte[b], Eric Bourillot[c], Loïc Briand[a], Eric Lesniewska[c], Jeannine Lherminier[b], Ece Aybeke[a], Francis Canon[a,*]

[a] Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS, INRA, Université de Bourgogne Franche-Comté, F-21000 Dijon, France
[b] INRA, UMR1347 Agroécologie, ERL CNRS 6300, Plateforme DImaCell, Centre de Microscopie INRA/Université de Bourgogne, F-21000 Dijon, France
[c] ICB UMR CNRS 6303, Université de Bourgogne Franche-Comté, F-21000 Dijon, France

ARTICLE INFO

Keywords:
TR146/MUC1 cells
Salivary mucins MUC5B
EgCG
IB5
Scanning Electron Microscopy
Atomic Force Microscopy
Astringency

ABSTRACT

The interaction of tannins with salivary proteins is involved in astringency. This paper focussed on saliva lining oral mucosae, the mucosal pellicle. Using a cell-based model, the impact of two dietary tannins (EgC and EgCG) on the mucosal pellicle structure and properties was investigated by microscopic techniques. The role of basic Proline-Rich-Proteins (bPRPs) in protecting the mucosal pellicle was also evaluated.

At low (0.05 mM) tannin concentration, below the sensory detection threshold, the distribution of salivary mucins MUC5B on cells remained unaffected. At 0.5 and 1 mM, MUC5B-tannin aggregates were observed and their size increased with tannin concentration and with galloylation. In addition, 3 mM EgCG resulted in higher friction forces measured by AFM. In presence of bPRPs, the size distribution of aggregates was greatly modified and tended to resemble that of the "no tannin" condition, highlighting that bPRPs have a protective effect against the structural alteration induced by dietary tannins.

1. Introduction

Astringency, mostly considered as an unpleasant sensory attribute, is an organoleptic tactile sensation (Green, 1993) mediated by the trigeminal nerve (Schobel et al., 2014). It is defined as the sensation of drying and puckering of oral mucosa. Astringency can be experienced during the consumption of plant food products, such as green tea, red wine or berries. In those products, proanthocyanidins, a group of tannins, have been identified as responsible for this sensation. Tannins are phenolic compounds (*syn.* polyphenols) and have the ability to precipitate proteins. They can be divided into three groups, proanthocyanidins (*syn.* condensed tannins), which are oligomers and polymers of flavan-3-ols, hydrolysable tannins, which are polyesters of sugars and gallic or ellagic acids, and complex tannins in which both types are covalently bound. Tannins are plant secondary metabolites, which play a role in plant defence mechanisms through their deleterious effects for plant predators (i.e. herbivores and omnivores). For instance in mammalian herbivores, tannins can reduce digestibility, damage the gastrointestinal mucosa and epithelium, and lead to kidney or liver failure or endogenous nitrogen loss (Shimada, 2006). In rodents, a tannin-rich diet induces weight loss, which is continuous in hamsters but reversible after three days in rats and mice. In parallel, feeding on tannins induces in rats and mice a dramatic increase in salivary proline-rich proteins (PRPs) within three days, while this is not observed in hamsters (Shimada, 2006).

Indeed in mammals, the presence of PRPs in saliva appears to be linked to the consumption of tannins. PRPs are particularly abundant in human saliva and may constitute up to 70% of parotid saliva proteins (Bennick, 1982). PRPs are classified in three groups depending on their isoelectric point and their degree of glycosylation: acidic, basic and glycosylated PRP (aPRP, bPRP and gPRP, respectively). aPRPs play a role in calcium binding and gPRPs in oral lubrication, while the main function of bPRP is the scavenging of tannins. Thus, investigations on tannin-protein interactions have shown that PRPs have a particular affinity for tannins (Shimada, 2006). Therefore, according to the Red Queen hypothesis, it can be postulated that bPRPs are part of a defence mechanism selected to protect organisms against the detrimental effects of tannins. Astringency is also probably a chemosensory signal for the detection of tannin-rich foods, leading to a shunning behaviour.

Astringency is thought to be due to a loss in the lubrication capacity

* Corresponding author.
E-mail addresses: martine.morzel@inra.fr (M. Morzel), christine.belloir@inra.fr (C. Belloir), aline.bonnotte@u-bourgogne.fr (A. Bonnotte), eric.bourillot@u-bourgogne.fr (E. Bourillot), loic.briand@inra.fr (L. Briand), lesniew@u-bourgogne.fr (E. Lesniewska), jeannine.lherminier@inra.fr (J. Lherminier), ece.aybeke@inra.fr (E. Aybeke), francis.canon@inra.fr (F. Canon).

https://doi.org/10.1016/j.foodchem.2018.01.141
Received 18 July 2017; Received in revised form 22 November 2017; Accepted 22 January 2018
Available online 03 February 2018

of salivary proteins and more particularly of the proteins composing the mucosal pellicle (Nayak & Carpenter, 2008). The mucosal pellicle is a thin biological structure, whose thickness can reach 100 nm (Morzel, Siying, Brignot, & Lherminier, 2014), made of salivary proteins anchored onto oral epithelial thanks to covalent and non-covalent bonds (Bradway et al., 1992; Gibbins, Yakubov, Proctor, Wilson, & Carpenter, 2014). The mucosal pellicle contains MUC5B, MUC7, cystatins and IgA (Gibbins et al., 2014) and also amylases and PRPs (Bradway et al., 1992). Among these salivary proteins, the mucins MUC5B have been identified as major components. Davies et al. have reported that tannins aggregate the salivary mucins MUC5B and MUC7 (Davies et al., 2014). Therefore, interaction of astringent compounds with adsorbed mucins could play an important role in astringency sensation (Biegler, Delius, Käsdorf, Hofmann, & Lieleg, 2016).

In this context, the two hypotheses tested in this study are that 1 – tannins aggregate the mucosal pellicle proteins, with an impact on lubricating properties of this structure and 2 – PRPs play a protective role by scavenging tannins, precluding their access to the mucosa and its consequent structural alteration. As a result, astringency would be perceived only when tannin concentration is high enough to overcome the protective capacity of PRPs. To test these hypotheses, an *in vitro* cell-based model of oral mucosa with a mucosal pellicle previously developed (Ployon et al., 2016) was used. First, to investigate the effect of tannins on the mucosal pellicle structure, the model was exposed to two dietary monomers of proanthocyanidins, Epigallocatechin (EgC) and Epigallocatechin gallate (EgCG), differing in their structure by the presence of a galloylated moiety on EgCG (Fig. 1) and which is detected sensorially at lower concentrations. The mucosal pellicle structure and properties were investigated using three complementary microscopic techniques, fluorescent immunostaining of salivary MUC5B, Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Second, in order to evaluate the role of bPRP in the protection of the oral mucosa, the model was covered by a liquid film containing IB5 (a human bPRP) prior to exposure to EgCG. The size of the aggregates with and without PRPs was evaluated by image analysis and compared.

2. Material and methods

2.1. Saliva collection

The study was performed in agreement with the guidelines laid out in the declaration of Helsinki. Written informed consent was obtained from the participants. Unstimulated saliva was obtained by the spitting method from fifteen volunteers who declared to be in good oral health. Subjects were instructed to refrain from smoking, eating or drinking for at least two hours before saliva collection. All samples were pooled and centrifuged at 14,000*g* for 20 min at 4 °C. The resulting pool of clarified saliva was divided into aliquots of 4 ml. Samples were immediately frozen at −80 °C.

2.2. Cell culture and formation of in vitro mucosal pellicle

TR146/MUC1 cells (Ployon et al., 2016) were grown in DMEM/F12–GlutaMAX medium (1:1) supplemented with 10% Foetal Bovine Serum (FBS), 1% penicillin/streptomycin (P/S) and 2.5 mg/ml of neomycin G418 (Gibco® by Life Technologies). Culture conditions were maintained at 37 °C and 7.5% CO_2. Cells were cultured in T75 flasks and the medium was changed every two days. Sub-cultures were prepared at 80% confluence using Trypsin-EDTA. Cells were seeded into eight-chamber glass slides for MUC5B immunostaining, and on 10 mm diameter glass slips for SEM and AFM imaging. All supports were coated with Cell-Tak™ (Corning) prior to seeding at a density of 4×10^6 cells/cm^2. In these conditions, confluence was reached in 48 h. Three days after confluence, a mucosal pellicle was deposited on the cells' surface by incubating cells for 2 h with clarified saliva diluted into growth medium (1:1) (Ployon et al., 2016). After incubation, samples were washed twice with PBS in order to eliminate the non-adsorbed saliva.

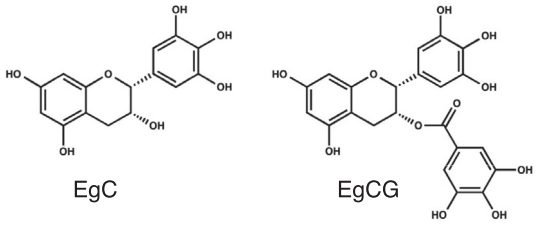

Fig. 1. Structure of Epigallocathechin (EgC) and Epigallocatechin gallate (EgCG).

2.3. Tannins solution

Epigallocatechin (EgC) and Epigallocatechin gallate (EgCG) were purchased from Santa Cruz Biotechnology (Texas, US). EgC and EgCG were diluted in PBS (pH 7.5) immediately before use to avoid compounds oxidation.

2.4. IB5 production

IB5 was produced and purified according to the method described by Boze et al. (2010) and was a kind gift from Dr. Cheynier (INRA Montpellier). The concentration of IB5 was adjusted to 0.66 mM in PBS.

2.5. Viability assay

Cells were cultured into 96-wells plates. When confluence was reached, cells were incubated for 2 h with clarified saliva diluted in growth medium as described above. After two washes with PBS, samples were exposed to tannins solutions (0.05–10 mM) or IB5 solution at 0.33 mM for 1 h. Cytotoxic effects were assessed using the Neutral Red assay, using a fluorimetric method. Briefly, cells were incubated for 3 h at 37 °C with 200 µl of medium containing neutral red at 50 µg/ml, washed twice with PBS and incubated at room temperature for 1 h in neutral red eluent (ethanol:H_2O:acetic acid, 50:49:1) with gentle agitation. Reading of the fluorescence was performed with Victor3V microplate reader (PerkinElmer) with excitation and emission wavelengths fixed at 544 nm and 595 nm, respectively. 80% of viability was considered as an indicator of non-cytotoxicity. Assays were performed in triplicate.

2.6. Exposure of the model to EgC and EgCG

Three concentrations of EgC and EgCG were tested for MUC5B immunostaining and SEM: 0.05, 0.5 and 1 mM. These concentrations were chosen taking into account the sensory detection thresholds of EgC and EgCG which were measured at 0.54 mM and 0.19 mM, respectively (Scharbert, Holzmann, & Hofmann, 2004). For AFM imaging, two concentrations of EgCG (1 and 3 mM) were tested. The cell-based model was covered with a tannin solution in PBS, or PBS alone as a control. After 5 min, the liquid was removed and cells were fixed prior to microscopical observations. Each condition was tested in triplicate.

2.7. Exposure of the model mucosa to EgCG in presence of IB5

The effect of EgCG at 1 mM was evaluated in presence of IB5 at 0.33 mM. The cell-based model was preliminarily covered by 62.5 µl of IB5 solution at 0.66 mM. Then, 62.5 µl of EgCG at 2 mM or 1 mM was added, resulting in final concentrations of 1 mM or 0.5 mM in EgCG and 0.33 mM in IB5. In the control condition, 62.5 µl of PBS were added to the 62.5 µl of IB5 solution.