SPR based studies for pentagalloyl glucose binding to α-amylase

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Abstract

Astringency is an organoleptic property resulting mostly from the interaction of salivary proteins with dietary polyphenols. It is of great importance to consumers but being typically measured by sensorial panels it turns out subjective and expensive.

The main goal of the present work is to develop a sensory system to estimate astringency relying on protein/polyphenol interactions. For this purpose, a model protein was immobilized on a sensory gold surface and its subsequent interaction with polyphenols was measured by Surface Plasma Resonance (SPR). α-amylase and pentagalloyl glucose (PGG) were selected as model protein and polyphenol, respectively. To ensure specific binding between these, various surface chemistries were tested. Carboxylic terminated thiol decreased the binding ability of PGG and allowed covalent attachment of α-amylase to the surface. The pH 5 was the optimal condition for α-amylase immobilization on the surface. Further studies focus on Localized SPR sensor and application to wine samples, providing objectivity when compared to a trained panel.

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1. Main text

Phenolic compounds comprise a great diversity of molecules that are present in a wide range of plants. Their different properties and capability to suffer several variations according to environmental conditions and maturation stages make them responsible for the majority of the organoleptic characteristics of food and beverages.

The consumption of fruits and vegetable with high polyphenols content has been highlighted for the reason that they are associated to health benefits such as the prevention of heart disease\(^1\), chronic inflammation\(^2\) and cancer\(^3\).

The biological effect of polyphenols is related not only to their antioxidant properties but also to their great capability to precipitate proteins. When proteins and polyphenols interact, protein/polyphenol complexes form and the growing size of these complexes may lead to precipitation\(^4\). This interaction starts inside the mouth, decreasing mouth lubrication and leading to a tactile sensation called astringency. This is traduced by the sensation of dryness, roughness or puckering of the oral cavity\(^5\). Understanding this protein/polyphenol interaction is important for accurate sensorial analysis and for understanding this effect in the biological system.

Several works based on precipitation and competition assays have been made to study polyphenol and protein interactions, but a method that simulates the mouth conditions is still missing. Therefore, we propose an easy and accurate tool to estimate astringency by mimicking protein/polyphenol interaction in the mouth. The base of the sensor device was the immobilization of protein on a gold surface and its interaction with polyphenols measured by SPR. \(\alpha\)-amylase was the salivary protein selected for this purpose since it represented 30% of human salivary proteins. The selected polyphenol was pentagalloyl glucose (PGG), very common in wine aging in oak barrels, tea and also because recent studies revealed that this particular compound has anti-tumorigenic activity\(^6\).

2. Experimental Parte

2.1. Materials

Buffers were prepared with MQ water (Milli Gradient, Millipore) and filtered through a 0.2 \(\mu\)m pore filter and degassed for 30 minutes with sonicator prior to use. The used buffer was sodium acetate 0.1 mM at pH 5.

\(\alpha\)-amylase from porcine pancreas (Type I-A, PMSF treated saline suspension) was purchased from Sigma-Aldrich. \(\alpha\)-amylase solutions were diluted in sodium acetate buffer with the suitable pH to the desired concentration. The purified PGG was kindly provided by CIQ.

2.2. Substracts

SPR chips (SiA-Au) were purchase from GE Health. The SPR surfaces were cleaned according to the most suitable way using: i) TL1 clean (1:5:5 volume ratio \(\text{H}_2\text{O}_2:\text{NH}_4:\text{H}_2\text{O}, 75^\circ\text{C}\)) for 10 min before their use, followed by thorough rinsing with MQ water or ii) solvent clean using first acetone, then ethanol and finally water, sonicated for 10 min each and followed by UV/Ozone clean for 1 h. Both cleaning procedures were made prior to surface modification by self-assemble of alkanethiols.

The alkanethiols used were amine (HSC\(_{16}\)H\(_32\)NH\(_2\) HCl), carboxyl (HSC\(_{15}\)H\(_{30}\)COOH), methyl (HSC\(_{16}\)H\(_{33}\)), alcohol (HSC\(_6\)H\(_2\)OH) and methyl polyethylene glycol (HSC\(_{11}\)H\(_{22}\)(OCH\(_2\)CH\(_2\))\(_3\)OCH\(_3\)) terminated thiols, purchased to Prochimia, Poland. Thiolation was performed in ethanol (p.a. grade, Merck) for a minimum of 12 h for the methyl terminated thiol (35 mM) and 24 h for the hydrophilic
thiols (2mM). Afterwards the surfaces were sonicated in ethanol, to remove unbound thiols and MQ water, both for 3 min and finally dried with nitrogen.

2.3. SPR measurements

The SPR measurements were performed with a Biacore X system at 23°C. The measurements to study the direct effect of PGG on the thiolated surface were carried out by using sodium acetate 0.1 mM as running buffer, with a flow rate of 10 μL/min, and injecting 100 μL of PGG 0.18 μmol/L, prepared in the running buffer, of pH 5, with 5% ethanol, after a stable base line was reached. Previous to the amylase binding, the surface was prepared by the amino-coupling method. The activation of the carboxylic groups was made through the injection of 35μL of a solution containing 100mM NHS (N-hydroxysuccinimide) and 400mM EDAC (N-ethyl-N’-dimethylaminopropyl-carbodiimide). α-amylase was immobilized by injecting 35 μL of a solution of 80.3 μg/mL, prepared in buffer with suitable pH, flowing at 5 μL/min. The remaining activated groups were blocked by injecting 35μL/min of glycine solution 0.8 mol/L, pH 8.4 or TRIS (tris(hydroxymethyl)aminomethane) 1 mol/L, pH 7 followed by 35μL of PGG.

3. Results and discussion

In general, polyphenols display affinity properties to a great range of compounds. Thus, before beginning the sensor assembly several studies were made in order to prevent PGG side-adsorption to the sensory surface and achieve a specific binding between α-amylase and PGG.

To avoid PGG side-adsorption to the sensory surface, several self-assembled monolayers on gold were tested using alkanethiols with different chemistries. The SPR was used to provide information about how PGG behaved in the presence of these surface chemistries. The alkanethiols selected for this purpose were amine (hydrophilic positively charged), carboxyl ((hydrophilic negatively charged), methyl (hydrophobic), alcohol (hydrophilic) and methyl polyethylene glycol (PEG-OMe) terminated thiols. All the surfaces were kept in contact for the same time with PGG and the results showed the smaller PGG binding when carboxylic terminated thiol was used (Figure 1). Overall, these results indicated that using a surface with carboxyl terminated thiol leads to less secondary bindings meaning that the ability of PGG to bind the surface is reduced.

![Fig. 1. SPR graphs showing the influence of different surface chemistries on PGG binding. A indicates the point of injection of PGG and B indicates the start of rinsing with buffer.](image-url)
For a covalent immobilization of α-amylase, the surface was activated by EDAC/NHS. Since not all activated carboxylic functions react with α-amylase, the un-reacted ones should be deactivated with a proper blocking agent. The selection of the blocking agents was also of great importance regarding the side PGG absorption (PGG should only establish a specific binding with α-amylase rather than the blocking agent). Tris and Glycine were the two blocking agents selected based on their chemistry and on the previous results. The SPR results showed that both agents allowed small PGG binding. The ΔRU values from the SPR (indicating the mass that remained on the surface) when the surface was not blocked were 1063±517, showing a high value for PGG binding. This occurred due to the strong electrostatic forces between the activated carboxylic groups and the PGG. However, when Tris and glycine were used, the ΔRU was 503±143 and 155±43, respectively. Comparing to when no blocking agent was used, the results for both agents indicated a mass reduction of 47 and 14% for PGG on the surface. This result was also consistent with the previous observations of the thiol layer, indicating again that carboxylic acid termination was better because it did not benefit the PGG binding. Therefore glycine was considered the suitable blocker.

The influence of the pH was also tested for the α-amylase immobilization using three different pHs, ranging from 5 to 8. The immobilization on the surface was made by activating the carboxylic layer followed by α-amylase injection. The pH 5 seemed to benefit α-amylase binding. To know the mass of α-amylase that was covalently attach to the surface glycine was injected, as blocking agent and also to remove the α-amylase adsorbed just by electrostatic forces. The mass of α-amylase that remained on the surface presented a ΔRU of 1001±380 and the binding interaction with PGG also seemed to increase, indicating a specific binding. This interaction seemed to mimic these interactions in the mouth simulating the action of drinking wine. Further tests using LSPR to allow the construction of an array of sensors and their application to monitor the astringency of wine samples will follow this approach, providing objectivity when compared to a trained panel.

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