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Interaction between sorghum procyanidin tetramers and the catalytic region of glucosyltransferases-I from *Streptococcus mutans* UA159

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ABSTRACT

Sorghum procyanidins (SPC) tetramers were reported to have inhibitory effects on the adhesion of *Streptococcus mutans* (*S. mutans*), which is one of the primary pathogenic bacteria that cause caries. To make clear the mechanism underlying the inhibitory effects of SPC tetramers on the adhesion of *S. mutans*, it is important to understand the interaction between SPC tetramers and the catalytic region of glucosyltransferases-I (GTF-I/CAT) from *S. mutans*. In this study, fluorescence quenching, UV–visible (UV–Vis) absorption and circular dichroism (CD) spectroscopies were applied to investigate the interaction between SPC tetramers and the GTF-I/CAT from *S. mutans* UA159. The fluorescence quenching results demonstrated that SPC tetramers combined with GTF-I/CAT protein on one binding site, and the fluorescence intensity of GTF-I/CAT protein was decreased regularly by static quenching with increasing concentrations of SPC tetramers. The UV–Vis absorption spectra of GTF-I/CAT protein were also changed with a red shift owing to the impact of SPC tetramers. In addition, the proportions of α-helix, β-sheet and random coil in the secondary structure of GTF-I/CAT protein were obviously altered by SPC tetramers. In conclusion, SPC tetramers have a strong affinity for GTF-I/CAT protein by altering its conformation and micro-environment. Our results suggest that SPC tetramers interact with GTF-I/CAT to interfere with the adhesion of *S. mutans*, which may facilitate the better application of SPC tetramers in the prevention of dental caries.

1. Introduction

As one of the most common bacterial infectious diseases, dental caries affects most adults around the world. *Streptococcus mutans* (*S. mutans*) has been identified as one of the primary pathogenic bacteria that cause dental caries (Bansal, Sharma, Grover, Sharma, & Sharma, 2013; Selwitz, Ismail, & Pitts, 2007; Simon-Soro, Belda-Ferre, Cabrera-Rubio, Alcaraz, & Mira, 2013). Glucosyltransferases (GTFs) from *S. mutans* are able to synthesize glucans from dietary sucrose, providing adhesion sites for *S. mutans* on tooth surface (Devulapalle et al., 2004). In particular, water-insoluble glucan synthesized by glucosyltransferases-I (GTF-I) is the most vital for the expression of the virulence factors of *S. mutans*. The dental plaque formed by the accumulation of bacteria on tooth surface will lead to the production of metabolic acids from sucrose, which will further induce the demineralization of tooth enamel and the initiation of dental caries (Hayacibara et al., 2004). Therefore, alterations of the structure and/or activity of GTFs can affect the bacterial adhesion to tooth surface and thus inhibit the development of dental caries (Yanagida, Isozaki, Shibusawa, Shindo, & Ito, 2004).

S. mutans can produce three forms of GTFs: GTF-I (water-insoluble glucan-synthesizing enzyme), GTF-S (water-soluble glucan-synthesizing enzyme) and GTF-SI (water-soluble and water-insoluble glucan-synthesizing enzyme). GTF-I, GTF-S and GTF-SI are respectively encoded by the genes *gtfB*, *gtfC* and *gtfD* (Argimon, Alekseyenko, DeSalle, & Caufield, 2013; Bowen & Koo, 2011; Inagaki et al., 2013), among which *gtfB* is pivotal for the adhesion of *S. mutans* to tooth surface (Hayacibara et al., 2004; Shiroza, Ueda, & Kuramitsu, 1987). With a total length of 4.8 Kb, *gtfB* gene can be divided into three functional fragments: catalytic region (CAT) located at 253–628 amino acids of N-terminal; glucan combination region with about 240 amino acid residues (glucan binding, GB or GLU); and 5 repeat sequence units, with each unit encoding 63–65 amino acid residues. The nucleotide sequence in *gtfB* gene encoding the activity of GTF-I from *S. mutans* has been identified by Shiroza et al. (1987). Xia et al. (2005) have correctly cloned the catalytic region of *gtfB* from *S. mutans* and its fusion protein was

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successfully expressed in *Escherichia coli* (*E. coli*), which lays a solid foundation for further studying the antagonists of GTF-I and its roles in the prevention of dental caries.

Procyanidins are generally a class of phenolic compounds widely present in fruits, vegetables, grains and some beverages, and they are characterized by a variety of bioactivities, such as antioxidant, anti-tumor and lipid-lowering activities as well as prevention of cardiovascular diseases. Sorghum (*Sorghum bicolor* L. Moench), the fifth most important food crop in the world after rice, wheat, corn and barley (Zhu, Shi, Yao, Hao, & Ren, 2017), is a good source of procyanidins. The procyanidins content of sorghum is 10.6–40.0 mg/g depending on the different varieties (Wu, Huang, Qin, & Ren, 2013), which has higher level of procyanidins than blueberry (Awika, Dykes, Gu, Rooney, & Prior, 2003).

It has been demonstrated that sorghum procyanidins (SPC) has the capability of restraining the growth and acid production of *S. mutans* Ingbritt (c) and *Streptococcus sobrinus* 6715 (Xu, Liu, Li, Tu, & Chen, 2011). Previous research has indicated that compared with SPC crude extract, SPC dimers and SPC trimers, SPC tetramers have better inhibitory effects on the adhesion of *S. mutans* in vitro (Huang, Cai, Yu, Tang, & Liu, 2014). However, it remains unclear whether SPC tetramers interact with GTF-I to interfere with the adhesion of *S. mutans* on tooth surface.

Therefore, in this study, the interaction between SPC tetramers and the catalytic region of glucosyltransferases-I (GTF-I/CAT) from *S. mutans* was investigated. The quenching type and combination mode of SPC tetramers with GTF-I/CAT protein were studied by fluorescence quenching assay. Moreover, CD and UV–Vis absorption spectra methods were used to investigate the structural changes of GTF-I/CAT protein resulting from the combination with SPC tetramers. The findings of the present study may provide important implications for better understanding the mechanism underlying the interactions between SPC tetramers and GTF-I/CAT protein, and lay a solid foundation for further studying the effects of SPC tetramers on the adhesion of *S. mutans* to tooth surface.

2. Materials and methods

2.1. Materials

Sorghum bicolor (L.) Moench (Hua Feng No. 1) was collected from Weifang, Shandong Province, China. GTF-I/CAT protein (molecular weight: about 44 KD, 80%purity) was obtained using gene cloning techniques collaboratively by our laboratory and PuJian biological technology company, and was subsequently identified through SDS-PAGE and Nano-LC-MS/MS (Yu, Tian, Li, Zhang, & Liu, 2015) (supplementary material). Reagents of analytical grade were purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Other chemicals with reagent purity were obtained from Sigma-Aldrich Chemical Co.. Toyopearl HW-40(s) gel was purchased from Tosoh Corporation (Tokyo, Japan).

2.2. Isolation and identification of SPC tetramers

2.2.1. Isolation of SPC tetramers

The procyanidins in sorghum were extracted for three times with ethanol (70:30, *v*/v) in water-bath at 80 °C for 90 min. The residue was removed by filtering, and the filtrates were concentrated in vacuum by a rotary evaporator. The extracts were loaded into an AB-8 macroporous resin column. The column was firstly eluted with distilled water to remove water-soluble impurities and then eluted by ethanol/water solution (40,60, v/v). The fraction eluted by ethanol/water solution was collected, condensed by vacuum rotary evaporation, and then lyophilized (Huang et al., 2014). This SPC mixture was further extracted by ethyl acetate. The extracts were isolated by Toyopearl HW-40(s) gel column (60 cm × 2.5 cm) and divided into five fractions, and the fifth of which was named as SPC-V.

2.2.2. HPLC-ESI-Q-TOF-MS/MS

SPC-V was identified by HPLC-ESI-Q-TOF-MS/MS using Agilent 1260 HPLC system equipped with Agilent 6520 Accurate-Mass Q-TOF MS/MS system (Agilent Technologies, Waldbronn, Germany). The sample was separated by a Waters SunFire™ C_{18} column (250 × 4.6 mm, 5 μm particle size, Ireland). Sample was dissolved in methanol to 2 mg/mL and filtered through 0.22 μm organic membrane (Nylon) before injection.

The mobile phase consisted of solvent A (0.1% acetic acid) and solvent B (acetonitrile). The gradient program was as follows: 0–5 min, 5–15% B; 5–6 min,15% B; 6–10 min, 15–17% B; 10–11 min, 17% B; 11–15 min, 17–18% B; 15–17 min, 18–5% B; 17–20 min, 5% B. The flow rate was set at 1 mL/min and the injection volume at 5 μL. The column temperature was 30 °C. The eluate was monitored at 280 nm with a diode-array UV detector. The conditions of MS/MS detector were as follows: negative mode; capillary voltage, 3500 V; collision energy, 40 V; nebulizer pressure, 40 psi; drying gas temperature, 350 °C; drying gas flow rate, 10 L/min and mass range, *m/z* 50–1700.

2.3. Fluorescence quenching assay

2.3.1. Preparation of stock solutions

The freeze-dried powder of SPC tetramers was dissolved with ultra-pure water to obtain solutions with concentrations ranging from 1.35×10^{-5}–1.73×10^{-3} mol/L, and the GTF-I/CAT protein was diluted to 1.82×10^{-3} mol/L with ultra-pure water. 3 mL GTF-I/CAT protein solution was mixed with 200 μL solutions of SPC tetramers at different concentrations. The mixtures were then allowed to react at 37 °C (human oral temperature) for 10 min. The GTF-I/CAT protein with only ultra-pure water was measured as blank control.

2.3.2. Fluorescence quenching analysis

F-4600 5 J2–0004 FL-SPECTOROPHOTOMET (Hitachi High-Technologies Corporation, Tokyo Japan) was used for fluorescence quenching measurements. The quenching effect of SPC tetramers on GTF-I/CAT protein was quantified by the fluorescence quenching method reported by Papadopoulou, Green, and Frazier (2005) with appropriate modifications. The excitation wavelength (λ_{ex}) was set at 295 nm, and the emission spectrum was recorded from 315 nm to 500 nm. Both slits were 5 nm.

The steady-state fluorescence quenching data were initially calculated on the basis of the classic Stern-Volmer equation (Eq. (1)):

$$F0/F = 1 + K\mathrm{sv}[\mathrm{Q}] = 1 + kq\tau 0[Q] \quad (1)$$

where [Q] is the concentration of the quencher (SPC tetramers in this study), F_0 is the fluorescence intensity of the fluorophore without quencher and F is the fluorescence intensity of the fluorophores with varying concentrations of quencher, k_q is the bimolecular quenching constant, τ_0 is the lifetime of the fluorophore in the absence of the quencher. Hence, based on Eq. (1), K_{SV} could be determined by linear regression of a plot of F_0/F vs. [Q]. Subsequently, the form of the classic Stern-Volmer equation (the Lehrer equation, Eq. (2)) was modified based on the understanding that the sample contained more than one fluorophore population with different accessibilities (Ujfalusi, Barko, Hild, & Nyitrai, 2010):

$$F0/F0 - F = 1/\alpha K\mathrm{sv}[Q] + 1/\alpha \quad (2)$$

where α is the fraction of the accessible fluorophore population.

By supposing that the quencher (Q) had some binding sites (n) on the protein, the number of binding sites could be calculated according to the results from Eq. (3):

$$\log(F0/F - 1) = \log KAC + n\log[Q] \quad (3)$$

where F_0 and F are the fluorescence intensities for the fluorophore