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Digestibility of fucosylated glycosaminoglycan from sea cucumber and its effects on digestive enzymes under simulated salivary and gastrointestinal conditions

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ARTICLE INFO

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D-Glucuronic acid (PubChem CID: 94715)
N-Acetyl-D-Galactosamine (PubChem CID: 35717)
Alpha-D-glucose (PubChem CID: 79025)
L-Fucose (PubChem CID: 17106)
D-Galactose (PubChem CID: 6036)
Acarbose hydrate (PubChem CID: 129837378)
Sodium Alginate (PubChem CID: 5102882)
Orlistat (PubChem CID: 3034010)
Heparin (PubChem CID: 772)

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ABSTRACT

The digestibility of fucosylated glycosaminoglycan (FG) and its effects on digestive enzymes were investigated using an in vitro digestion model. Results showed that the molecular weight and the reducing sugar content of FG were not significantly changed, and no free monosaccharides released from FG were detected after the salivary, gastric and intestinal digestion, indicating that both the backbone and the sulfated fucose branches of FG are resistant to be cleaved in the saliva and gastrointestinal tract environments. Furthermore, FG can dose-dependently inhibit digestive enzymes such as α-amylase, pepsin and pancreatic lipase in different degrees under the simulated digestion conditions due to the sulfate and carboxyl groups. These physiological effects of FG may help control the postprandial glucose concentration and have the potential in the prevention or treatment of reflux disease and obesity. The findings may provide information on the digestibility and beneficial physiological effects of FG as a potential natural product to promote human health.

1. Introduction

Polysaccharides derived from natural products have showed many biological functions such as anti-hyperlipidemia, anti-hyperglycemia and antithrombotic activities following oral administration; however, how the biomacromoleculars exert their activities in gastrointestinal tract is still a puzzle (Fonseca & Mourão, 2006; Wu et al., 2016; Zhao et al., 2012). Therefore, digestion of bioactive polysaccharides and their effects on gastrointestinal related physiological functions have received increasing attention among researchers in recent years (Chen et al., 2016).

Sea cucumbers, belonging to Echinodermata, Holothuroidea, are marine invertebrates found in most benthic marine habitats across the world. These animals have been a traditional tonic food in Asian countries such as Japan, Korea, Philippines and China for centuries as they contain high level of nutritional contents including vitamins, minerals, proteins and polysaccharides (Katzman & Jeanloz, 1969). Fucosylated glycosaminoglycan (FG), one of the polysaccharides from the body wall of sea cucumbers, has shown many pharmacological activities including antitumor, anti-hyperlipidemia, anti-hyperglycemia, antivirus, anti-inflammatory, anticoagulant and antithrombotic activities (Pomin, 2014). The activities were greatly affected by its molecular size and sulfated fucose (Fuc) branched units, which has been confirmed by the literature and our previous studies (Chen et al., 2013; Wu et al., 2012; Zhao et al., 2013). Intriguingly, recent studies also showed that FG can increase the content of probiotics bacteria in vitro in achieving health-enhancing effect and exert antithrombotic and anti-hyperlipidemic effects by the oral routes to experimental animals (Fonseca, Sucupira, Oliveira, Santos, & Mourão, 2017; Fonseca & Mourão, 2006; Liu, Ko, & Hu, 2002; Wei et al., 2017; Wu et al., 2016).

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Fonseca et al. (2017) hypothesised that FG is depolymerized and its sulfated Fuc branches are removed by the acidic gastric environment, which diminishes the anticoagulant activity of orally administered FG. However, there is little information available regarding the salivary, gastric or intestinal digestion of FG and the effects of FG on various digestive enzymes in gastrointestinal tract, because of the technical difficulty to track the metabolism of polysaccharides in vivo.

In our previous study, a FG from *Stichopus variegatus* was obtained and confirmed to have a structure mainly composed of a β1,4–linked trisaccharide repeating unit −{(L-Fuc$_{2S4S}$-α1,3-)D-GlcA-β1,3- D-GalNAc$_{4S6S}$}- (where GlcA and GalNAc represent glucuronic acid and N-acetyl-galactosamine residues) (Zhao et al., 2015). Fuc$_{4S}$ and Fuc$_{3S4S}$ residues α1,3-linked to GlcA exist in the FG. In this study, an in vitro digestion model of the FG was developed to investigate the digestion of FG. Furthermore, we studied the effects of FG on the activities of various digestive enzymes to evaluate the in vitro physiology effects of FG potentially taking place in gastrointestinal tract.

2. Materials and methods

2.1. Materials and chemicals

The fucosylated glycosaminoglycan (FG) with Mw 77 KDa was prepared from sea cucumber *Stichopus variegatus* according to our reported method (Zhao et al., 2015). Galactose (Gal), glucose (Glc), GlcA, 1-phenyl-3-methyl-5-pyrazolone (PMP), α-amylase from human saliva (Type IX-A, 1000–3000 U/mg protein), lipase from porcine pancreas (Type II, 100–500 U/mg protein), pancreatin from porcine pancreas, bile salts and pepsin from porcine gastric mucosa (2500 U/mg protein) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). GalNAc, Fuc, starch from potato, soybean oil, acarbose hydrate, sodium alginate and orlistat were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Trypsin (250 U/mg protein) was purchased from Shanghai Solarbio Bioscience & Technology Co., Ltd. (Shanghai, China). Amberlite FPA98Cl ion exchange resin was obtained from Rohm and Haas Company (Philadelphia, USA). Deuterium oxide (D_2O) and D_2O containing 0.05 wt.% 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid (TSP) sodium salt was purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Determination of uronic acid, sulfate group and intrinsic viscosity of FG

Uronic acid was measured by the carbazole method, with GlcA as the standard (Dische, 1947), sulfate group by the $BaCl_2$/gelatin method (Saito, Yamagata, & Suzuki, 1968) as described previously, and SO_3^- to COO^- molar ratios by a conductimetric titration method (Casu & Gennaro, 1975). The intrinsic viscosity [η] was determined in a 0.1 M NaCl solution at 25 °C using an Ubbelohde-type capillary viscometer according to the method in Pharmacopoeia of the People's Republic of China (Luo et al., 2013; Yang et al., 2010).

2.3. Preparation of desulfated and carboxyl-reduced derivatives of FG

The desulfated FG (dsFG) and carboxyl-reduced FG (crFG) were prepared as previously described (Vieira & Mourão, 1988; Wu et al., 2015). The obtained derivatives of FG were converted to their sodium salt by passage through a cation-exchange column (Dowex 50W × 8 Na^+ form) and lyophilized. The desulfation degree and reduction rate of FG were determined by the conductometric titration method.

2.4. Simulated salivary digestion of FG

2.4.1. Saliva preparation

The fresh saliva sample was collected from a healthy nonsmoking volunteer (Buettner, 2002). Subject had to be free of oral disease, fever and/or cold, malignant disease and maintain a good oral hygiene. The donor also had not been treated with antibiotics for at least 3 months before the experiment. Volunteer was asked to refrain from eating and drinking 1.5 h prior to saliva collection.

The spitting method for collecting human whole saliva samples was developed according to the method of Navazesh, 1993. The collected saliva was centrifuged at 3040*g* for 20 min to remove impurities and cells, and the supernatant was stored at −20 °C. The human saliva amylase activity was determined according to the reported method (Ruth & Roozen, 2000).

2.4.2. in vitro salivary digestion of FG

FG was subjected to in vitro salivary digestion according to the method (Zhang, Zhang, Zhang, Decker, & McClements, 2015). Test tube A contained 2 mL of FG solution (2 mg/mL) and 2 mL of saliva, test tube B contained 2 mL of deionized water and 2 mL of saliva, and test tube C contained 2 mL of deionized water and 2 mL of FG solution (2 mg/mL). All the test tubes were incubated in the incubator shaker at 37 °C for 2 h to mimic the agitation in the mouth. The process was set to be much longer than the time that a fluid product would spend in the mouth to determine the possible digestibility of FG. Samples were taken at different digestion time (0, 0.5, 1 and 2 h) and boiled for 10 min to inactivate human salivary amylase. All digestions were done in duplicates.

2.5. Simulated gastrointestinal digestion of FG

The electrolyte stock solutions of simulated gastric fluid (SGF) and intestinal fluid (SIF) were prepared and simulated gastrointestinal digestion of FG was established according to the method previously described (Minekus et al., 2014).

The SGF electrolyte stock solution contained 6.9 mM KCl, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 47.2 mM NaCl, 0.1 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$ and 0.15 mM $CaCl_2{\cdot}2H_2O$ was adjusted to pH 2.5 with 1 M HCl. The SIF electrolyte stock solution was prepared containing 6.8 mM KCl, 0.8 mM KH_2PO_4, 85 mM $NaHCO_3$, 38.4 mM NaCl, 0.33 mM $MgCl_2(H_2O)_6$ and 0.6 mM $CaCl_2{\cdot}2H_2O$, and the pH was adjusted to 7.0 by using 1 M NaOH.

Simulated gastrointestinal digestion was performed in two consecutive steps mimicking first gastric digestion followed by intestinal digestion. Briefly, 10 mL of FG aqueous solution (4 mg/mL) was mixed with 7.5 mL of SGF electrolyte stock solution, 1.6 mL pepsin stock solution of 25 000 U/mL made up in SGF electrolyte stock solution, 4.5 μL of 0.03 M $CaCl_2$, 1 M HCl to adjust pH to 2.5 and rest of volume with water to obtain a final ratio of FG solution to SGF of 1:1 (v/v). The digestion was performed at 37 °C for 6 h in a shaking bath with continuous shaking at 150 rpm. The pH of the digestion solution was kept at 2.5 with 1 M HCl. The intestinal digestion was then started by increasing the pH of gastric digested solution to 7.0 (drop-wise addition of 1 M $NaHCO_3$) and subsequently adding 4.5 mL of SIF electrolyte stock solution, 2.0 mL of a pancreatin solution 800 U/mL made up in SIF electrolyte stock solution based on trypsin activity, 1 mL fresh bile (160 mM in fresh bile), 7.5 μL of 0.03 M $CaCl_2$, 1 M NaOH to adjust pH to 7.0 and the rest of volume with water to obtain a final ratio of gastric chyme to SIF of 1:1 (v/v). The intestinal mixture was incubated at 37 °C for another 6 h with continuous shaking at 150 rpm. Samples (2 mL) were taken from the mixture at 0, 0.5, 1, 2, 4, and 6 h during gastric and gastrointestinal digestion and boiled for 10 min to inactivate enzymes for further analysis. All the experiments were performed in triplicate.

2.6. Determination of molecular weight change of FG

The digestion samples were through a 0.22 μm membrane and the molecular weight of FG was analyzed on a Shimadu LC-20A HPLC system (Shimadzu, Japan) coupled with evaporative light scattering detector (ELSD, Shimadzu, Japan). A TSK-gel G4000 PWXL (7.8 × 300 mm, 10 μm) was used at 35 °C with an injection volume of