Carbohydrate Polymers 143 (2016) 296–300

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Influences of acidic reaction and hydrolytic conditions on monosaccharide composition analysis of acidic, neutral and basic polysaccharides

Qing-Chi Wang [a], Xia Zhao [a,b,*], Jiang-Hua Pu [a], Xiao-Hong Luan [a]

[a] Key Laboratory of Marine Drugs, Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China
[b] Shandong Provincial Key Laboratory of Glycoscience and Glycoengineering, Ocean University of China, Qingdao 266003, China

ARTICLE INFO

Article history:
Received 19 November 2015
Received in revised form 28 January 2016
Accepted 8 February 2016
Available online 10 February 2016

Keywords:
Monosaccharide composition
Hydrolytic condition
Alginate
Starch
Chitosan
Chondroitin sulfate

ABSTRACT

Monosaccharide composition analysis is important for structural characterization of polysaccharides. To investigate the influences of acidic reaction and hydrolytic conditions on monosaccharide composition analysis of polysaccharides, we chose alginate, starch, chitosan and chondroitin sulfate as representative of acidic, neutral, basic and complex polysaccharides to compare the release degree of monosaccharides under different hydrolytic conditions. The hydrolysis stability of 10 monosaccharide standards was also explored. Results showed that the basic sugars were hard to release but stable, the acidic sugars (uronic acids) were easy to release but unstable, and the release and stability of neutral sugars were in between acidic and basic sugars. In addition, the hydrolysis process was applied to monosaccharide composition analysis of *Hippocampus trimaculatus* polysaccharide and the appropriate hydrolytic condition was accorded with that of the above four polysaccharides. Thus, different hydrolytic conditions should be used for the monosaccharide composition analysis of polysaccharides based on their structural characteristics.

1. Introduction

Monosaccharide composition analysis plays an important role in the structural characterization of polysaccharides. The qualitative and quantitative analysis of sugars is often based on the release of monosaccharides from polysaccharides (Wu, Jiang, Lu, Yu, & Wu, 2014). Trifluoroacetic acid (TFA) hydrolysis has been commonly accepted as a routine method for monosaccharide release (Honda et al., 1989; Harazono et al., 2011; Wang et al., 2015; Stepan & Staudacher, 2011). However, the release degree of monosaccharide depends on the cleavage of glycosidic bonds of polysaccharides under hydrolytic condition (Harazono et al., 2011; Albersheim, Nevins, English, & Karr, 1968; Zhang & Shen, 2013), resulting monosaccharide composition analysis may be incorrect if a same hydrolytic condition was used to different polysaccharides. Thus, it is necessary to explore suitable hydrolytic conditions for monosaccharide composition analysis of polysaccharides with different structural characteristics.

The reversed-phase HPLC method using 1-phenyl-3-methyl-5-pyrazolone (PMP) as a pre-column derivatization reagent has been widely used for the monosaccharide composition analysis of polysaccharides (Honda et al., 1989; Dai et al., 2010). Alginate, starch, chitosan and chondroitin sulfate (CS) are common polysaccharides in nature with different structure characteristics. In this study, the four polysaccharides were chosen as representative of acidic, neutral, basic and complex polysaccharides to compare the release degree of monosaccharides under different hydrolytic conditions. In addition, the hydrolysis stability of 10 monosaccharide standards was also explored. The appropriate hydrolysis temperature, time and concentration of TFA were investigated with the aim to provide a reference for monosaccharide composition analysis of polysaccharides.

2. Experimental

2.1. Materials and reagents

Alginate, chitosan, chondroitin sulfate (CS) and *Hippocampus trimaculatus* polysaccharides (HTP) were provided by Marine Biomedical Research Institute of Qingdao (China). Starch was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Monosaccharide standards of glucose (Glc),

* Corresponding author at: Ocean University of China, Key Laboratory of Marine Drugs, Yushan Road 5, Qingdao 266003, China. Tel.: +00 86 53282031560; fax: +00 86 53282031560.
E-mail addresses: zhaoxia@ouc.edu.cn, 1184748799@qq.com (X. Zhao).

http://dx.doi.org/10.1016/j.carbpol.2016.02.023
0144-8617/

galactose (Gal), glucosamine (GlcN), xylose (Xyl), mannose (Man), rhamnose (Rha), fucose (Fuc), galactosamine (GalN), glucuronic acid (GlcA) and galacturonic acid (GalA) were purchased from Sigma-Aldrich (USA). HPLC-grade acetonitrile was purchased from Merck KGaA (Germany). 1-phenyl-3-methyl-5-pyrazolone (PMP) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (USA).

2.2. Hydrolysis of alginate, starch, chitosan and CS

Single-factor experiments of hydrolysis temperature, time and concentration of TFA were designed firstly to investigate the influences of hydrolytic conditions on monosaccharide composition analysis of alginate, starch, chitosan and CS. Then orthogonal L_9 (3^4) experiments were designed to determine the appropriate hydrolytic conditions of the above 4 polysaccharides. Orthogonal experiment is a common method to study multiple factors and multiple levels which can optimize the process by analyzing the typical experimental results (Liu, Zhang, Wen, & Tang, 2010). The hydrolysis of 4 polysaccharides was carried out as follows: Polysaccharide sample powder (10 mg) was dissolved in TFA (0.5–4.0 mol/L) at a concentration of 10 mg/mL and hydrolyzed in a sealed glass ampoule at 105–120 °C for 1–6 h. The TFA was removed by rotary evaporation and the reaction residue was washed out of the evaporation flask with 1 mL water (Dai et al., 2010). The hydrolysis temperature (A), hydrolysis time (B), concentration of TFA (C) of the orthogonal L_9 (3^4) experiment were listed in Table 1. All experiments were operated in parallel three times, and all hydrolysates were diluted to the same concentration before derivatization.

2.3. Derivatization procedure

PMP derivatization procedure was carried out as described previously (Wu et al., 2014b; Wang, Zhao, Yu, Li, & Hao, 2009). Briefly, the hydrolyzed polysaccharide sample or monosaccharide standard was mixed with 0.3 mol/L aqueous NaOH (100 μL) and 0.5 mol/L methanolic solution of PMP (120 μL) in a small sample tube with lid. The whole mixture was heated to 70 °C and reacted for 1 h. The reaction mixture was neutralized with 0.3 mol/L HCl (100 μL) after it was cooled to room temperature, and then extracted with chloroform (0.5 mL) three times. The aqueous layer was filtered through a 0.22 μm micron membrane filter before HPLC analysis.

2.4. HPLC analysis

The Agilent 1260 HPLC system consisted of a quaternary pump, an autosampler, a variable wavelength detector (VWD) and a system controller. The data were collected using an Open LAB CDS Chemstation Edition (version C.01.05) provided by the Agilent Company (USA). A reversed phase Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm) was purchased from the Agilent Company (USA). Chromatographic separation of PMP derivatives was carried out using 0.1 mol/L phosphate buffer (pH 6.8) and acetonitrile at a ratio of 83:17 (v/v, %) as a mobile phase at a flow rate of 1.0 mL/min. The temperature of the column was maintained at 30 °C and detected by VWD at 245 nm (Wu et al., 2014b).

Table 1
Factors and levels of the orthogonal L_9 (3^4) experiment.

Factors/levels	Alginate	Starch	Chitosan	CS
Temperature (°C, A1)	100	100	110	110
Temperature (°C, A2)	105	105	115	115
Temperature (°C, A3)	110	110	120	120
Hydrolysis time (h, B1)	2	2	2	2
Hydrolysis time (h, B2)	3	3	4	4
Hydrolysis time (h, B3)	4	4	6	6
Concentration of TFA (mol/L, C1)	1.0	1.0	2.0	1.5
Concentration of TFA (mol/L, C2)	1.5	1.5	3.0	2.0
Concentration of TFA (mol/L, C3)	2.0	2.0	4.0	2.5

2.5. Control degradation of monosaccharide standards solution

To explore the stability of monosaccharide standards under different hydrolytic conditions, the equimolar mixture of 10 monosaccharide standards were hydrolyzed in an ampoule with 2 mol/L TFA at 110 °C for 2, 4 and 6 h, respectively. Then the TFA was removed by rotary evaporation and washed out by 1 mL water (Dai et al., 2010).

2.6. Application to the monosaccharide composition analysis of HTP

The hydrolysis process was applied to monosaccharide composition analysis of *H. trimaculatus* polysaccharide (HTP), which has a complex monosaccharide composition contained acidic, neutral and basic sugars. The hydrolysis of HTP was carried out as the orthogonal L_9 (3^4) experiment, namely, the hydrolysis temperature (A) at 100, 110 and 120 °C; hydrolysis time (B) at 2, 4 and 6 h; concentration of TFA (C) at 2, 3 and 4 mol/L; and the operation was same as above mentioned.

3. Results and discussion

3.1. Hydrolysis of alginate, starch, chitosan and CS

The influence of hydrolytic conditions on the release degree of monosaccharide from 4 polysaccharides was shown in Fig. 1. Take the influence of hydrolysis temperature on the release degree of monosaccharide for example (Fig. 1C). For alginate, the peak areas (the unit is "mAu × min", refers to the integral value of peak height and retention time) of the mannuronic acid (M) and guluronic acid (G) derivatives reached the maximum at 105 °C. The loss of M and G increased with the temperature going up, and the loss ratios of M and G at 120 °C were 22.6% and 44.6%, respectively, indicating that M and G were easily released and easily destroyed. For starch, the maximum peak area of Glc derivatives was 126500.9 mAu × min at 105 °C, and the peak area decreased slightly with temperature increasing from 105 °C to 120 °C (the loss ratio was 8.0%), indicating that Glc was released completely at 105 °C and stable in hydrolysis solution. For chitosan, the peak area of GlcN derivative increased with the rising temperature and reached the maximum at 120 °C, indicating that the GlcN of chitosan was hard to release and stable. For CS, The peak area of GalNAc derivatives increased with the temperature increasing from 105 °C to 120 °C, and reached the maximum (20899.2 mAu × min) at 120 °C. However, the peak area of GlcA derivatives reached the maximum at 115 °C, and then decreased by 19.3% at 120 °C. This phenomenon indicated that the GalNAc was released hardly and stable while the GlcA was released easily and unstable. The influences of hydrolysis time and concentration of TFA (as shown in Fig. 1A and B) were analyzed similarly by single-factor experiments. The results of single-factor experiments indicated that the uronic acids of alginate were easy to release but unstable, the amino sugar of chitosan was hard to release but stable, and the release and stability of the neutral sugar of starch was in between that of alginate and chitosan.

The orthogonal L_9 (3^4) experiment was performed further to investigate the appropriate hydrolytic conditions based on the results of single-factor experiments. Take the optimization of hydrolytic condition of chitosan for example. As listed in Table 2, the hydrolysis time and temperature were the major factors to affect the release degree of GlcN from chitosan, while the minor factor