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Modification of polygalacturonic acid hydroxyls with trimethylammonium- and/or sulfonate-2-hydroxypropyl group

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ABSTRACT

Polygalacturonic acid (PGA) was quaternized with 2,3-glycidyltrimethylammonium chloride (GTMAC) and/or sulfonated with 3-chloro-2-hydroxy-1-propanesulfonate (CHPS) under vacuum or at ambient pressure in the absence or presence of NaOH. The aim was to find out what optimal ratio of reactants are required to obtain the highest possible degree of substitution (DS), yield and molar masses. The highest DS of PGA obtained (calculated on a fully substituted derivate) for quaternized sample was around one according to elemental analyses. At ambient pressure the DS was lower than under a vacuum treatment. Lower DS and yield (36%) of PGA were obtained when PGA was sulfonated. At ambient pressure the sulfonation has not taken place. Repeated quaternization of PGA has not increased the DSs of PGA, while sulfonation was beneficial for increase of DS. When PGA was modified simultaneously with both alkylating agents, the yield of PGA was further improved (up to 52%). Further, the DS of the ammonium substituent was higher than that of the sulfonate group, and the yield of molar mass of the modified PGA was the highest from all tested experiments. At ambient pressure the DS of PGA treated with both reagents was lower than under vacuum. The treatment of PGA with NaOH at the absence of GTMAC or CHPS caused M_w decrease to 11,150 g/mol, while when quaternized in the absence of NaOH the values were between 20,940 and 32,540 g/mol. The repeated quaternization caused even more dramatic decrease of molar mass (to 6400 g/mol). According to NMR both C-2 and C-3 positions of PGA were substituted equally due to a use of the alkylating reagent or a certain charge.

1. Introduction

Pectin is a family of complex polysaccharides present in all plant primary cell walls (Ridley, O'Neill, & Mohnen, 2001). Pectic polysaccharides are important components of food products (Savary, Hotchkiss, Fishman, Cameron, & Shatters, 2003; Thakur, Singh, & Handa, 1997). Their solubility in water depends uppon the degree of esterification, acetyl content, pH, etc. In our previous work we have prepared water-insoluble PGA derivatives with ion-exchanging properties (Šimkovic, 1997; Šimkovic, Hricovíni, & Sasinková, 2002). The derivatives were suitable as stationary phases for purification of glycanases (Ondrášová, Omelková, Schubertová, & Šimkovic, 2002). In the present work we have focused on the preparation of water-soluble PGA derivatives using monofunctional quaternizing or sulfonating reagents. We have employed repeated quaternizing as well as applied sulfonating procedures, and have further used combinations of these modification procedures. The substrate itself contains carboxyl groups in a salt form and is not soluble in water without a pretreatment with base. Due to the fact that, in water/NaOH environment the alkylating agents are hydrolyzed to correspoding alcohols, which are innactive reaction side-products, we developed a modification procedure under vacuum to minimize the hydrolysis of the agent. The products were characterized by NMR and light scattering techniques and complemented by elemental analyses. The aim of the work was to prepare water-soluble polysaccharides in highest possible yields and molar masses. We believe that they could find uses in contolled drug delivery, film or suspension preparations and other composite applications (Liu, Fishman, & Hicks, 2007; Sinitsya, Čopíková, Prutzanov, Skoblza, & Machvič, 2000).

2. Experimental

2.1. Materials

PGA sodium salt (Sigma, batch #: 053K3780; C, 32.16; H, 5.13), GTMAC (Fluka) and CHPS (Aldrich) were used as a substrate or ionization reagents. All other chemicals used were without further purification (Merck).

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2.2. Analysis

The elemental analysis were run on a Fisons EA-1108 instrument, while NMR measurements were performed on INOVA-600 VARIAN spectrometer at 25 °C in D_2O with 3-(trimethylsilyl)-propionic acid (TSP) as an internal standard at a relaxation delay of 1 s. For HSQC the mixing time was 0.5 s; acquisition time 0.23 s; width 6000.6 Hz; 2D width 35987.4 Hz; 256 repetitions; 2 × 128 increments; power 34 dB, on during acquisition, off during delay. For HMBC the acquisition time was 0.175 s with adiabatic pulses. The size exclusion chromatography–multiple angle laser light scattering (SEC–MALLS) experimental conditions were: Dawn DSP multi-angle laser light scattering photometer from Wyatt (Santa Barbara, CA, USA) on line to a Alliance 2690 size exclusion chromatography system from Waters (Milford, MA, USA) by using two Waters Ultrahydrogel columns (1000–250 Å) and 0.05 M acetic buffer at pH 4.0 or 0.1 M $NaNO_3$ at pH 6.0 or 0.2 M NaCl + 0.1 M Tris buffer at pH 8.0; 35 °C as elution systems with 0.8 mL/min flow rate; dn/dc = 0.160 g/mL and KMX-16 differential refractometer from Milton Roy (Riviera Beach, FL, USA). To eliminate the aggregation effect of samples the experiments were repeated at pH 10.0 (0.1 M carbonate buffer as a mobile phase) using two TSK PW (G4000–G3000) columns setting.

Table 1
Quaternization/sulfonation of PGA

Sample #	Molar ratios of reaction components				Yield[a] (%)	X[b] (%)
	PGA	Y[c]	NaOH	H_2O		
1	1	4[d]	0	0–1000[e]	39	1.94[f]
2	1	10[d]	0	0–200[e]	43	3.02[f]
3	1	20[d]	0	0–200[e]	45	3.41[f]
4	1[g]	10[d]	0	0–200[e]	17	0.98[f]
5	0.5[h]	1[d]	0	0–1000[e]	30	2.86[f]
6	1	4[d]	4	0–1000[e]	40	3.36[f]
6[*]	1	4[d]	4	1000	43	2.10[f]
7	1	4[d]	10	0–1000[e]	32	2.28[f]
8	1	0	10	0–1000[e]	78	0
9	1	5[i]	10	0–1000[e]	34	1.83[j]
10	0.3[k]	1[i]	10	0–1000[e]	36	3.10[j]
11	1	10[i]	20	0–1000[e]	35	1.45[j]
11[*]	1	5[i]	10	1000	16	0[j]
12	1	5[d]/5[i]	10	0–1000[e]	52	3.27[f]/1.39[j]
13	1	10[d]/10[i]	20	0–1000[e]	40	3.13[f]/1.92[j]
13[*]	1	10[d]/10[i]	20	1000	37	1.81[f]/0.25[j]

[a] Water-soluble part was calculated on a fully substituted product.
[b] Nitrogen or sulfur content.
[c] Ionizing agent.
[d] GTMAC.
[e] See Section 2.
[f] Nitrogen content.
[g] Repeated quaternization of sample 2.
[h] Repeated quaternization of sample 3.
[i] CHPS.
[j] Sulfur content.
[k] Repeated sulfonation of sample 9.
[*] Experiments performed under ambient pressure.

2.3. Derivatization

The molar ratios of reagents are listed in Table 1. The PGA was mixed with water/NaOH solution, treated on vacum rotary evaporator for 1 h at 60 °C and subsequently the alkylating agent was added and the mixture was further modified on vacum rotary evaporator at 60 °C for 3 h under vacuum (3.3–4 kPa) or at ambient pressure. The reaction was stopped by dilution with water, neuralized to pH 7 when necessary, dialyzed (1 kDa MWCO; Spectra/Por®), filtered through 0.2 μm filter (PALL Gelman Laboratory) and lyophilized.

3. Results and discussion

3.1. Modification of PGA with glycidyltrimethylammonium chloride

The first quaternization experiments were performed without NaOH to find out how the reaction would proceed. The degree of substitution could be increased by changing the PGA/GTMAC mo-

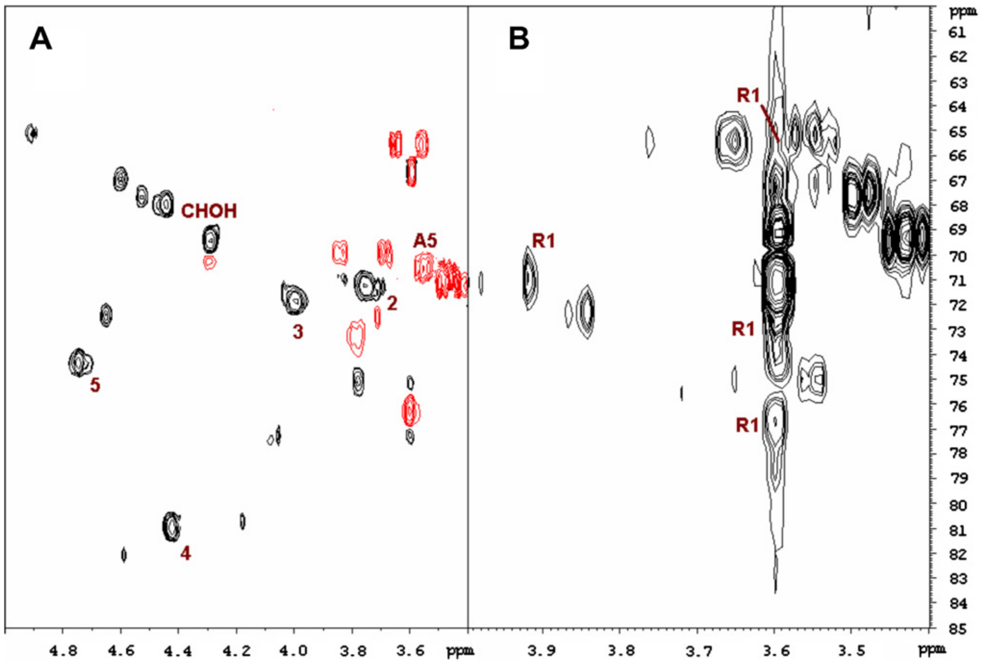

Fig. 1. The HMQC-DEPT (A) and HMBC (B) spectra of sample 3. The numbers assigned to peaks indicate C/H atoms in galacturonic acid residues; A5 is C/H-5 signal of arabinose; CHOH are signals of the TMAHP-substituent and R1 are HMBC multiplets confirming the correlation of the substitunet with C/H-2 and 3 signals of HSQC.