

Sucrose-Branched Polymer Synthesized by Protease from *Bacillus Subtilis*

Maurício Rodrigues Borges,* Rosangela Balaban

Summary: Vinyl sugar ester, 1'-O-vinyladipoyl-sucrose, was synthesized using sucrose and DVA (divinyl adipate) in organic medium (dimethylformamide), catalyzed by protease from *Bacillus subtilis*. Several reaction conditions were examined by means of qualitative analysis (TLC). The monomer obtained has undergone chemical polymerization with potassium persulfate and hydrogen peroxide to produce an amphiphilic polymer having sugar branches. The monomer was characterized by ^{13}C -NMR and FTIR spectra. The IR analysis confirmed the synthesis of the poly (1'-O-vinyladipoyl-sucrose).

Keywords: enzymatic synthesis; poly (1'-O-vinyladipoyl sucrose); sucrose ester

Introduction

Nowadays sucrose is becoming a very important raw material for chemical transformations. According to specialists, the world production of sugar cane reached around 162 million tons in 2006, due to the increase of the production mainly in Brazil and India. Consequently the sucrose's price should fall for less than US\$ 0.10/ pounds weight in New York in 2007.^[1] Sucrose presents interesting physical, chemical and rheological properties^[2] and enzymatic synthesis is a new strategy to keep almost the same structure of the sugars after transformation. This way is possible, for example, by connecting hydrophobic molecules from vegetable oil or derivatives with sucrose, aiming to obtain amphiphilic esters containing sucrose branched.^[3,4] These carbohydrate based products are biocompatible, biodegradable and non-toxic and consist of two inexpensive, renewable and easily accessible starting agricultural materials.^[5] Sucrose-branched polymers are very attractive materials in many application fields, for example in biofunctional materials, anti-

biotics, surfactants, cosmetics and so on.^[6] Herein we report the successful enzyme-catalyzed modification of an organic solvent-soluble disaccharide, sucrose, using anhydrous DMF as the reaction medium. The transesterification of DVA with sucrose was catalyzed by an alkaline protease from *Bacillus subtilis*. The vinyl sucrose ester obtained was polymerized by chemical catalyst to obtain a biodegradable polymer containing sucrose moiety.

Experimental Part

Materials

Alkaline protease B72, B79 from *Bacillus subtilis* (100 U/mg) was kindly supplied by Biovet (Bulgaria), DVA was purchased by TCI (Japan), DMF was obtained from Quimex (Brazil), potassium persulfate and hydrogen peroxide were purchased from CRQ (Brazil).

Analytical Methods

The progress of DVA transesterification with sucrose was monitored by HPLC with a refractive index detector and a carbohydrate analysis column, MetaCarb 87 P (Varian) that is packed with an exchange polymeric material in the Pb^{++} ionic form. Therefore, only distilled, de-ionized and

Laboratory of Biopolymers, Federal University of Rio Grande do Norte, Natal-RN, Brazil, C.P. 1662.

Fax: +55 (84) 3215-3803;

E-mail: mauriborges@hotmail.com

filtered (0.5 μm) water was used as the mobile phase. Aiming to de-gassing and make the water bacteria-free, the mobile phase was exposed to vigorous heating by microwave stove for 15 minutes immediately before use. The flow rate used was 1.0 mL/min. The conversion rate of sucrose to sucrose ester was calculated through the decrease of sugar concentration in comparison to the blank (reaction medium without enzyme). TLC qualitative analysis on silica gel 60 plates from Merck with an eluent mixture consisting of ethyl acetate/methanol/water (17/2/1 by vol.) presented spots after to be sprayed with H_2SO_4 (10%) followed by heating.

Infrared spectra of the monomer and respective polymer were obtained by FTIR spectrophotometer (ABB Bomen). The samples were dissolved in de-ionized water (5mg/ mL) and an aliquot of 40 μL of them were applied on zinc selenite optical plates and dried under reduced pressure to give homogeneous films. The position of acylation in the sucrose ester was determined by ^{13}C -NMR (Varian Mercury 200 MHz), using $\text{DMSO-}d_6$ as solvent.

Gel permeation chromatography was performed with a Varian 9002-SDS equipped with a Rheodyne injector 7125, refractive index detector Star 9040, interface Star 800 and a column TOSOH, TSK-GEL GMPWXL (300 \times 7.8)mm. The eluent was NaCl aqueous solution (0.1M) at flow rate of 0.6mL/min and 50 °C. A calibration curve was obtained with dextran standards in the number-average molar mass (M_n) range from 3,325 Da to 1,500 kDa. Both polymer sample and dextran standards were diluted to 5.0 mg/mL, using the eluent solution, followed by filtration in 0.22 μm Millipore membrane.

Transesterification Reaction

At first, sucrose (6.42g) was dissolved in DMF at 50 °C, followed by addition of DVA (14.87g). The reaction was initiated by addition of enzyme (6.0 g) and the suspension was placed in a shaking incubator with a stirring speed of 150 rpm for 5 days. The reaction was stopped by filtering

off the enzyme (catalyst), using a 0.22 μm centrifugal filter device. The DMF was evaporated off in a rotary evaporation system. The product was purified by silica gel chromatography with an eluent consisting of chloroform/methanol (9:1, by vol.).

Polymerization

The polymerization of the monomer 1'-O-vinyladipoyl-sucrose was carried out by dissolving 0.40g of divinyl sucrose ester in 2.0 mL H_2O and the solution was sparged with N_2 for 10 minutes. Afterwards, potassium persulfate (4mg) and H_2O_2 (4.0 μL) were added and the solution was stirred at 60 °C for 24h, in inert atmosphere [3]. The resulting product was precipitated in acetone, filtered and dried (45 °C, 24 hours).

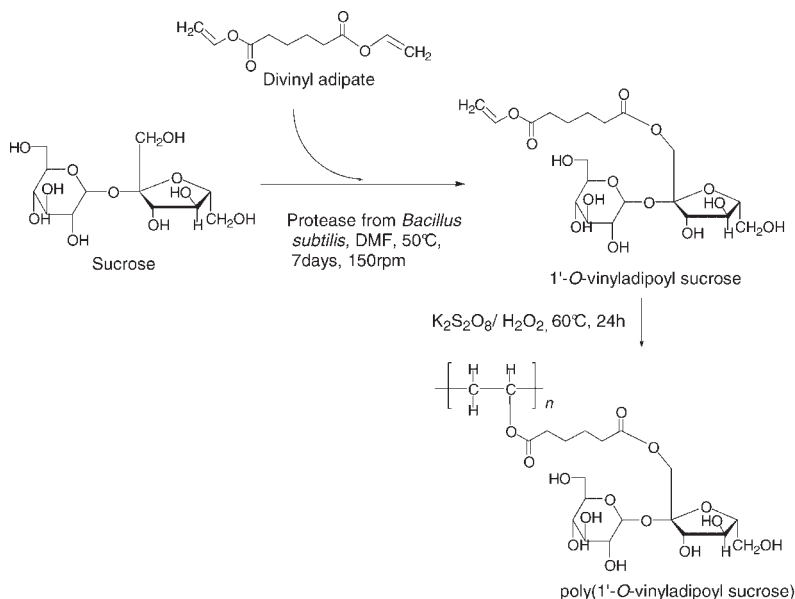
The Figure 1 presents the chemoenzymatic synthesis of poly (1'-O-vinyladipoyl-sucrose).

Results and Discussion

Strategy for Sugar Branched Polymer Development

The influence of the molar ratios between sucrose and DVA (1/1; 1/2; 1/4) using 40 mg/ mL of protease from *Bacillus subtilis* as catalyst at 50 °C in DMF was studied. At these reaction conditions the degree of transesterification of DVA was enhanced for the largest DVA amounts. The conversion rate of sucrose to sucrose ester reached more than 90% for molar ratio of sucrose/ DVA equal to 1/4, while for molar ratio of sucrose/ DVA equal to 1/1 the conversion ratio was only about 37%, after 5 days of reaction. The Figure 2 presents the conversion rate of sucrose to sucrose ester.

The TLC analysis of the synthesized sucrose ester on silica gel plates with an eluent consisting of ethyl acetate/ methanol/ water (17/2/1) confirmed that only one product was detected in the reaction mixture, after revelation with H_2SO_4 (10%) spray followed by heating. The purification of the sucrose ester was carried out by silica gel chromatography to separate the excess of DVA and sucrose residues.

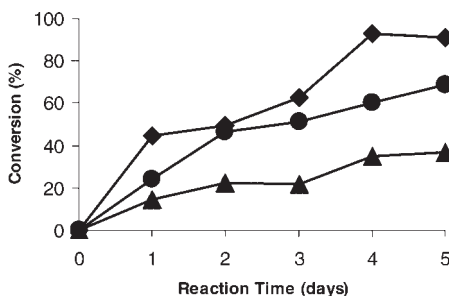
**Figure 1.**

Chemoenzymatic synthesis of poly (1'-O-vinyladipoyl-sucrose).

¹³C-NMR Characterization of the Monomer 1'-O-Vinyladipoyl Sucrose

The structure of vinyl sucrose was verified by ¹³C-NMR analysis, as shown in Table 1. The general method was the same as described by Yoshimoto *et al.*^[4] In other words, the acylation of a hydroxyl group of sugar results in a downfield shift of the peak corresponding to the *O*-acylated carbon and an upfield shift of the peak corresponding

to the neighbor one. In this case, ¹³C-NMR revealed that vinyl sucrose was substituted at C-1' position of sucrose, because C-1' carbon of sucrose presented a downfield chemical shift from 62.67 to 64.08 ppm and C-2' presented an upfield chemical shift from 104.50 to 102.23 ppm. These results indicate that alkaline protease from *Bacillus subtilis* shows an effective regioselectivity in the transesterification of DVA with sucrose.

**Figure 2.**

Effect of molar ratio between sucrose and DVA on conversion rate of sucrose to sucrose ester: sucrose/ DVA = 1/4 (♦), sucrose/ DVA = 1/2 (●), sucrose/ DVA = 1/1 (▲). The alkaline protease from *Bacillus subtilis* concentration used was 40 mg/ mL and the suspension was shaken at 50 °C and 150 rpm.

Table 1.

Chemical shift of ¹³C-NMR of sucrose and sucrose-DVA.

Position	Sucrose	Sucrose-DVA	$\Delta\delta$
	δ (ppm)	δ (ppm)	
C ₁	92.34	92.58	-0.24
C ₂	72.14	71.74	+0.40
C ₃	73.32	73.28	+0.04
C ₄	70.35	70.14	+0.22
C ₅	73.45	73.28	+0.17
C ₆	61.06	60.85	+0.21
C _{1'}	62.67	64.08	-1.41
C _{2'}	104.50	102.23	+1.92
C _{3'}	77.59	77.00	+0.59
C _{4'}	82.93	82.98	-0.05
C _{5'}	74.82	73.75	+1.07
C _{6'}	61.75	62.34	-0.59

FTIR Characterization of the Monomer 1'-O-Vinyladipoyl Sucrose and the Polymer Poly (1'-O-Vinyladipoyl Sucrose)

The Figure 3 presents the infrared spectra of the monomer and the respective polymer. The interest frequency region to accompany the polymerization is between 1640 cm^{-1} and 1680 cm^{-1} ($\nu_{\text{C}=\text{C}}$). The absorption band of vinyl group disappears during the polymerization. It can be noticed that the absorption band at 1643 cm^{-1} ($\nu_{\text{C}=\text{C}}$), relative to the monomer, disappeared in comparison to the polymer. This spectroscopic result has confirmed the free radical polymerization in the vinyl group.

GPC analysis of the Poly (1'-O-Vinyladipoyl Sucrose)

The Figure 4 presents the chromatogram of the sucrose polymer. The obtained values of the number-average molar mass $\langle M_n \rangle$, weight-average molar mass $\langle M_w \rangle$ and

dispersivity $\frac{\langle M_w \rangle}{\langle M_n \rangle}$ were $6.4 \times 10^3\text{ g/mol}$, $4.2 \times 10^4\text{ g/mol}$ and 6.57, respectively.

The polymerization of vinyl compounds can be initiated by free radicals, e.g. by azo initiators in organic solvents, or with redox initiators in water.^[3,6] Chen *et al.* obtained polymers of sucrose acrylate in aqueous solution with number-average molar mass $\langle M_n \rangle$ of 10^6 g/mol , using redox initiators in the polymerization. The initiator systems used were Fe^{+2} , $(\text{NH}_4)_2\text{SO}_4$ and H_2O_2 .^[7] Lu *et al.* synthesized 1'-O-vinyladipoyl sucrose in pyridine and polymerized this vinyl sugar ester with a redox mechanism, using potassium persulfate and hydrogen peroxide as initiator in water to give the poly (1'-O-vinyladipoyl sucrose) with number-average molar mass $\langle M_n \rangle$, weight-average molar mass $\langle M_w \rangle$ and dispersivity $\frac{\langle M_w \rangle}{\langle M_n \rangle}$ of $3 \times 10^4\text{ g/mol}$, $5 \times 10^4\text{ g/mol}$ and 1.61, respectively.^[3] Tokiwa *et al.* polymerized the vinyl sugar ester 6-O-vinyladipoyl D-glucose using ferrous sulfate and hydrogen peroxide as redox-initiator in water at two different conditions: in vacuum and at atmospheric pressure, to give polymers with number-average molar mass $\langle M_n \rangle$ of $3 \times 10^4\text{ g/mol}$ and $2 \times 10^4\text{ g/mol}$, respectively.^[8] In a general way, these examples show that the use of redox-initiators in water instead of free radicals initiators in organic solvents can present good results and the advantage of being an environmental friendly alternative.

Conclusion

Alkaline protease from *Bacillus subtilis* is an effective catalyst to synthesize sucrose ester with DVA, showing high selectivity at the hydroxyl group from primary carbon of the sucrose. Smaller values of sugar/ DVA molar ratio implicate in larger conversion values of sucrose to sucrose vinyl ester. It was possible to reach more than 90% of conversion using sucrose/ DVA molar ratio equal to 1/4. The redox-initiator system used in water ($\text{K}_2\text{S}_2\text{O}_8$ and H_2O_2) allowed vinyl group polymerization of the enzymatically prepared monomer to give poly

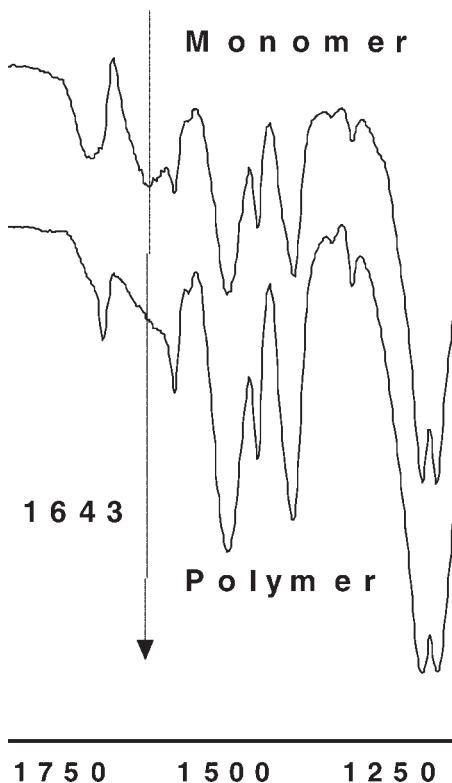


Figure 3.
FTIR spectra of sucrose-DVA and its polymer.

Chromatogram

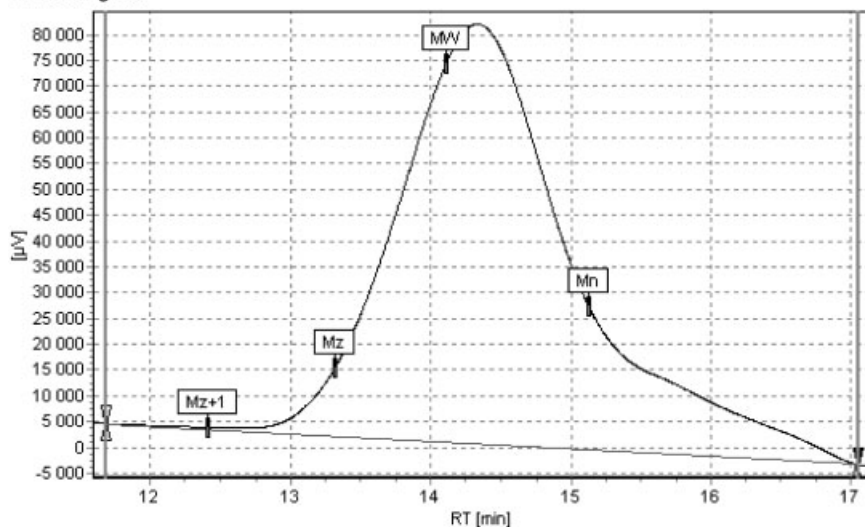


Figure 4.

GPC profile of poly (1'-O-vinyladipoyl sucrose). R_t is the retention time [min] at a constant flow of 0.6 mL/min and 50 °C. The labels indicate the corresponding averages of the molar mass.

(1'-O-vinyladipoyl sucrose) with number-average molar mass (M_n) of 6.4×10^3 g/mol. This catalytic condition called attention due to its environmental friendly proceeding. Finally, sugar branched polymers can be obtained by using different combinations of sugars and vinyl esters in order to obtain a wide range of materials with desired physicochemical properties.

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