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Note

Action of *Azotobacter vinelandii* poly-β-D-mannuronic acid C-5-epimerase on synthetic D-glucuronans

Pahn S. Chang [1], Rupendra Mukerjea, D. Bruce Fulton [2], John F. Robyt *

Laboratory of Carbohydrate Chemistry and Enzymology, 4254 Molecular Biology Building, Iowa State University, Ames, IA 50011, USA

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Abstract

Eleven different glucans (wheat starch, potato amylopectin, potato amylose, pullulan, alternan, regular comb dextran, α-cellulose, microcrystalline cellulose, CM-cellulose, chitin, and chitosan) that had their C-6 primary alcohol groups oxidized to carboxyl groups by reaction with 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion (TEMPO), were reacted with *Azotobacter vinelandii* poly-β-(1→4)-D-mannuronic acid C-5-epimerase. All of the oxidized polysaccharides reacted with the C-5-epimerase, as evidenced by comparing: (1) differences in the relative viscosities; (2) differences in the carbazole reaction; (3) differences in their susceptibility to acid hydrolysis, and (4) differences in their ability to form calcium gels, before and after reaction. We further show the formation of L-iduronic acid from D-glucuronic acid for oxidized and epimerized amylose by 2D NOESY and COSY + ^{1}H NMR.

Keywords: C-5-Epimerase; *Azotobacter vinelandii*; Alginate; Polyuronic acids; D-Glucuronic acid; L-Iduronic acid

1. Introduction

Alginate is a naturally occurring, linear (1→4)-linked copolymer of β-D-mannuronic acid and α-L-guluronic acid [1–5] that can be obtained from various species of brown seaweeds [6] and by fermentation of *Azotobacter vinelandii* and *Pseudomonas aeruginosa* [7–10]. These organisms first synthesize poly-(1→4)-β-D-mannuronic acid. The organisms elaborate a C-5-epimerase that catalyzes the inversion of the C-6 carboxyl groups of some of the β-D-mannuronic acid residues to give α-L-guluronic acid and the formation of alginate [11–13]. The inversion changes the configuration of the glycosidic linkage from β to α and the configuration of the uronic acid from D to L [13]. The amount of α-L-guluronic acid in alginates can vary between 33 and 66%. A total of eight genes that encode a family of C-5-epimerases in *A. vinelandii* have been cloned and expressed in *E. coli* [14,15]. A couple of these cloned C-5-epimerases have been shown to invert the carboxyl groups of D-mannuronic acids to give different distributions and sequences of L-guluronic acids in alginates [15].

* Corresponding author. Tel.: +1-515-2941964; fax: +1-515-2940453.

E-mail address: jrobyt@iastate.edu (J.F. Robyt).

[1] Present address. Department of Food Science & Technology, Seoul National Polytechnic University, 172 Kongenung-Dong, Seoul, South Korea.

[2] Present address. Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50011, USA.

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Alginates are known to form stable gels by complexing with divalent cations [16]. The affinity of the alginate for the divalent cations and the formation of gels is proportional to the amount of L-guluronic acid present in the alginate [17,18]. Alginates have many commercial applications as thickeners, emulsifiers, and stabilizers and their calcium complexes for forming gels, films, and encapsulating agents [19,20]. Alginates can be tailor-made with different amounts of L-guluronic acid by reacting them in vitro with C-5-epimerase [21].

In this study, we report the scope of the specificity of *A. vinelandii* C-5-epimerase preparation by studying its reaction with 11 different, synthetic poly-D-glucuronic acids that were produced by the oxidation of the C-6-primary hydroxyl groups of both α- and β-glucans with 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion (TEMPO) to carboxyl groups [22].

2. Experimental

Materials

C-5-epimerase. The enzyme was obtained by culturing *A. vinelandii* NRRL-14647 and isolating it from the culture supernatant, as described by Haug and Larsen [11]. The organism was cultured in 1-L flasks and the cells were removed by centrifugation. The C-5-epimerase was obtained by precipitation of the 30–80% (w/v) $(NH_4)_2SO_4$ fraction from the culture supernatant. The precipitate was dissolved in 100 mL of 50 mM collidine buffer (pH 6.8) and dialyzed for 24 h at 4 °C against the salt solution of the culture medium with the omission of $CaCl_2$. The dialyzate was freeze-dried to give a preparation of enzyme powder [23]. Assay of the C-5-epimerase activity was by the acid hydrolysis/phenol H_2SO_4 procedure, using alginate as the substrate [23]. One unit of enzyme activity is defined as the number of μmol of β-D-mannuronic acid inverted to α-L-guluronic acid per min. The specific activity of the enzyme was 375 mIU/mg of protein.

Polyglucuronic acids. Eleven different poly(D-glucuronic acids) were obtained by oxidizing wheat starch, potato amylose, potato amylopectin, alternan, *Leuconostoc mesenteroides* B-742 regular comb dextran, pullulan, α-cellulose, microcrystalline cellulose, CM-cellulose, chitin, and chitosan with TEMPO, as described by Chang and Robyt [22]. Sodium alginate (medium viscosity, No. A2033) was obtained from Sigma Chemical Co., St. Louis, MO.

Methods

Reaction of C-5-epimerase with the poly(D-glucuronic acids). Poly(D-glucuronic acid) (500 mL, 2.5 mg/mL), 50 mM collidine buffer (pH 6.8, 2 L), 25 mM $CaCl_2$ (350 mL), and C-5-epimerase (1000 IU, 500 mL) were mixed together and the reaction allowed to proceed for 72 h at 20 °C. The reaction solution was concd to 500 mL under vacuum and the polysaccharides were precipitated by the addition of two volumes of EtOH, followed by centrifugation. The precipitates were triturated with 100 mL of anhyd acetone (5–6 times) and then by a final treatment with 100 mL of anhyd EtOH. The polysaccharides were then dried at 50 °C under continuous vacuum for 15 h, giving a free-flowing powder.

Determination of the percent of inversion. The glycuronans (3 mg) that had been reacted with the C-5-epimerase were suspended in 3.0 mL of 0.3 M HCl and heated at 100 °C; 0.50 mL aliquots were removed at various times (12, 24, 48, and 72 h), centrifuged, and 25 μL of the supernatant taken in triplicate for determining the amount of uronic acid that was solubilized, using the phenol–H_2SO_4 procedure [23]. Glycuronans that had not been reacted with C-5-epimerase were also hydrolyzed and analyzed in an identical manner. From the amount of uronic acid (UA) released from the epimerized polysaccharide (EP) and from the unepimerized polysaccharide (UP), the percent of inversion (%I) for the action of the C-5-epimerase on α-linked polyuronic acids and β-linked polyuronic acid are given, respectively, by the following equations:

$$(\%I)_\alpha = \frac{(\text{UA from UP}) - (\text{UA from EP})}{(\text{UA from UP})} \times 100 \quad (1)$$

$$(\%I)_{\beta} = \frac{(\text{UA from UP}) - (\text{UA from UP})}{(\text{UA from EP})} \times 100 \quad (2)$$

Carbazole reaction with oxidized polysaccharides and oxidized and epimerized polysaccharides.—The Dische reaction [24] was adapted for use as a micro procedure with 98-well plates. The oxidized polysaccharides and the oxidized and epimerized polysaccharides (10.0 mg each) were suspended in 1.0 mL of water, mixed and then placed into an ice bath; 6.0 mL of concd H_2SO_4 was added to the chilled solution of polysaccharide, which was thoroughly mixed until a clear solution was obtained. Aliquots (25 μL) of each sample were then taken in triplicate and added to a 98-well plate; 175 μL of 6:1 H_2SO_4–water was added, mixed, and then heated at 80 °C for 35 min. The samples were then allowed to cool to 20–21 °C and 6 μL of carbazole reagent (1 mg/mL in EtOH) was added and the solutions kept for 2 h to develop the maximum color [24]; the absorbance was then measured at 490 nm, using a microplate colorimeter. The means of the triplicate measurements and the mean deviations were computed.

Table 1
Relative viscosities (η/η_o) for oxidized and oxidized and epimerized D-glucans at 25 °C

Relative viscosity (η_r) [a]		
Original polysaccharide	Oxidized	Epimerized
Alternan	10.98 ± 0.04	72.04 ± 0.27
Potato amylopectin	10.62 ± 0.01	71.10 ± 0.39
Wheat starch	9.95 ± 0.05	70.25 ± 0.51
Pullulan	9.82 ± 0.10	67.58 ± 0.48
Regular comb dextran	9.74 ± 0.10	65.92 ± 0.44
Chitosan	9.32 ± 0.02	57.27 ± 0.40
Microcrystalline cellulose (Avicel)	11.11 ± 0.01	53.61 ± 0.43
α-Cellulose	10.59 ± 0.06	49.97 ± 0.35
Chitin	10.52 ± 0.01	49.09 ± 0.51
Potato amylose	9.42 ± 0.03	38.04 ± 0.27
Carboxymethyl cellulose	9.97 ± 0.05	26.49 ± 0.16
Alginate [b]		98.01 ± 0.62

[a] Relative viscosity, $\eta_r = (\eta/\eta_o)$, where η = time of flow of the polysaccharide solution (2 mg/mL) through the Ostwald viscometer and η_o = time of flow of the solvent, water, through the viscometer.

[b] Alginate from brown seaweed is a copolymer of β-D-mannuronic acid and α-L-guluronic acid formed by the reaction of C-5-epimerase with poly β-D-mannuronic acid.

Determination of the viscosity. The viscosities of the polyuronic acids were measured with 2 mg/mL samples in water at 25 °C, using an Ostwald viscometer. Samples were analyzed in triplicate and the mean and mean deviation computed for the relative viscosities (η/η_o), where η is the time of flow of the polysaccharide solution in the viscometer and η_o the time of flow of the solvent, water, in the viscometer.

Proton NMR spectroscopy.—C-6-Oxidized amylose and oxidized and epimerized amylose were each dissolved to their maximum capacity (~ 2 mg/mL) in 600 μL of 1 M NaOH in 9:1 water–D_2O and placed in standard 5 mm NMR tubes. Each of the samples was made 5 mM in 4,4-dimethyl-4-silapropane sulfonate (DSS), which was used as the internal chemical shift standard. The concentrations of the polysaccharides were not sufficient to obtain a natural abundance ^{13}C NMR spectrum, and so only ^{1}H NMR experiments were performed. The NMR data were obtained by using a Bruker DRX500 spectrometer, operating at 499.865 MHz, with a sample temperature of 25 °C. For each of the samples 1D ^{1}H, 2D ^{1}H NOESY [25] and 2D DFQ-COSY [26] spectra were obtained. In addition, a 1D proton spectrum of 50 mM D-glucose and a 2D ROESY [27] spectrum of 50 mM maltose, both in D_2O, were obtained to assist in the spectral assignments. Solvent suppression was achieved by use of the WATERGATE method [28] for the NOESY experiments.

3. Results and discussion

Comparison of the relative viscosities of oxidized and oxidized and epimerized polysaccharides.—The reaction of the C-5-epimerase with the oxidized polysaccharides gave products that had significantly increased viscosities (Table 1). The relative viscosities increased from a high of 10.98 to 72.04 for oxidized alternan to a low of 9.97 to 26.49 for CM-cellulose, giving changes of a high of 7.1-fold for oxidized wheat starch and oxidized regular comb dextran to a low of 2.7-fold for oxidized CM-cellulose, with an average increase in the viscosity for the 11 glycuronans of 5.6-fold

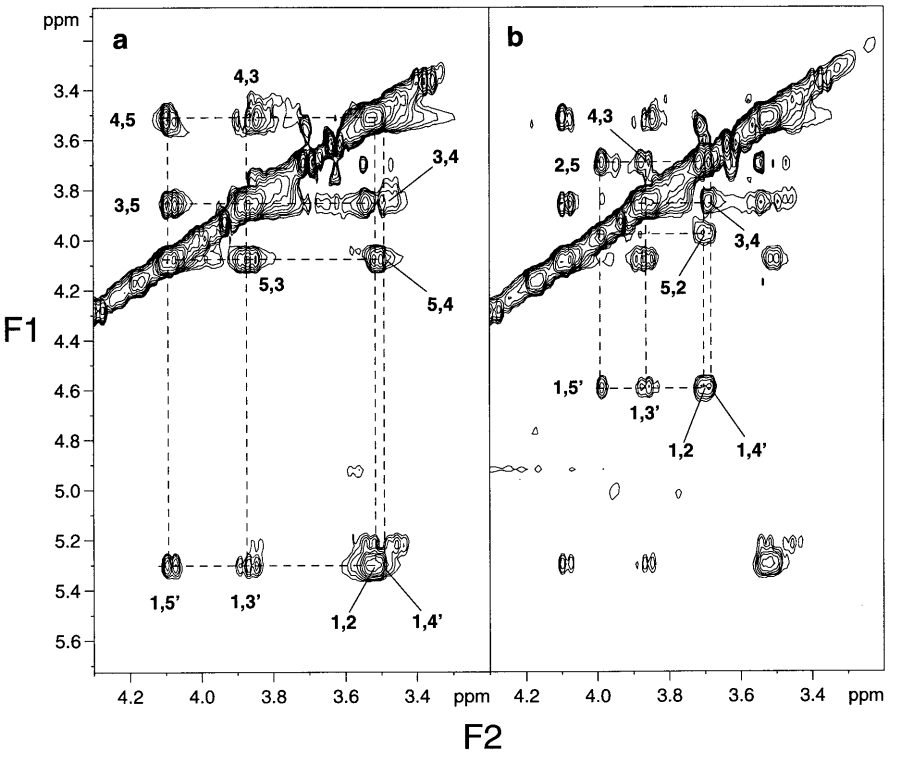

Fig. 1. 2D NOESY proton spectra of (a) oxidized amylose and (b) oxidized and epimerized amylose. In (a) assignments for oxidized amylose are shown using the standard numbering system for pyranose ring protons. Inter-pyranosyl NOEs (i.e., those between an anomeric hydrogen and hydrogens on the adjacent ring) are indicated by a prime on the number of the second proton. In (b), assignments for oxidized and epimerized amylose are numbered, all other peaks are the same as those in (a), arising from oxidized amylose.

(Table 1). These large differences in the relative viscosities indicate that definite structural changes had occurred for each of the 11 glycuronans after reaction with the C-5-epimerase.

^{1}H NMR of oxidized and oxidized and epimerized amylose.—2D NOESY and DQF-COSY ^{1}H NMR spectra were obtained for C-6-oxidized amylose and C-6-oxidized amylose that was reacted with the C-5-epimerase. Although the lines were quite broad due to the high molecular weight of the polysaccharides, the spectra were of adequate quality to obtain assignments. Fig. 1 shows the region of the NOESY spectrum containing NOEs involving the anomeric hydrogens (lower region), which includes NOEs between protons within the same pyranosyl residues (upper region of Fig. 1(a)) and NOEs involving the anomeric hydrogens (lower region), which includes NOEs between successively linked pyranosyl residues. Splittings due to scalar coupling are observed in the F2 direction. Due to the broad linewidths only the relatively large three-bond couplings between trans hydrogens (hydrogens axially disposed on adjacent carbons) can be resolved in the NOESY spectra. In the COSY spectra, however, all expected *J* couplings were observed either as active or passive couplings (data not shown). Using through-space connectivities (NOESY), through-bond connectivities (COSY), coupling patterns, and chemical shifts, the spectrum of oxidized amylose was assigned, as shown in Fig. 1(a) and Table 2. The H-5 signal is a doublet in Fig. 1(a) and is shifted downfield from 3.75 ppm in D-glucose (data not shown) to 4.10 ppm, indicating that C-6 was a carboxyl group. An additional group of weak lines (lower region of Fig. 1(a) and (b)) was observed at 5.25 ppm in F1 and between 3.4 and 3.9 ppm in F2. These are due to a small amount of unoxidized D-glucose in the oxidized amylose, which was confirmed with the ROESY spectrum of maltose (data not shown).

Fig. 1(b) shows the corresponding region of the NOESY spectrum of oxidized and epimerized amylose. All resonances observed in Fig.

1(a) are also present, indicating that D-glucuronic acid residues are present in the epimerized polysaccharide. However, a number of new peaks are present that can be unambiguously assigned to C-5 epimerized residues. The H-5 resonance is now a singlet (see middle of Fig. 1(b)), showing that H-5 is no longer trans to H-4, and therefore inversion of the carboxyl group at C-5 has occurred.

The observed scalar coupling-patterns show that the pyranose rings of both oxidized amylose and oxidized and epimerized amylose exist predominantly in the $^4C_1$ chair conformation with H-2, H-3, and H-4 in axial dispositions. The carboxyl group in oxidized amylose is equatorial and the hydrogen on C-5 is axial, giving a doublet (lower part of Fig. 1(a)), whereas the carboxyl group in oxidized and epimerized amylose is axial and the hydrogen on C-5 is equatorial, giving a singlet, as shown in the middle part of Fig. 1(b). With the carboxyl group axial in oxidized and epimerized amylose, H-1 is subject to anisotropic shielding. In contrast, the carboxyl group in oxidized amylose is in the equatorial position and therefore anisotropic shielding will not occur. This is shown by the upfield shift of H-1 from 5.31 to 4.61 ppm (Table 2), which is observed in oxidized and epimerized amylose. Inversion of the carboxyl group to the axial position will cause significant changes in the steric and electrostatic properties of the polysaccharide, as is evident from changes in the relative viscosity after epimerization.

Carbazole reaction of the oxidized and the oxidized and epimerized polysaccharides.—It is further demonstrated that the C-5-epimerase inverts the C-6 carboxyl group of all of the oxidized polysaccharides by comparing the absorbances of the carbazole reaction for the oxidized polysaccharides with the absorbances of the carbazole reaction of the exact same amounts of oxidized and epimerized polysaccharides, with the exception of chitin and chitosan, as they do not undergo the reaction. Inversion of the carboxyl group of D-glucuronic acid gives L-iduronic acid. If L-iduronic acid is being formed, the absorbance in the carbazole reaction would be higher for the oxidized polysaccharides than for the corresponding oxidized and epimerized polysaccharides, because the extinction coefficient of L-iduronic acid is 29% of the extinction coefficient of D-glucuronic acid [29]. The product of the reaction of uronic acids with H_2SO_4 is 5-formylfuroic acid [30], and this product is formed more slowly from L-iduronic acid than from D-glucuronic acid, especially at 80 °C [31] and, thus, L-iduronic acid has a lower extinction coefficient than D-glucuronic acid in the carbazole reaction. All of the oxidized and epimerized polysaccharides had lower absorbances than the oxidized polysaccharides (Table 3), showing that the C-5-epimerase was indeed inverting the C-6 carboxyl groups to give α-L-iduronic acid.

Table 2
Proton chemical shifts and multiplicities for ring protons of oxidized amylose and oxidized and epimerized amylose determined by 2D NOESY proton NMR

Polysaccharides	Proton	Chemical shift (ppm) [a]	Multiplicity
Oxidized amylose	1	5.31	1
	2	3.54	2
	3	3.89	3
	4	3.53	3
	5	4.10	2
Oxidized and epimerized amylose	1	4.61	1
	2	3.72	2
	3	3.87	3
	4	3.71	2
	5	4.00	1

[a] Chemical shifts are relative to the internal standard, 4,4-dimethyl-4-silapropanesulfonate (DSS).

Table 3
Absorbances for the carbazole reaction with equal amounts of unepimerized and epimerized glucuronans

Original polysaccharide	Unepimerized	Epimerized [a]
Wheat starch	0.658 ± 0.024	0.347 ± 0.027
Potato amylose	0.643 ± 0.025	0.312 ± 0.027
Potato amylopectin	0.667 ± 0.024	0.290 ± 0.012
α-Cellulose	0.381 ± 0.040	0.207 ± 0.016
Pullulan	0.294 ± 0.010	0.277 ± 0.036
Alternan	0.364 ± 0.016	0.276 ± 0.018

[a] Absorbances were measured at 490 nm for triplicate samples and the mean and the mean deviation calculated for equal amounts of each polysaccharide.

Comparison of the acid hydrolysis of oxidized and oxidized and epimerized polysaccharides.—In general, it is recognized that β-glycosidic linkages are more resistant to acid hydrolysis than are α-glycosidic linkages [32], and it has specifically been shown that the α-linkages of L-guluronic acid residues in alginates are hydrolyzed more easily and rapidly than the β-linkages of D-mannuronic acid residues [11,23]. The α-linked glycuronans, when reacted with C-5-epimerase, will have some of the α-linkages converted to β linkages and thus will not be hydrolyzed as fast as the unreacted α-glycuronans. Likewise, the β-linked glycuronans, when reacted with C-5-epimerase, have some of the β linkages converted into α linkages and are thus hydrolyzed faster than the unreacted β-glycuronans.

Fig. 2(A–F) presents the comparison of the acid hydrolysis curves for the α-linked glycuronans (C-6-oxidized wheat starch, potato amylose, potato amylopectin, pullulan, alternan, and regular comb dextran) and for their C-5-epimerase reacted counterparts. All of these α-glycuronans show that the C-5-epimerase had inverted some of the carboxyl groups, giving β-linked residues, as the reacted glycuronans were hydrolyzed more slowly and to a lesser degree than the unreacted ones. This is exactly what would be expected if C-5-epimerase were inverting the C-6 carboxyl groups, converting α linkages into β linkages.

Fig. 2(G–H) presents the comparison of the acid hydrolysis curves for the β-linked glycuronans (C-6-oxidized α-cellulose and *O*-carboxymethylcellulose) and for their C-5-epimerase-reacted counterparts. These β-glycuronans show that the C-5-epimerase had inverted some of the carboxyl groups, giving α-linked residues, as the reacted glycuronans were hydrolyzed faster and to a greater degree than the unreacted ones. This is exactly what would be expected if C-5-epimerase were inverting the C-6 carboxyl groups, converting β linkages into α linkages.

The differences in the amounts of uronic acids that are released from the oxidized polysaccharides can, thus, be used to give the amount of inversion by the action of the C-5-epimerase. The differences after 72 h of hydrolysis between the unreacted and the reacted glycuronans can be converted into the percent of uronic acids inverted by using Eqs. (1) and (2). Table 4 gives the percent of D-glucuronic acid converted into L-iduronic acid for nine of the C-6 oxidized polysaccharides that were reacted with C-5-epimerase, but the percent inversion could not be determined for chitin and chitosan by the acid hydrolysis and phenol–H_2SO_4 procedure as they do not give a colored product because of the acetamido group and the amino group, respectively, at C-2. Chitin and chitosan also do not undergo the carbazole reaction for the same reason. Nevertheless, C-6-oxidized chitin and chitosan do undergo reaction with C-5-epimerase, as judged by the increases in their relative viscosities after reaction (see Table 1) and differences in their ability to form gels with calcium ions (Table 5).

Structures of the C-6-oxidized and epimerized polysaccharides.—Based on the results of the percent of D-glucose oxidized for the 11 polysaccharides given in Ref. [20] and on the results given in Table 4, the C-6-oxidized and epimerized polysaccharides have unique structures. Wheat starch had 100% of its available D-glucose residues oxidized to D-glucuronic acid and the C-5-epimerase inverted 60% of these carboxyl groups. Potato amylose had 96% of its available D-glucose residues oxidized and the C-5-epimerase inverted 72% of them.

Pullulan consists of maltotriose units linked α-(1→6) to each other, giving two D-glucose residues out of three with C-6-hydroxyl groups, which were 100% oxidized. C-5-

epimerase inverted 48% of them, giving approximately one carboxyl group inverted in every three residues.

Alternan consists of D-glucose residues alternately linked α-(1→6) and α-(1→3) with 10% α-(1→3) branch linkages. All of the free C-6-hydroxyl groups were oxidized and C-5-epimerase inverted 51% of them.

Regular comb dextran has single D-glucose residues linked α-(1→3) to every D-glucose residue in the α-(1→6)-linked dextran chain. Of these single D-glucose residues, 92% were oxidized and C-5-epimerase inverted 62% of them.

α-Cellulose had 87% of the D-glucose residues oxidized to D-glucuronic acid and

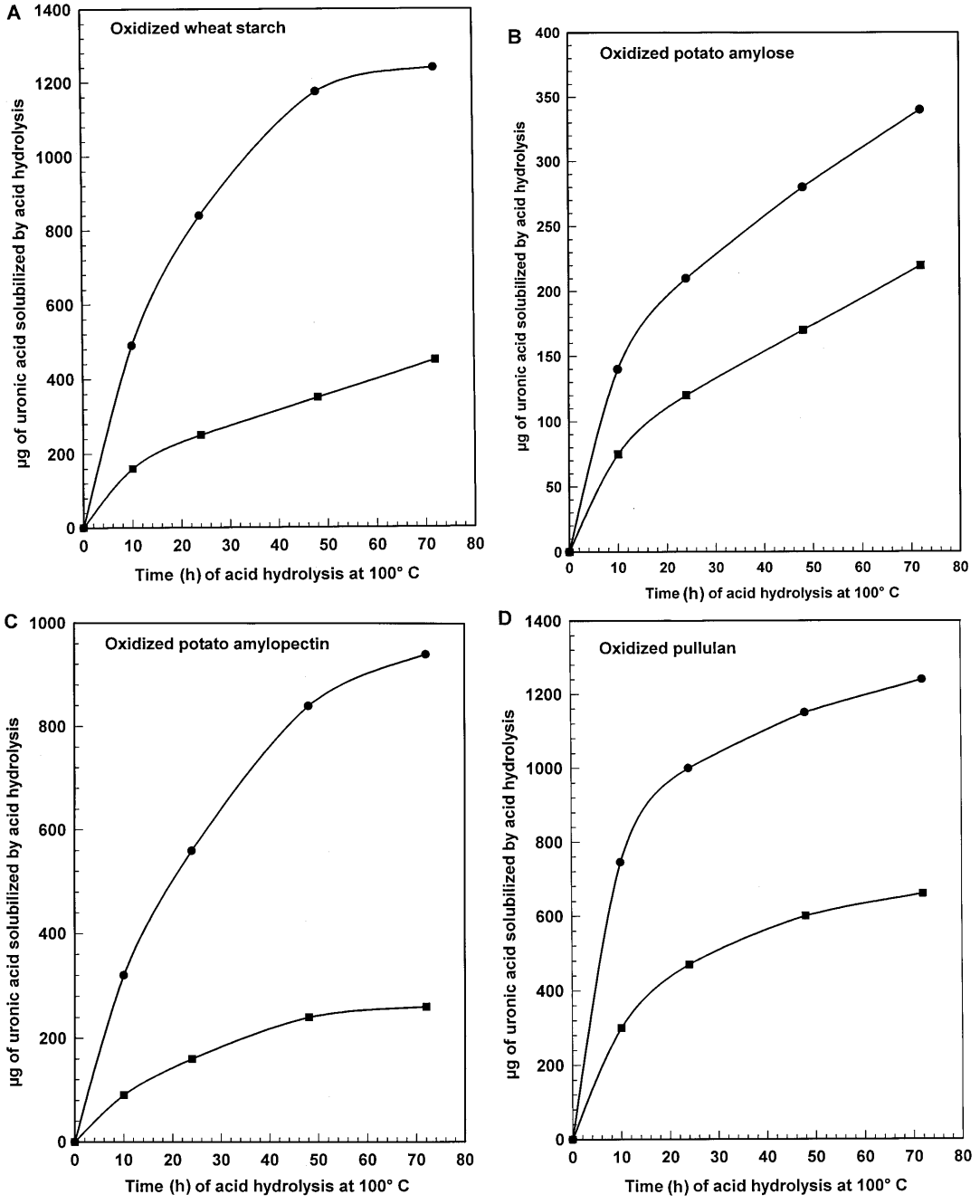

Fig. 2. Acid hydrolysis and phenol–H_2SO_4 analysis of the released (solubilized) uronic acid from unepimerized (●) and epimerized (■) polyuronic acids. (A) C-6-oxidized wheat starch; (B) C-6-oxidized potato amylose; (C) C-6-oxidized potato amylopectin; (D) C-6-oxidized pullulan; (E) C-6-oxidized alternan; (F) C-6-oxidized regular comb dextran; (G) C-6-oxidized microcrystalline cellulose (Avicel), and (H) C-6-oxidized CM-cellulose.

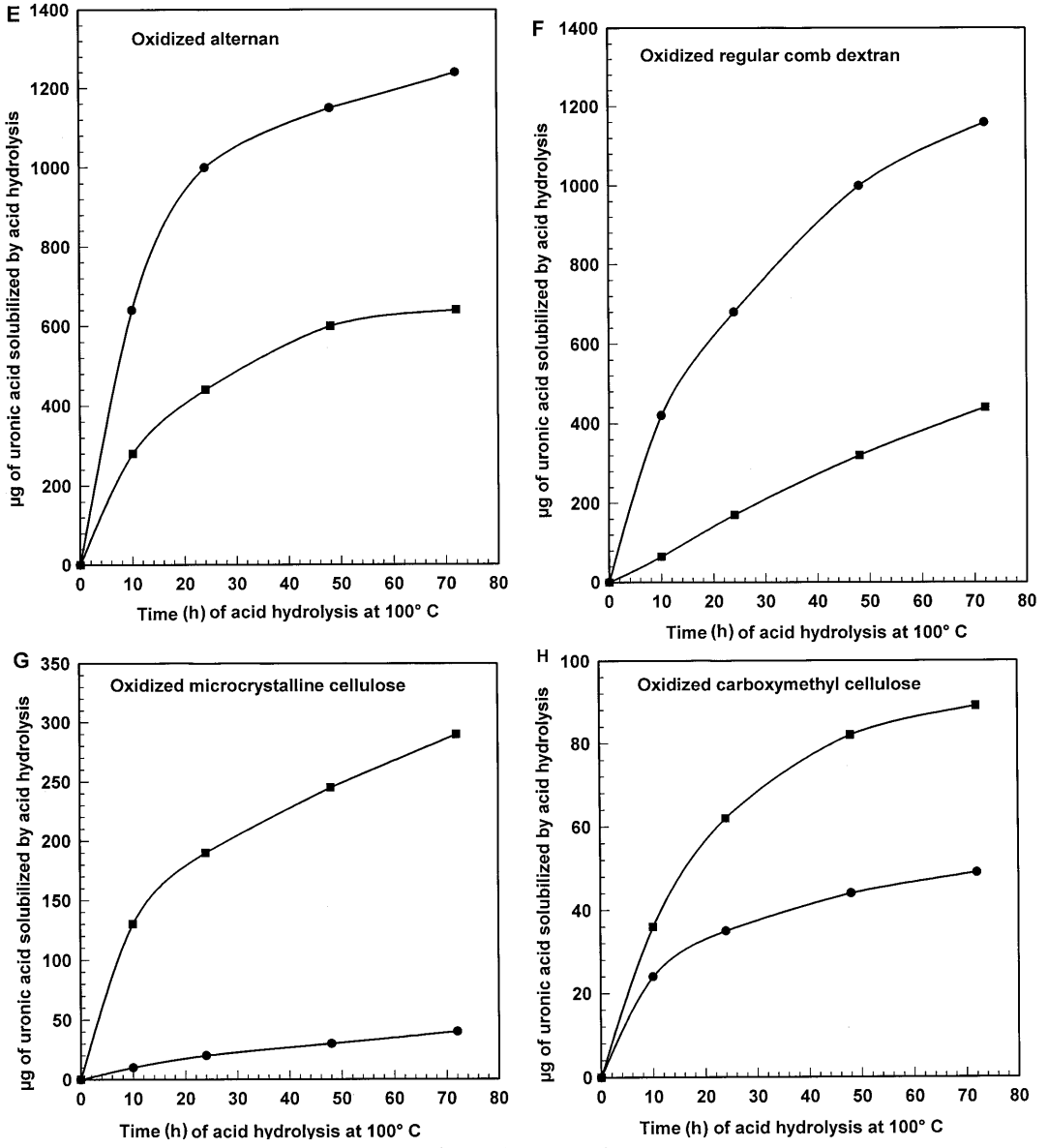

Fig. 2. (*Continued*)

C-5-epimerase inverted 72% of them. *O*-Carboxymethylcellulose had 90% of its D-glucose residues oxidized, and C-5-epimerase inverted 44% of them.

Formation of Ca^{2+}–gels.—A qualitative comparison of the formation of Ca^{2+}–gel beads by the C-6-oxidized polysaccharides and epimerized polysaccharides is presented in Table 5. Five oxidized polysaccharides did not form Ca^{2+}–gels at all, but on epimerization all five formed gels — three of them from pullulan, alternan, and regular comb dextran formed very good gels, and two of them from chitin and chitosan, formed moderate gels. Two of the oxidized polysaccharides from wheat starch and potato amylopectin formed good gels, and after epimerization, they formed very good gels. Oxidized potato amylose formed a good gel, and after epimerization formed a moderate gel. Oxidized CM-cellulose formed a moderate gel, and after epimerization, it formed a poor gel.

4. Summary and conclusions

A C-5-epimerase preparation from *A. vinelandii* inverted the carboxyl groups of 11 C-6-oxidized glucans that comprised both α- and β-linked glucans and diverse structures. We demonstrated these inversions by compar-

Table 4
Percent of D-glucuronic acid converted to L-iduronic acid by the action of C-5-epimerase on various glycuronans, as determined by acid hydrolysis [a]

Glycuronan obtained from	% Converted [a]
Microcrystalline cellulose (Avicel)	87
α-Cellulose	72
Potato amylopectin	72
Regular comb dextran	62
Wheat starch	60
Alternan	51
Pullulan	48
CM-cellulose	44
Potato amylose	33

[a] Percent of D-glucuronic acid inverted determined from 72 h of acid hydrolysis of α-linked glycuronans by using Eq. (1) and from 72 h of acid hydrolysis of β-linked glycuronans by using Eq. (2) in the text.

Table 5
Qualitative comparison of the formation of calcium gels with unepimerized and epimerized glycuronans

Polysaccharides	Unepimerized [a]	Epimerized
Wheat starch	good	very good
Potato amylopectin	good	very good
Potato amylose	good	moderate
α-Cellulose	moderate	moderate
Chitin	none	moderate
Chitosan	none	moderate
Pullulan	none	very good
Alternan	none	very good
Regular comb dextran	none	very good
CM-cellulose	moderate	poor

[a] From Ref. [20].

ing: (1) differences in the relative viscosities; (2) differences in the carbazole reaction; (3) differences in their susceptibility to acid hydrolysis, and (4) differences in their ability to form calcium gels, before and after reaction. We further show the formation of L-iduronic acid from D-glucuronic acid for oxidized and epimerized amylose by 2D NOESY and COSY ^{1}H NMR.

The C-5-epimerase displayed high specificity for the inversion of equatorially linked carboxyl groups to axially linked carboxyl groups. The configuration of the hydroxyl group at C-2 did not seem to be a factor, D-glucuronic acid being inverted, as well as D-mannuronic acid. Substitution of an equatorial acetamido group at C-2 did not have an effect. The configuration of the glycosidic linkage did not have an effect, α-linked D-glucuronic acid being inverted, as well as β-linked D-glucuronic acid. Further, the structural location of the D-glucuronic acid in the polysaccharide was not a factor, with alternating α-(1→3)-linked D-glucuronic acid and α-(1→6)-linked D-glucose (C-6-oxidized alternan), two consecutive α-(1→4) linked D-glucuronic acids and α-(1→6)-linked D-glucose (C-6-oxidized pullulan), and α-(1→3)-linked single D-glucuronic acid residues to an α-(1→6)-linked dextran chain, all had about 50% of the D-glucuronic acid residues inverted by C-5-epimerase.

The inversion reaction forms new glycuronan copolymers composed of D-glucuronic acid and L-iduronic acid, some of which can form divalent cation gels, similar to alginate. These gels have properties that should make them potential materials for applications in thickening, suspending, emulsifying, stabilizing, and encapsulating materials in foods, pharmaceuticals, and agricultural products.

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