Complexation of Borate with Poly(1-(acrylamido)-1-deoxylactitol): Dilute Solution Viscosity, ^{11}B NMR, and ^{13}C NMR Studies

Mark E. Wilson,[†] Samir Najdi,[⊥] John M. Krochta,[‡] You-Lo Hsieh,[§] and Mark J. Kurth*[,†]

Department of Chemistry, Department of Food Science and Technology, and Department of Textiles and Polymer Science, University of California, Davis, California 95616, Department of Chemistry, Al-Quds University, East Jerusalem, Israel

Received February 18, 1998; Revised Manuscript Received May 8, 1998

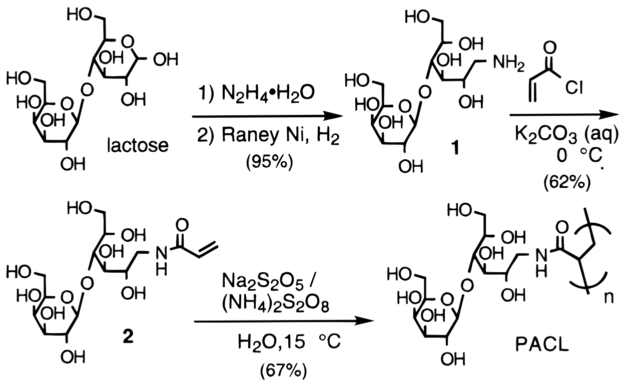
ABSTRACT: The interaction of borate ions with poly(1-(acrylamido)-1-deoxylactitol) (PACL) in aqueous media was investigated. Dilute solution viscometry revealed that low concentrations of Borax ($Na_2B_4O_7$) increased the intrinsic viscosity of PACL, while higher concentrations reduced it. ^{11}B NMR spectra of borate complexes of methyl β-D-galactopyranoside (M$_\beta$DGP) (taken to model the C-1′ through C-6′ portion of the PACL repeating unit) and the monomer 1-(acrylamido)-1-deoxylactitol (**2**) showed evidence for α,β-bidentate, α,β-dimer, and α,γ-bidentate borate complexes. The ^{11}B NMR spectrum of PACL−borate showed α,β-bidentate and α,β-dimer only. The binding constants for the α,β-bidentate and α,β-dimer complexes were determined for M$_\beta$DGP and **2** by integration of the ^{11}B NMR signals. ^{13}C NMR was used to identify the putative sites of complexation on PACL by comparing the spectra of borate complexes of M$_\beta$DGP and **2** with PACL−borate. Complexation was found to occur predominantly at the C-6′, C-4′, and C-3′ positions.

Introduction

The complexation of borate ions with hydroxyl-containing polymers is a process of considerable practical interest. Aqueous solutions of poly(vinyl alcohol),[1−3] poly(glyceryl methacrylate),[4] and poly(saccharide)s,[5] for example, form mobility-controlled viscous fluids and thermoreversible hydrogels in the presence of borate. While borate complexes of diols,[6] small polyols,[7] and disaccharides[8] are relatively well understood, the exact nature of complexation in polysaccharides is rarely clarified. Most investigations to date have involved linear[9] and lightly cross-linked[5] or modified[10] natural polysaccharides. The interaction of borate ions with semisynthetic polymers such as poly(vinylsaccharide)s has not been investigated.

The present work examines borate complexation with poly(1-(acrylamido)-1-deoxylactitol) (PACL), a poly(vinylsaccharide) of the amide type. Poly((acrylamido)-saccharide)s derived from several mono- and disaccharides were reported by Klein,[11,12] but the analogous lactose-based polymer has not appeared in the literature. Our interest in polymers derived from lactose[13] prompted us to prepare PACL and investigate its interaction with aqueous borate. We prepared the requisite monomer 4-*O*-β-D-galactopyranosyl-1-(acrylamido)-1-deoxyglucitol (**2**) from lactose in three steps (Scheme 1). Redox-initiated free-radical polymerization in water afforded PACL. We examined the interaction of PACL with borate in the sol range to obtain a basic understanding of the rheological properties and the nature of the complexes involved. By using dilute solution viscometry, we examined the influence of Borax on the specific and intrinsic viscosity. Through ^{11}B NMR, we elucidated the types of borate complexes formed and quantitated their binding constants where possible. Using ^{13}C NMR, we identified the putative sites of complexation on the disaccharide ligand of PACL.

Scheme 1. Synthesis of PACL from Lactose



Experimental Section

General. All NMR spectra were obtained with a QE-300 spectrometer (General Electric, Fremont, CA). Infrared spectra were collected with a Galaxy 3000 FTIR (Mattson, Madison, WI). Anhydrous Borax ($Na_2B_4O_7$; Fisher Scientific) was pulverized and baked 48 h at 130 °C prior to use. Methyl β-D-galactopyranoside (Aldrich) was used as received.

4-O-β-D-Galactopyranosyl-1-amino-1-deoxyglucitol (1). To a solution of α-lactose (200 g, 0.581 mol) in 300 mL of H_2O was added 85 mL (1.66 mol) of 98% (w/w) hydrazine hydrate, and the mixture was stirred for 24 h at 25 °C. The resulting hydrazone solution was hydrogenated using Raney nickel (40 mL of 50% w/w) and H_2 (1000 psi at 25 °C) in a 1-L high-pressure reactor (Autoclave Engineers, PA) at 50 °C for 24 h. The amber solution was filtered through Celite (2-cm pad) and then treated with diacetyl dioxime to remove residual Raney nickel. The pink slurry was filtered, decolorized with Darco G-60, and filtered again through Celite (2-cm pad). The water was removed in vacuo to give 190.6 g (95%) of **1** as a yellow

---

[†] Department of Chemistry, University of California.

[‡] Department of Food Science and Technology, University of California.

[§] Department of Textiles and Polymer Science, University of California.

[⊥] Department of Chemistry, Al-Quds University.

S0024-9297(98)00243-5 CCC: $15.00

Table 1. SEC-MALLS, Viscometric, and Thermal Data for PACL

property	mean value[a]
M_w	10^6 Da
M_w/M_n	2.2 (0.01)
$\langle RG \rangle^2$	38 nm
dη/dC	0.169 (0.001)
[η]	0.52
T_g	148 °C

[a] Average of three analyses.

foam. ^{13}C NMR (75 MHz, D_2O; dioxane reference) δ 103.1, 79.1, 75.3, 72.9, 72.7, 71.2, 70.81, 70.8, 68.9, 62.3, 61.1, 43.2. HRMS (FAB) *m/z* for (M $^+$) $C_{12}H_{26}NO_{10}$, calcd 344.1557, obsd 344.1572.

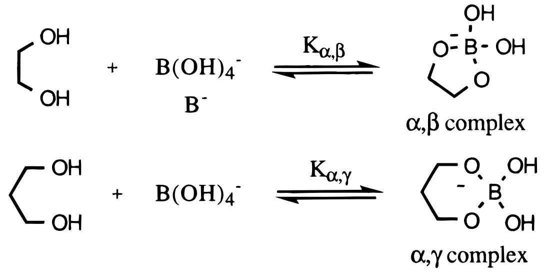
4-O-β-D-Galactopyranosyl-1-(acrylamido)-1-deoxyglucitol (2). To a rapidly stirring solution of **1** (50 g, 145 mmol) in 100 mL of 2 M aqueous K_2CO_3 at 0 °C was slowly added acryloyl chloride (23.6 mL, 300 mmol) so as to avoid raising the temperature above 2 °C. The solution was stirred an additional 8 h at 10 °C and then allowed to warm to 25 °C, where it was stirred an additional hour. The pH was adjusted to 1 with 10% aqueous HCl, and the solution was extracted with diethyl ether (3 × 200 mL). The aqueous fraction was made basic (pH 10) with aqueous NaOH and extracted with diethyl ether (3 × 200 mL). The aqueous fraction was neutralized to pH 7 with 2 M aqueous HCl, and the water was removed in vacuo. The amber residue was dissolved in 500 mL of methanol and filtered through a 2-cm pad of Celite. The filtrate was dried over Na_2SO_4 and dropped slowly into 1.5 L of rapidly stirring acetone to precipitate **2**. The precipitate was washed with acetone and dried in a vacuum desiccator for 8 h to yield 36.2 g (62.4%) of **2** as a white powder. IR (KBr pellet) 1654 cm^{-1}. ^{13}C NMR (75 MHz, D_2O; dioxane reference) δ 168.6, 129.9, 127.5, 102.8, 79.3, 74.8, 72.4, 71.0, 70.8, 70.6, 69.7, 68.5, 62.0, 60.7, 41.9. HRMS (FAB) *m/z* for (M + K$^+$) $KC_{15}H_{27}NO_{11}$, calcd 436.1221, obsd 436.1210.

Solution Polymerization.[14] A solution of **2** (5.6 g, 14.1 mmol) in 42 mL of H_2O was deoxygenated by three freeze–pump–thaw cycles and placed in a 50-mL reaction flask equipped with an immersed magnetic stirrer in a constant-temperature bath at 15 °C. A solution of the initiators $Na_2S_2O_5$ (9.5 mg, 0.05 mmol) and $(NH_4)_2S_2O_8$ (11.4 mg, 0.05 mmol) in 0.2 mL of degassed H_2O was added all at once, and the reaction was stirred under nitrogen for 24 h. The polymer was precipitated from 300 mL of 1% methanolic sodium acetate and dried 8 h in a vacuum oven at 40 °C to give 3.7 g (67%) of PACL as a white powder. A small aliquot of PACL (0.6 g) was dialyzed (1000 MW cutoff) against deionized H_2O, precipitated from methanol, and dried 8 h in a vacuum oven at 40 °C prior to use in the borate binding studies.

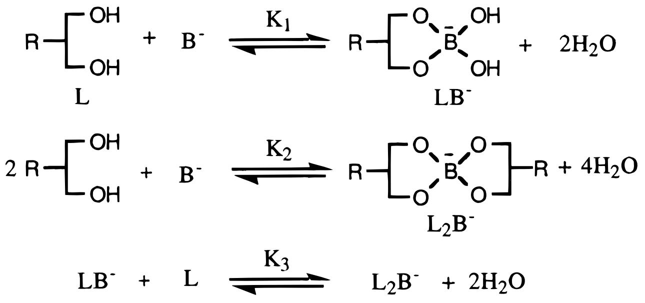
Polymer Characterization. Light-scattering measurements were performed using a Dawn DSP-F laser photometer (Wyatt Technologies, Santa Barbara, CA) using a He laser source (638 nm). The DRI detector was an Anspec ERC-7512 (ERMA CR. Inc, Tokyo, Japan). SEC-MALLS (size-exclusion chromatography with multiple-angle laser light scattering detection) was performed at a flow rate of 0.68 mL/min using a Waters HPLC with two TosoHaas 5-mm × 30-cm columns in series: (1) G6000PW, (2) G5000PW in a heat jacket at 40 °C. Molecular weight and polymer conformation calculations were performed using ASTRA-enhanced Beta software, version 1.1.7 (Wyatt Technologies). Thermal analysis was accomplished using a Shimadzu DSC-50 (Shimadzu, Japan). Dilute solution viscosity was performed using a Cannon–Ubbelohde continuous dilution viscometer (Cannon Instruments, State College, PA). The results are summarized in Table 1.

^{11}B NMR. The samples were prepared in the concentrations indicated by adding Borax solution ($Na_2B_4O_7$ in 20% D_2O:H_2O) to the ligand in a volumetric flask just short of the mark. The pH was adjusted to 11 with a small amount of 1 M aqueous NaOH or 1 M aqueous HCl, and more Borax solution was added to the mark. The solution was left to stand at 25 °C overnight. A 600-μL aliquot of the sample solution was placed in a quartz NMR tube (5-mm o.d.). The tube was sealed and allowed to equilibrate in the spectrometer at the set temperature (±1 °C) for 1 h prior to data collection. Spectra were recorded at 96.46 MHz with the chemical shifts referenced to $BF_3 \cdot Et_2O$ external standard by the substitution method.[15] The sweep width was 2 kHz and the pulse width was 90° (12 μs). There was a 300-μs delay between the pulse and data acquisition to allow the broad background from the probe to dephase. Data acquisition time was 2 s, with a relaxation delay of 50 ms. Typically, 35 K scans were collected. Apodization was exponential, with 10-Hz line broadening prior to Fourier transform.

Scheme 2. Formation of Bidentate Cyclic Borate Complexes from Diols and Tetrahydroxyborate (Borate) Ions



Scheme 3. Equilibria between Borate Ions and Diols in Aqueous Media



^{13}C NMR. The samples were prepared in the same manner as described above. Spectra were recorded at 75.48 MHz with the chemical shifts referenced to 1,4-dioxane internal standard. The pulse width was 30° (4 μs), the acquisition time was 800 ms, and the relaxation delay was 1 s. Apodization was exponential, with 1-Hz line broadening prior to Fourier transform.

Results and Discussion

The PACL–Borate System. The PACL repeating unit consists of 4-*O*-β-D-galactopyranosyl-1-amido-1-deoxy glucitol attached to a vinyl backbone (Scheme 1). The hydroxyl groups can serve as ligands provided that geometrical considerations allow for the interactions. Tetrahydroxyborate (borate) reacts reversibly with these hydroxyls to form borate complexes, which can be five-membered (α,β-bidentate coordination) or six-membered (α,γ-bidentate coordination) ring structures (Scheme 2).[16] The chemical equilibria for the formation of these borate complexes involves borate (B$^-$), borate monocomplex (both α,β- and α,γ-types) (LB$^-$), and borate dicomplex (both α,β- and α,γ-types) (L_2B^-) (Scheme 3). The source of borate used in this study is Borax ($Na_2B_4O_7$), which dissociates into equal amounts of boric acid (B) and borate according to

$$B_4O_7^{2-} + 7H_2O \rightleftharpoons 2B(OH)_3 + 2B(OH)_4^- \qquad (1)$$

Viscometric Studies. The rheological properties of aqueous PACL solutions were profoundly altered by

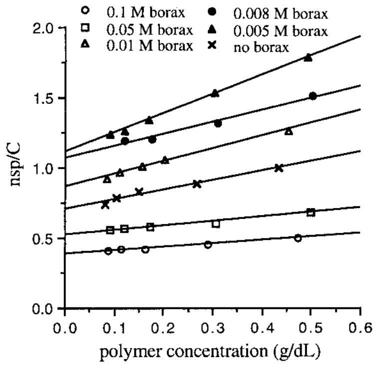


Figure 1. Reduced viscosity of PACL as a function of polymer concentration for solutions containing various amounts of borax.

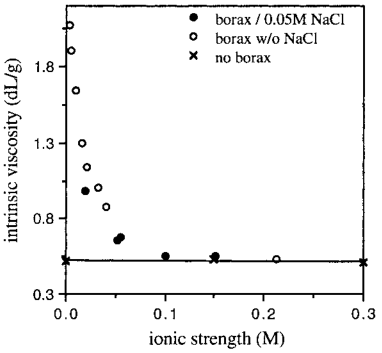


Figure 2. Dependence of the intrinsic viscosity of PACL on ionic strength in solutions containing varying amounts of borax ($Na_2B_4O_7$) and NaCl. The lowest data point on the abscissa of the "borax/0.05 M NaCl" plot is actually 0.02 M NaCl.

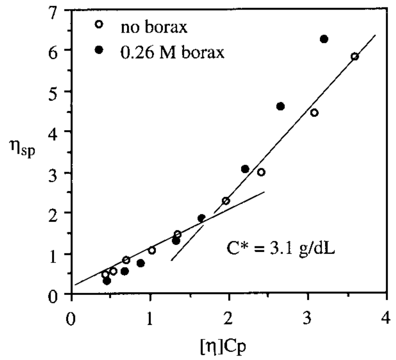


Figure 3. Specific viscosity (η_{sp}) of PACL as a function of reduced polymer concentration ($[\eta]Cp$) in the presence and absence of added borax.

borate (Figure 1). Low concentrations of Borax increased the reduced viscosity (η_{sp}/C) of PACL over the pure aqueous PACL solution, but higher concentrations of Borax caused the reduced viscosity to fall below that of the pure polymer solution.

This rheological behavior has been observed in other polymer—borate systems and has been interpreted as a balance between polymer coil expansion and contraction.[17] Expansion of the mean coil dimensions of PACL could arise either through a steric effect (i.e., an increase in the steric bulk of the pendent disaccharide ligand upon borate complexation)[18] or from the buildup of charge along the polymer chains (Coulombic repulsion). The contraction of the average molecular dimensions at higher Borax concentrations is likely due from the formation of intrachain cross-links, e.g., an L_2B^- complex between two ligands separated by several residues.

The Coulombic repulsion hypothesis discussed above was not supported by the linearity of the plots in Figure 1; solutions of polymer—ion polyelectrolytes typically give curved plots.[19] To further assess the invalidity (or validity) of the Coulombic repulsion mechanism, we examined the intrinsic viscosity of PACL—borate solutions in the presence and absence of added salt. We reasoned that an increase in the dielectric of the medium should decrease or even mask the effects brought about through electronic repulsion. Figure 2 shows that the intrinsic viscosities of PACL—borate solutions with NaCl were similar to those without NaCl. Even at low ionic strength (0.02 M), the NaCl-containing PACL—borate solutions exhibited nearly the same intrinsic viscosity as pure PACL—borate. Thus, Coulombic repulsion does not appear to be the predominant mechanism responsible for the increased intrinsic viscosities observed at low borate concentrations.

A quantity of practical and theoretical interest in the study of polymer solution rheology is the overlap concentration (C^*). This is the minimum concentration of polymer at which the chains begin to overlap. Since interpolymer cross-links (e.g., chain entanglements, ion-complexes, etc.) are enhanced above C^*, the viscosity of polymer solutions typically increase dramatically above this concentration. The relationship between C^* and the intrinsic viscosity $[\eta]$ of a polymer is[20]

$$C^* \approx \epsilon/[\eta] \tag{2}$$

where the value of ϵ may vary from one polymer to another. Accordingly, C^* can be obtained by examining the viscosity as a function of reduced polymer concentration ($[\eta]C_p$). The value of C^* is considered as the point at which the viscosity increases more sharply (i.e., $C^* = C_p$ when $[\eta]C_p = \epsilon$).

The specific viscosity (η_{sp}) of aqueous solutions of PACL as a function of $[\eta]C_p$ appears in Figure 3. While η_{sp} can be seen to increase with increasing $[\eta]C_p$, the effect becomes more pronounced above a reduced concentration of ~1.6. As the intrinsic viscosity of PACL is 0.52 dL/g, this corresponds to an overlap concentration of 3.1 g/dL. Thus, the value of the ϵ in eq 2, while found to be ~1.4 for other polymers used in borate-binding studies,[21] appears to be ~1.6 for PACL. That interchain borate complexation was increased above a reduced concentration of 1.6 can be seen by the increase in the viscosity as a function of polymer concentration in the 0.26 M Borax-containing solutions above this point.

^{11}B NMR Studies. ^{11}B NMR is a well-established tool for studying the structures of borate—ligand complexes.[22] The method relies on the principle that the interconversion of the borate—ligand complexes is slow enough to allow for the detection of discrete complexes without excessive lifetime broadening or signal averaging.[23] The rate of exchange between boric acid (B) and free borate (B^-) is rapid ($k \approx 10^{10}$ M^{-1} s^{-1}),[24] however. So, the uncomplexed borate signal (i.e., B/B^- peak) appears as a weighted average of the boric acid resonance at 19.2 ppm (relative to $BF_3{\cdot}Et_2O$) and borate at 0.6 ppm. ^{11}B NMR spectra are normally line-broadened because ^{11}B has a quadrupole moment. Additional peak broadening can occur with increasing molecular radii and increasing solution viscosity.[25] Accordingly, the spectra of small polyol—borate systems are reasonably

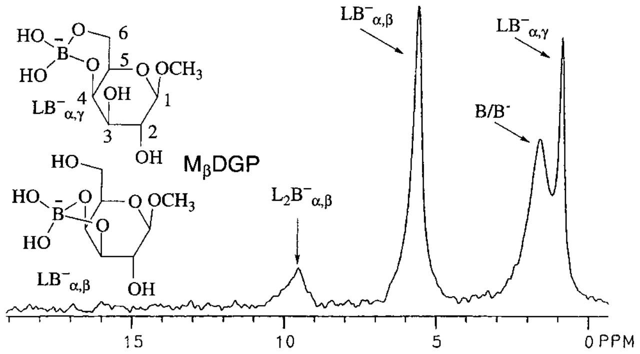


Figure 4. ^{11}B NMR spectrum (20% D_2O, 96 MHz, $BF_3.Et_2O$ external standard) of methyl β-D-galactopyranoside (M_βDGP) in 4.9 mM borax at pH 11 and 25 °C. Stoichiometric ratio of ligand to total boron (B_{total}) is 5:1.

Table 2. ^{11}B NMR Chemical Shifts[a] for Borate Complexes at pH 11 and 25 °C

ligand	diol	chem shift LB⁻	lit.[b]	L₂B⁻	lit.[b]
MβDGP	1,2	5.6	5.6	9.5	9.5
	1,3	0.9	1.0		
2	1,2	5.7	5.6	9.6	9.5
	1,2	4.9		9.6	9.5
	1,3	1.1	1.0		
PACL	1,2	5.7		9.7	
	1,3	1.0	1.0		

[a] Relative to $BF_3{\cdot}Et_2O$. [b] From ref 7b.

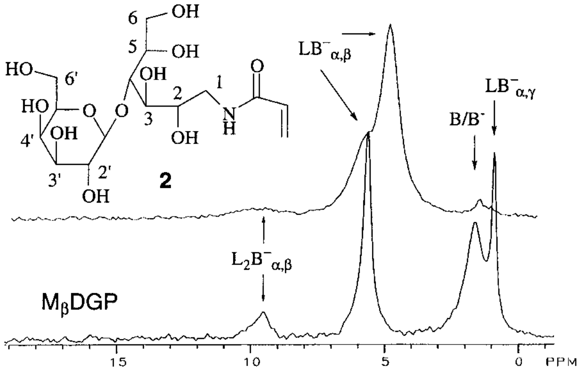


Figure 5. ^{11}B NMR spectrum (20% D_2O, 96 MHz, $BF_3{\cdot}ET_2O$ external standard) of M_βDGP (bottom) and 2 (top) in aqueous borax solutions at pH 11 and 25 °C. Stoichiometric ratio of ligand to B_{total} is 5:1 for M_βDGP and 1.8:1 for **2**.

well resolved, but polymer–borate systems tend to give broadened spectra, which make peak assignments difficult.

To assist in the assignment of the complexes that form in the PACL–borate system, we examined the complexes obtained with methyl β-D-galactopyranoside (M_βDGP). This methyl glycoside was taken to represent the galactosyl portion of the PACL repeating unit, i.e., C-1′ through C-6′. The ^{11}B NMR spectrum of M_βDGP (Figure 4) revealed that three different borate complexes formed in 4.9 mM Borax at pH 11. On the basis of literature shift ranges[7] and approximate line widths,[26] we assigned these peaks as $L_2B_{\alpha,\beta}$ (9.5 ppm), $LB_{\alpha,\beta}$ (5.6 ppm), and $LB_{\alpha,\gamma}$ (0.8 ppm). Table 2 summarizes the observed chemical shifts and literature shifts for the various borate complexes.

The ^{11}B NMR spectrum of **2** in 5.6 mM Borax (Figure 5) showed the same $L_2B_{\alpha,\beta}$ and $LB_{\alpha,\beta}$ peaks as the borate–M_βDGP system. Thus, the broad peak centered at 9.6 was assigned as an $L_2B_{\alpha,\beta}$ complex and the peak at 5.2 ppm was assigned as an $LB_{\alpha,\beta}$ complex (likely involving C-3′, C-4′). The peak at 4.9 ppm was also assigned as an $LB_{\alpha,\beta}$ complex involving one or more of the following ligand sets: C-2:C-3, C-5:C-6, C-2′:C-3′, or C-2:N-amide. The diol C-2′:C-3′ is unlikely, however, because borate complexes of anti-1,2 diols are generally not observed in pyranoses. Ligands C-2:C-3 and C-5:C-6 afford a threo-1,2 complex and an exocyclic-1,2 borate complex, respectively. While the larger area of the peak at 4.9 ppm is consistent with the formation of either borate complex (both are known to have a higher formation constant than an erythro-1,2-diol complex,[26] i.e., the C-3′, C-4′ $LB_{\alpha,\beta}$ complex at 5.2 ppm), assignment of the peak at 4.9 ppm based upon chemical shift alone was not possible.

The relatively small size of the B/B⁻ peak in the spectrum of **2**–borate is interesting considering that a higher total boron (B_{total}) to ligand ratio was used in these samples than for the borate–M_βDGP samples. This suggests that borate–complex formation is more favored for ligand **2** relative to M_βDGP. Also interesting is the reduced $LB_{\alpha,\gamma}$ peak in the spectrum of **2**–borate relative to that in M_βDGP–borate. While 1,3 complexes typically have smaller formation constants than 1,2 complexes, this difference appears to be more pronounced in the **2**–borate system.

The ^{11}B NMR spectrum of PACL in 4 mM Borax at pH 11 (Figure 6) showed the presence of $LB_{\alpha,\beta}$ and $L_2B_{\alpha,\beta}$ complexes. Despite the use of a 2-fold excess of borate, no $LB_{\alpha,\gamma}$ complex was detected. The broadness of the resonance centered at 5.7 ppm precluded assignment of the $LB_{\alpha,\beta}$ complex (whether endo- or exocyclic). When lower concentrations of borate were used, the signal-to-noise ratio was too small. When higher borate and PACL concentrations were used ([Borax] > 0.5 M; [PACL] > 10 g/dL), gelation occurred.

The half-height peak widths observed in the ^{11}B spectra of M_βDGP and **2**, which ranged from 35 to 100 Hz for the LB⁻ complexes and 155 to 165 Hz for the LB_2^- complexes, were broad enough to mask closely overlapping resonances. Accordingly, the possible presence of interconverting LB⁻ and LB_2^- conformers or the exchange of LB⁻ and LB_2^- complexes with polyborate [e.g., $B_3O_3(OH)_4^-$ and $B_4O_5(OH)_4^{2-}$] cannot be ruled out. Gorin and Mazurek suggested that interconverting borate conformers were partially responsible for the broadening of peaks in the ^{13}C NMR spectra of polyol–borate systems, and they demonstrated that polyborate exchange contributed significantly to increased line

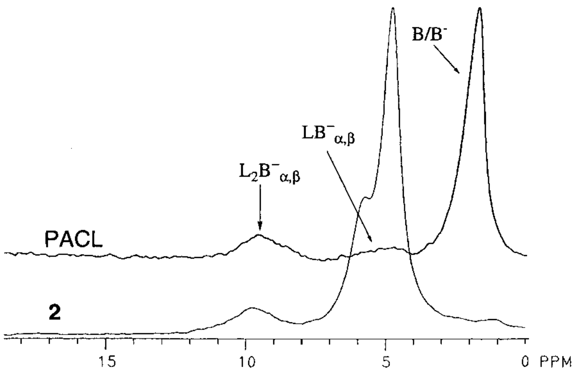


Figure 6. ^{11}B NMR spectrum of PACL (20% D_2O, 96 MHz, $BF_3{\cdot}Et_2O$ external standard) in 4 mM borate at pH 11 and 25 °C. Stoichiometric ratio of ligand to β_{total} is 1:2.

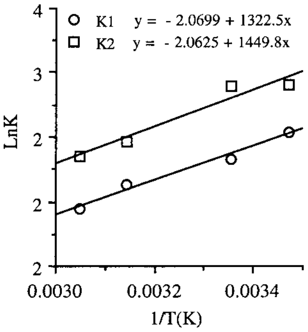


Figure 7. van't Hoff plot for the M$_\beta$DGP−borate system. Conditions are 0.05 M M$_\beta$DGP in 5 mM borax at pH 11.

widths.[27] Polyborate species are known to exist at high pH's (albeit in low concentrations when the total boron concentration is below ∼0.1 M), and their interconversion is too rapid for the detection of discrete species by NMR.[24]

The integrated peak areas of the ^{11}B NMR spectra are directly proportional to the concentration.[28] Taking advantage of this fact affords a convenient means of determining the formation constants of borate complexes. Using the mass balance equations for the ligand and borate species,

$$[\mathrm{L}]_\mathrm{T} = [\mathrm{L}] + \sum[\mathrm{LB}^-] + 2\sum[L_2B^-] \qquad (3)$$

$$[\mathrm{B}]_\mathrm{T} = [\mathrm{B}] + [\mathrm{B}^-] + \sum[\mathrm{LB}^-] + \sum[\mathrm{L_2B}^-] \qquad (4)$$

the concentrations of the borate complexes can be obtained by multiplying their relative peak areas (A_i) by the total boron concentration ($[\mathrm{B}]_\mathrm{T}$). Similarly, the concentration of B^- can be determined by taking into account the concentration of boric acid through the relation $\mathrm{B}^-/\mathrm{B} = 10^{-9.2}/10^{-\mathrm{pH}}$. Each equilibrium constant can then be determined through the appropriate substitutions:

$$K_1 = \frac{A_{\mathrm{LB}^-}}{A_{\mathrm{B}^-}[\mathrm{L}]} \qquad (5)$$

$$K_2 = \frac{A_{\mathrm{L_2B}^-}}{A_{\mathrm{B}^-}[\mathrm{L}]^2} \qquad (6)$$

We determined the formation constants and thermodynamic parameters of M$_\beta$DGP−and **2**−α,β-borate complexes in order to gain insight into the complexing properties of the PACL repeating unit. The ^{11}B NMR spectrum of M$_\beta$DGP in 5 mM Borax solution at pH 11 was measured at several different temperatures, and the log's of the formation constants for the LB$_{\alpha,\beta}$ and L$_2$B$_{\alpha,\beta}$ complexes were plotted against inverse temperature to afford a van't Hoff plot (Figure 7). The enthalpy and entropy were determined from the slope and intercept, respectively. The corresponding van't Hoff plot for the **2**−borate complexes appears in Figure 8. The formation constants at 25 °C and the thermodynamic parameters are summarized in Table 3.

The value of the formation constant for the LB$_{\alpha,\beta}$ complex of M$_\beta$DGP was similar to the value reported by van den Berg et al. (10 M^{-1}).[8] The formation constant for the L$_2$B$_{\alpha,\beta}$ complex was similar to that reported by Pezron et al. for the α-anomer of M$_\beta$DGP, as were the thermodynamic parameters for the mono- and dicomplexes of M$_\beta$DGP.[20] The value of K_1 for the **2**−borate complex LB$_{\alpha,\beta}$ was larger than the value of K_1 for the corresponding M$_\beta$DGP complex. However, because the peaks for the **2**−borate complexes (endo- and exocyclic LB$_{\alpha,\beta}$) were unresolved, the areas (and thus the equilibrium constant and corresponding thermodynamic parameters) represent both species. The large value of K_2 for the **2**−borate L$_2$B$_{\alpha,\beta}$ complex supports the notion that the pendent disaccharide moiety serves as a good cross-linking ligand in the PACL-borate system.

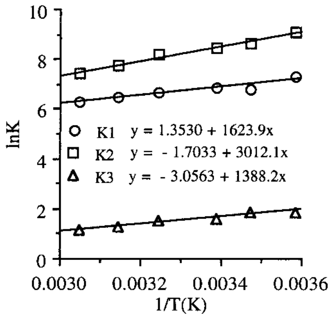


Figure 8. van't Hoff plot for the **2**−borate system. Conditions are 0.04 M **2** in 5.6 mM borax at pH 11.

Table 3. Formation Constants[a] and Thermodynamic Parameters[b] for Ligands M$_\beta$DGP and 2

ligand	complex	K_i	log K_i	ΔH, kJ mol^{-1}	ΔS, J mol^{-1} K^{-1}
M$_\beta$DGP	LB$_{\alpha,\beta}$	K_1, M^{-1}	0.9(0.1)	−1	−17
	L$_2$B$_{\alpha,\beta}$	K_2, M^{-2}	1.2(0.2)	−12	−17
2	LB$_{\alpha,\beta}$	K_1, M^{-1}	3.0(1.5)	−13	11
	L$_2$B$_{\alpha,\beta}$	K_2, M^{-2}	3.6(1.7)	−25	−14

[a] At 25 °C. [b] Determined using the relations ln $K_\mathrm{i} = -\Delta G/RT$ and $\Delta G = \Delta H - T\Delta S$.

^{13}C NMR Studies. ^{13}C NMR is an established tool for identifying the sites of chelation in polyols.[29] Complexation with borate causes a downfield shift of 0.5 to 10 ppm for the carbon bearing the hydroxyl group. Because borate complexation is not quantitative, the chelating sites are identified by a reduced (relative) peak size and the appearance of a new peak downfield. The spectra of small polyol−borate complexes are reasonably well resolved. Polysaccharide−borate systems, however, tend to give spectra that are more difficult to interpret because of the large number of relatively broad peaks. This general line broadening is attributed to the increased viscosity of polysaccharide−borate solutions and from the possible presence of LB$^-$ and L$_2$B$^-$ conformers or the exchange of these species with polyborate.[30,31] To gain insight into the probable chelating sites of PACL, we examined the spectra of the ligands themselves, i.e., M$_\beta$DGP and **2**, in the presence of borate and compared these spectra with the spectra of PACL−borate.

The ^{13}C NMR spectrum of M$_\beta$DGP in 0.5 M aqueous Borax and the reference spectrum of M$_\beta$DGP in 25% D$_2$O−H$_2$O appear in Figure 9. Chelation at C-6 was evidenced by a reduction in peak size and the appearance of a new peak downfield (Δppm = 0.7 ppm). Downfield shifts were also noted for C-4 and C-3. That there was no obvious peak shift for C-2 is consistent with the observation that borate complexes of anti-1,2 diols are generally not observed in pyranoses. Accordingly, C-4 is likely involved in an LB$_{\alpha,\beta}$ complex along with C-3 as well as an LB$_{\alpha,\gamma}$ complex with C-6 (see Figure 4).

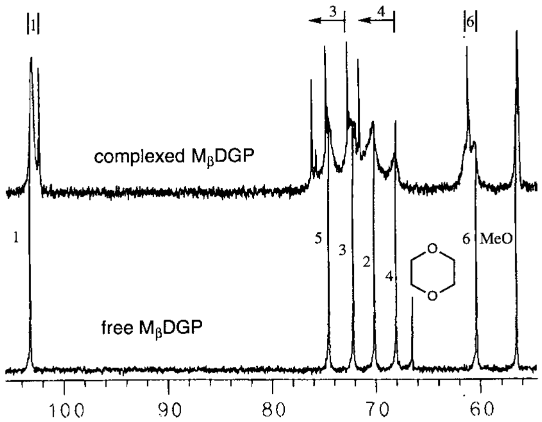


Figure 9. ^{13}C NMR spectrum of M$_\beta$DGP (20% D_2O:H_2O, 75 MHz, dioxane internal standard); [M$_\beta$DGP] = 0.5 M in (top) 0.5 M borax and (bottom) 20% D_2O in H_2O at 25 °C.

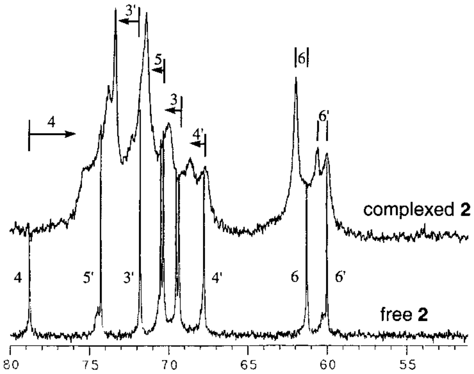


Figure 10. ^{13}C NMR spectrum of 0.5 M **2** (20% D_2O:H_2O, 75 MHz, dioxane internal standard) in (top) 0.5 M borax and (bottom) 20% D_2O in H_2O at 25 °C.

The ^{13}C NMR spectrum of **2** in 0.5 M Borax (Figure 10) showed some of the same peak shifts as were observed in the spectrum of M$_\beta$DGP−borate. The amide carbonyl (not shown) gave a slightly broadened peak in the presence of Borax. However, no new carbonyl peak was observed to support chelation of the amide group with borate. As observed for M$_\beta$DGP, the C-6′ position of **2** was shifted downfield (Δppm = 0.6) in the presence of borate. Ligand C-6 was also involved in chelation. However, while the C-6′ position of **2** was shifted downfield to give a peak of comparable intensity, C-6 was reduced substantially, affording a new peak downfield at 62 ppm that was quite large. Borate complexes of 1,2 diols are typically more stable than those of 1,3 diols.[16] Thus, it is reasonable that complexation at C-6 would be greater than at C-6′. Moreover, the inclusion of C-5 as the other hydroxyl involved in this 1,2 diol−borate complex allows us to assign the large peak at 4.9 ppm in the ^{11}B NMR spectrum of **2** (Figure 5) as an exocylic LB$_{\alpha,\beta}$ complex involving the diol set C-5:C-6.

The hydroxyls on carbons 4′, 3, 5, and 3′ of **2** also appeared to be involved in complexation, as evidenced by their decreased relative peak sizes and the appearance of new peaks downfield. Interestingly, C-4 and C-1′ (not shown) shifted upfield in the presence of borate. A similar upfield shift was also observed for C-1 of M$_\beta$DGP (Figure 9) and is likely attributed to a shielding effect brought about through neighboring group complexation.

The ^{13}C NMR spectrum of PACL−borate, shown in the expanded region from 50 to 80 ppm, appears in Figure 11. Interestingly, the C-6 position was only slightly involved in complexation (as evidenced by a small shoulder downfield of the uncomplexed shift position). Why the C-6 hydroxyl in PACL is less complexed than the C-6 hydroxyl in **2** is not known. One explanation is that the proximity of this position to the polymer backbone introduces steric constraints. The hydroxyl at C-6′ appeared to be more involved in complexation, affording a new peak ~0.6 ppm downfield. Although the peaks corresponding to C-3′ and C-4′ appeared to be slightly decreased, the severe line broadening precluded clear identification of these sites or any other additional sites as chelating ligands. Nonetheless, considering the chelating sites in **2**, the sites of chelation likely in PACL are C-6′, C-4′, and C-3′. Ligands C-6, C-5, C-3, and C-2 may also be involved in complexation.

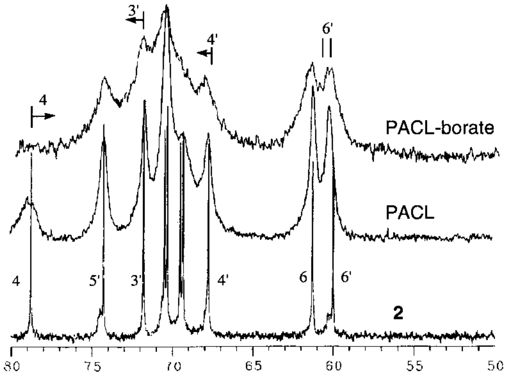


Figure 11. ^{13}C NMR spectrum (20% D_2O in H_2O, 75 MHz, dioxane external standard) of PACL (2.7 g/dL) in (top) 0.5 M borax and (middle) 20% D_2O in H_2O at 25 °C. Bottom spectrum is **2** in 20% D_2O in H_2O.

Conclusions

The rheological properties of dilute aqueous solutions of PACL were altered by borate. At low concentrations of borate, the intrinsic viscosity increased 4-fold over pure PACL solutions. At higher concentrations of borate, the viscosity fell below that of pure PACL solution. The chain overlap concentration, which was determined to be 3 g/dL, was corroborated by the observation that a gel was obtained when a solution of PACL (10 g/dL) and Borax (0.5 M) was prepared.

The ^{11}B NMR spectra of M$_\beta$DGP and **2** in the presence of borate showed that three types of complexes formed: an α,β-bidentate LB$_{\alpha,\beta}$ borate complex, the corresponding dimer L$_2$B$_{\alpha,\beta}$, and a 1,3 diol−borate complex LB$_{\alpha,\gamma}$. While all three complexes were readily formed in the M$_\beta$DGP−borate system, **2**−borate showed two types of LB$_{\alpha,\beta}$ complexes, the corresponding dimer L$_2$B$_{\alpha,\beta}$, and only a small amount of LB$_{\alpha,\gamma}$. PACL−borate showed a broad LB$_{\alpha,\beta}$ peak as well as the dimer L$_2$B$_{\alpha,\beta}$.

^{13}C NMR studies of M$_\beta$DGP, **2**, and PACL in the presence of borate showed that the hydroxyls on the PACL ligand most likely to be involved in chelation are at C-6′, C-4′, and C-3′. The hydroxyls at C-6, C-5, C-3, and C-2 could also be involved in complexation.

Acknowledgment. We thank Professor Charles Shoemaker (Department of Food Science and Technology, University of California, Davis) for help with the SEC-MALLS and Paul Bruins (Department of Chemistry, University of California, Davis) for help with the NMR experiments. We are grateful for financial support from the Wisconsin Milk Marketing Board and the California Milk Advisory Board.

References and Notes

(1) Sibayama, M.; Sato, M.; Kimura, Y.; Fujiwara, H.; Nomura, S. *Polymer* **1988**, *29*, 336.

(2) Sinton, S. W. *Macromolecules* **1987**, *20*, 2430.

(3) Nickerson, R. F. *J. Appl. Polym. Sci.* **1971**, *15*, 111.

(4) Pezron, E.; Leibler, L.; Ricard, A.; Lafuma, F.; Audebert, R. *Macromolecules* **1989**, *22*, 1169.

(5) Yoshimura, K.; Miyazaki, Y.; Sawada, S.; Waki, H. *J. Chem Soc., Faraday Trans.* **1996**, *92* (4), 651.

(6) Pizer, R.; Ricatto, P. *J. Inorg. Chem.* **1994**, *33*, 2402.

(7) (a) van Duin, M.; Peters, J. A.; Kieboom, A. P. G.; van Bekkum, H. *Tetrahedron* **1984**, *40*, 2901; (b) **1985**, *41*, 3411.

(8) van den Berg, R.; Peters, J. A.; van Bekkum, H. *Carbohydr. Res.* **1994**, *253*, 1.

(9) Pezron, E.; Ricard, A.; Leibler, L. *J. Polym. Sci., Polym. Phys. Ed.* **1990**, *28*, 2445.

(10) Sato, T.; Tsujii, Y.; Fukuda, T.; Miyamoto, T. *Macromolecules* **1992**, *25*, 5, 5970.

(11) Klein, J. *J. Makromol. Chem.* **1987**, *188*, 1217.

(12) Klein, J. *J. Makromol. Chem.* **1989**, *190*, 2527.

(13) (a) Wilson, M. E.; Hu, M.; Kurth, M. J.; Krochta, J. M.; Hsieh, Y. L. *J. Appl. Polym. Sci.* **1996**, *59*, 1759. (b) Zhou, W. J.; Wilson, M. E.; Kurth, M. J.; Hsieh, Y.-L.; Krochta, J. M. *Macromolecules* **1997**, *30*, 7063.

(14) A full account of the synthesis and characterization of PACL and the related poly(1-(methacrylamido)-1-deoxylactitol) is given in the Ph.D. dissertation of Mark E. Wilson, University of California, Davis, 1997.

(15) Brevard, G.; and Granger, P. *Handbook of High-Resolution Multinuclear NMR*; John Wiley: New York, 1981; p 40.

(16) Takeda, T.; Oi, T. *Bull. Chem. Soc. Jpn.* **1994,** *67*, 1485.

(17) Kurokawa, H.; Shibayama, M.; Ishimaru, T. *Polymer* **1992**, *33* (10), 2182.

(18) Plots of the mass-average molecular weight of PACL versus the *z*-average radius of gyration, $\langle R_G \rangle^{1/2}$ had an average slope of 0.52, indicating that the conformation of PACL in water in the absence of borate is a random coil.[14] Accordingly, we adopt a model where the relationship between the intrinsic viscosity, $[\eta]$ (the *y* intercepts in Figure 1), and the molecular conformation of PACL is given by:

$$[\eta] = K_\theta M^{0.5}\,\alpha^3 \qquad K_\theta = \Phi\left(\frac{\langle r \rangle}{M}\right)^{1.5}$$

where α is the expansion factor of the polymer coil, Φ is a universal constant, M is the molecular weight, and $\langle r \rangle$ is the unperturbed mean-square end-to-end distance of the polymer chain.

(19) Bolewski, V. K.; Rychly, B. *Kolloid Z. Polym.* **1968**, *228*, 48.

(20) Pezron, E.; Leibler, L.; Ricard, A.; Lafuma, F.; Audebert, R. *Macromolecules* **1988**, *21*, 1126.

(21) The ϵ value for *O*-(2,3-dihydroxypropyl)cellulose[10]and galactomannan[20] were both shown to be ∼1.4.

(22) Henderson, W. G.; How, M. J.; Kennedy, G. R.; Mooney, E. F. *Carbohydr. Res.* **1973**, *28*, 1.

(23) The energy levels absorbed by interconverting species are blurred by ΔE according to $\Delta E = h/(2\pi\tau)$, where τ is the lifetime of each discrete species. In NMR, this translates to a collapse of spectral structure (peak coalescence) when $\tau < 1/(2\pi\Delta\delta\upsilon)$, where $\Delta\delta\upsilon$ is the chemical shift difference (in Hz) between the interconverting species. Accordingly, two borate complexes separated by 1 ppm at 96 MHz will appear as two distinct peaks when $\tau > 1.7$ ms but will coalesce to one peak when $\tau < 1.6$ ms. As $\Delta\delta\upsilon$ decreases, the τ of each respective borate complex must increase in order to retain peak resolution.

(24) Anderson, J. L.; Eyring, E. M.; Whittaker, M. P. *J. Phys. Chem.* **1964**, *68*, 1128.

(25) The line widths are influenced by the molecular radii (r) and the solution viscosity (η) through the approximate relation:

$$\frac{1}{T_2} = \frac{3}{2}\pi^2 \frac{2I+3}{I^2(2I-1)}(e^2qQ/h)^2\left(1 + \frac{\zeta 2}{3}\right)\frac{4\pi r^3\eta}{3kT}$$

where e^2qQ/h is the quadrupolar coupling constant (in Hz) and ξ is the electric-field-gradient asymmetry. The increasing molecular radii of the LB_2^- complexes relative to the LB^- complexes is why the LB_2^- peaks appear broader. The broadened peaks in the NMR spectra of PACL−borate are likely due from the high solution viscosity.

(26) Chapelle, S.; Verchere, J.-F. *Tetrahedron* **1988**, *44*, 4469.

(27) Gorin, P. A. J.; Mazurek, M. *Can. J. Chem.* **1973**, *51*, 3277.

(28) This is true provided the relaxation times of the borate complexes lie within the pulse repetition time. The half-height widths ($\Delta\upsilon_{1/2}$) of the ^{11}B peaks in the present study ranged from 35 to 165 Hz. Thus, the approximate effective quadrupolar relaxation times, estimated through: $(\pi\Delta\upsilon_{1/2})^{-1}$, are from 9.1 to 1.9 ms, which is well within the pulse repetition time used (50 ms). For more studies on the proportionality between ^{11}B peaks area and concentration, see: (a) Pizer, R. D.; Ricatto, P. J.; Tihal, C. A. *Polyhedron* **1993**, *12*, 2137. (b) Dawber, J. G.; Green, S. I. E.; Dawber, J. C.; Garail, S. *J. Chem Soc., Faraday Trans. 1* **1988**, *84*, 41.

(29) Makkee, M.; Kieboom, A. P. G.; van Bekkum, H. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 230.

(30) The slow interconversion of borate complexes could cause lifetime broadening in the NMR spectra.[23] For ^{13}C NMR at 75 MHz, two peaks separated by 1 ppm will appear as one peak if $\tau < 2.1$ ms. If τ is near this value, the peak will be broadened. The ^{13}C NMR half-height peak widths of borate complexes of M_βDGP and **2**, which ranged from 30 to 80 Hz, suggest either the interconversion of two or more conformers or the equilibration of different complexes (possibly involving polyborate ions) that is too rapid for each discrete complex to be detected. The extreme broadening in the ^{13}C NMR of PACL−borate is likely due from the high viscosity of the solution.

MA9802430