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^{11}B NMR investigation of the complexation behavior of borate with polysaccharides in aqueous solution

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Abstract

The complexation of borate with linear dextrans consisting of pyranoside residues and their monomer derivatives (α-methyl-D-glucopyranoside and α-methyl-D-mannopyranoside) was investigated in detail by using ^{11}B NMR spectroscopy. For the monomer systems five kinds of borate complexes with α,β-diols and α,γ-diols of the pyranosides were present: that is, (α,β)-monochelate, (α,γ)-monochelate, (α,β)(α,β)-bischelate, (α,γ)(α,γ)-bischelate and (α,β)(α,γ)-bischelate complexes. Borate reacts with dextran to form inter-chain (α,β)(α,β)- and (α,β)(α,γ)-bischelate complexes in the 1:2-complexation in addition to the (α,β)- and (α,γ)-monochelate complexes. The formation of intra-chain bischelate complexes can be negligible for a rather stiff polymer as dextran. Formation constants of the (α,γ)-monochelate complex are almost constant among dextrans with different chain lengths except Dextran 3000 which is the shortest polymer examined. On the other hand, the stability of (α,β)-monochelate complexes decreases with an increase in the chain length. The complexation behavior for polysaccharides with various chain lengths was discussed in correlation with hydrodynamic and steric hindrance of the polymer ligands.

Keywords: Borate–polysaccharide complexes; Speciation; Polymer length; Conformation; ^{11}B NMR

1. Introduction

Biological and environmental processes are taking place in rather complicated heterogeneous media which contain various macromolecules [1,2]. In many cases, these processes have been studied in a biomimetic system by using synthetic polymers or gels as a model material [3,4]. Several well-established techniques have been worked out for complexation equilibria in both homogeneous and heterogeneous solutions [5–7], however, studies on the equilibria with these synthetic macromolecules of course still need further considerations. The polymer complexation systems exhibit some peculiarities arising from the fact that ligands are fixed on the polymer chain or the cross-linked polymer network, which greatly limits the analogy with the complexation between small molecules. In the case of ion condensation on polymers the polyelectrolyte effect [8–10] should be considered. Furthermore, we must take inter-chain and intra-chain complexes into consideration for the 1:2 complexation [11–13]. Hydrodynamic and steric hindrance of the polymer ligands may be important factors for such complexation.

Previously, we investigated the interaction of boric acid–borate with dextran (linear α-1,6-linked polysaccharide) and Sephadex (dextran gel covalently cross-linked by epichlorohydrin) by using ^{11}B NMR spectroscopy. We clarified that the adsorption of boron onto Sephadex gels is a consequence of complex formation between borate and diol moieties of glucopyranoside residues in the cross-linked dextran [14,15]. In this paper, the complexation behavior between borate and

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dextrans with various chain lengths were examined in detail. One of our main objectives of this work is to clarify the effects of the polymer chain length on the complexation. The complexation with their monomer derivatives, α-methyl-D-glucopyranoside and α-methyl-D-mannopyranoside, was also studied. A comparison of the complexation behaviors between the dextrans and the monomer derivatives will provide useful information about the effects.

2. Experimental

2.1. Chemicals

All chemicals were of analytical grade and used without further purification. Dextran 3000 was donated by Dextran Products Limited (Canada). Dextran T10, T40, T70 and T500 were obtained from Pharmacia Biotech (Sweden). Characteristics of dextrans used are summarized in Table 1.

2.2. Sample preparation

Boric acid–borate solutions ($I = 0.1$, NaCl) containing various amounts of dextran were prepared. To avoid the formation of polyborate, total concentrations of boric acid–borate were controlled below 0.02 mol dm^{-3} [16,17]. The concentration of dextran was expressed in monomol dm^{-3} on the basis of the pyranoside residue. The pH of the solution was adjusted with a little amount of HCl or NaOH solution. All pHs were measured by using a Horiba F-24C pH meter with a 3 mm o.d. glass electrode (Horiba 6069-10C).

2.3. NMR measurements

^{11}B NMR measurements were performed on a JEOL JNM-GSX 500 spectrometer with a 10 mm multinuclear probe at 298 ± 1 K at the Center of Advanced Instrumental Analysis, Kyushu University. The standard NMR parameters were as follows: an observed frequency of 160.0 MHz, the flip angle of approximately 90° (36 μs), the pulse repetition time of 1 s and the spectral width of 31 kHz. Sample tubes (10 mm o.d.) made of PTFE (Poly(tetrafluoroethylene)) were employed for the measurements. The field frequency lock was achieved with the ^2H resonance of D_2O in a 3 mm o.d. plastic concentric tube. The chemical shifts were reported with respect to a 0.1 mol dm^{-3} boric acid solution as an external reference. On this scale the signal of $Et_2O \cdot BF_3$, which is the generally adopted reference, came at −19.4 ppm. Overlapping NMR signals were resolved into individual peaks by a Lorentzian curve-fitting method [15,18].

Table 1
Characteristic parameters of dextrans with different chain lengths

	M_w	N
Dextran 3000	3000	18.5
Dextran T10	9500	58.6
T40	36 800	227
T70	69 100	427
T500	507 000	3130

3. Results and discussion

Borate can bind with pyranosides to form various kinds of complexes. The interaction of borate with polysaccharides and their monomer derivatives could well be understood with the knowledge of simple diol–

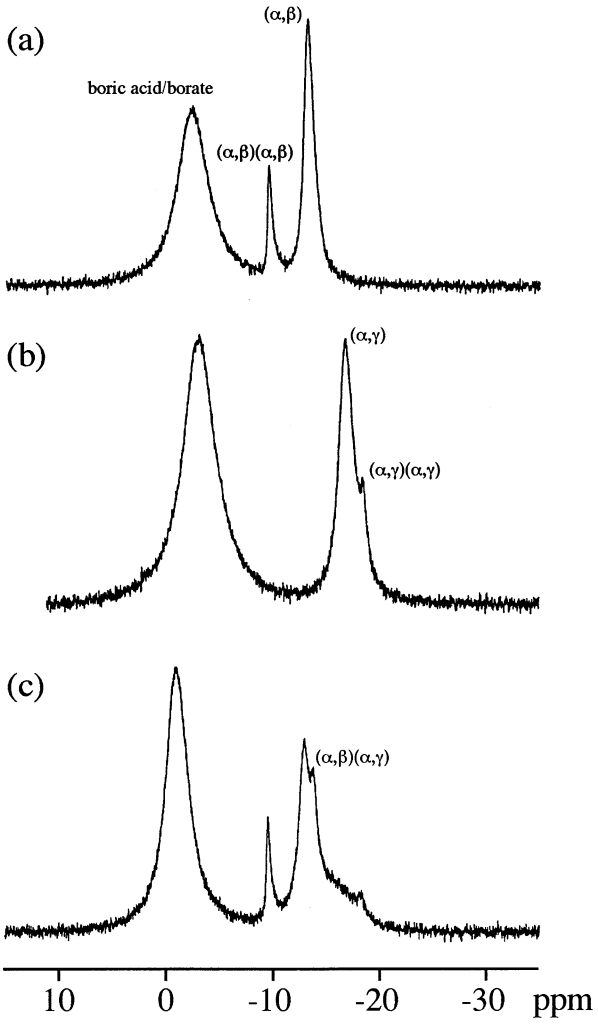
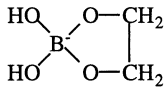
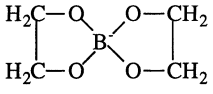


Fig. 1. ^{11}B NMR spectra of 0.02 mol dm^{-3} boric acid–borate solutions containing (a) 1.1 mol dm^{-3} 1,2-ethanediol at pH 8.55, (b) 1.1 mol dm^{-3} 1,3-propanediol at pH 8.75 and (c) 1.1 mol dm^{-3} 1,2-ethanediol and 1.1 mol dm^{-3} 1,3-propanediol at pH 8.44.

borate complex systems. Fig. 1 shows ^{11}B NMR spectra for 1,2-ethanediol and 1,3-propanediol systems. Since the complexation between borate and the diols is slow relative to the ^{11}B NMR time-scale, new NMR signals due to the complexes are observed besides the signal of free boric acid–borate [19]. Each complex has a characteristic chemical shift [20], so that one can determine binding structures of the borate complexes on the basis of their shift values. Signals at −13.3 and −9.9 ppm for the 1,2-ethanediol system are ascribed to the 1:1 and 1:2 complexes with α,β-diol moieties (hydroxyl groups on adjacent carbon atoms), respectively.

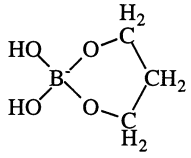


(α,β)-monochelate complex

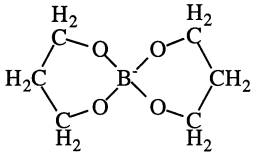


(α,β) (α,β)-bischelate complex

For the 1,3-propanediol system, signals at −16.7 and −18.5 ppm are ascribed to the 1:1 and 1:2 complexes with α,γ-diol moieties (hydroxyl groups on alternative carbon atoms), respectively.

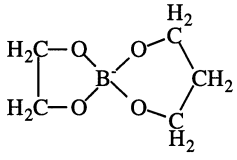


(α,γ)-monochelate complex



(α,γ) (α,γ)-bischelate complex

A new signal is observed at −14 ppm for the 1,2-ethanediol and 1,3-propanediol mixed system. This signal should be assigned to the 1:2 complex with 1,2-ethanediol and 1,3-propanediol as follows.



(α,β) (α,γ)-bischelate complex

Since α-methyl-D-glucopyranoside, α-methyl-D-mannopyranoside and dextran have both α,β- and α,γ-diol groups, borate can react with these pyranosides to form at least five kinds of chelate complexes, that is, (α,β)-monochelate, (α,β)(α,β)-bischelate, (α,γ)-monochelate, (α,γ)(α,γ)-bischelate and (α,β)(α,γ)-bischelate, and following equations should be taken into consideration for the complexation equilibria:

$$B(OH)_3 + H_2O \rightleftarrows B(OH)_4^- + H^+ \quad (1)$$

$$K_a = [B(OH)_4^-]\,[H^+]/[B(OH)_3] \quad (2)$$

$$B(OH)_4^- + \text{Pyranoside} \rightleftarrows (\alpha,\ \beta)\ \text{monochelate} + 2\ H_2O \quad (3)$$

$$\beta_{(\alpha,\beta)} = [(\alpha,\ \beta)]/([B(OH)_4^-]\,[\text{Pyranoside}]) \quad (4)$$

$$B(OH)_4^- + 2\ \text{Pyranoside} \rightleftarrows (\alpha,\ \beta)(\alpha,\ \beta)\ \text{bischelate} + 4\ H_2O \quad (5)$$

$$\beta_{(\alpha,\beta)(\alpha,\beta)} = [(\alpha,\ \beta)(\alpha,\ \beta)]/([B(OH)_4^-]\,[\text{Pyranoside}]^2) \quad (6)$$

$$B(OH)_4^- + \text{Pyranoside} \rightleftarrows (\alpha,\ \gamma)\ \text{monochelate} + 2\ H_2O \quad (7)$$

$$\beta_{(\alpha,\gamma)} = [(\alpha,\ \gamma)]/([B(OH)_4^-]\,[\text{Pyranoside}]) \quad (8)$$

$$B(OH)_4^- + 2\ \text{Pyranoside} \rightleftarrows (\alpha,\ \gamma)(\alpha,\ \gamma)\ \text{bischelate} + 4\ H_2O \quad (9)$$

$$\beta_{(\alpha,\gamma)(\alpha,\gamma)} = [(\alpha,\ \gamma)(\alpha,\ \gamma)]/([B(OH)_4^-]\,[\text{Pyranoside}]^2) \quad (10)$$

$$B(OH)_4^- + 2\ \text{Pyranoside} \rightleftarrows (\alpha,\ \beta)(\alpha,\ \beta)\ \text{bischelate} + 4\ H_2O \quad (11)$$

$$\beta_{(\alpha,\beta)(\alpha,\gamma)} = [(\alpha,\ \beta)(\alpha,\ \gamma)]/([B(OH)_4^-]\,[\text{Pyranoside}]^2) \quad (12)$$

where [Pyranoside] is the concentration of free pyranoside residue. These formation constants can be evaluated from ^{11}B NMR signal intensities as discussed below.

3.1. Monomer system

Fig. 2 shows the ^{11}B NMR spectrum for α-methyl-D-mannopyranoside. Complexes formed have shown identical chemical shift values with those for other polyhydroxy compounds [14,15,19,20], however, signals from (α,β)-monochelate and (α,β)(α,γ)-bischelate and ones from (α,γ)-monochelate and (α,γ)(α,γ)-bischelate might overlap each other. That is, the signal at −13 ppm should be ascribed to (α,β)-monochelate and/or (α,β)(α,γ)-bischelate, that at −9 ppm to (α,β)(α,β)-bischelate, and that around −18 ppm to (α,γ)-monochelate and/or (α,γ)(α,γ)-bischelate. One can easily estimate values of $([(\alpha,\beta)]+[(\alpha,\beta)(\alpha,\gamma)])/([B(OH)_4^-][\text{Pyranoside}])$ and $([(\alpha,\gamma)]+[(\alpha,\gamma)(\alpha,\gamma)])/([B(OH)_4^-][\text{Pyranoside}])$ from corresponding NMR signal intensities and the pK_a value of boric acid (pK_a = 9.05 [18]). These quotients are expressed as follows:

$$([(\alpha,\ \beta)] + [(\alpha,\ \beta)(\alpha,\ \gamma)])/([B(OH)_4^-][\text{Pyranoside}]) = \beta_{(\alpha,\beta)} + \beta_{(\alpha,\beta)(\alpha,\gamma)}[\text{Pyranoside}] \quad (13)$$

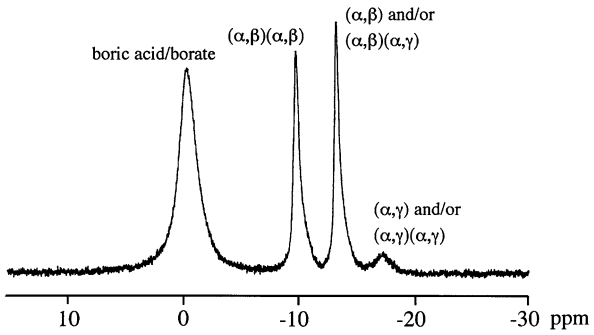


Fig. 2. ^{11}B NMR spectrum of 0.02 mol dm^{-3} boric acid–borate solution (I = 0.1, NaCl) containing 0.4 mol dm^{-3} α-methyl-D-mannopyranoside at pH 7.9.

$$([(\alpha,\ \gamma)] + [(\alpha,\ \gamma)(\alpha,\ \gamma)])/([B(OH)_4^-][\text{Pyranoside}]) = \beta_{(\alpha,\gamma)} + \beta_{(\alpha,\gamma)(\alpha,\gamma)}[\text{Pyranoside}] \quad (14)$$

According to above equations a plot of the quotient of the left-hand side versus the free ligand concentration [Pyranoside] should give a straight line.

Fig. 3(a) shows a plot of the relevant parameters of Eq. (13) for the α-methyl-D-mannopyranoside system. A good linear relationship is clearly obtained. From the slope and the intercept, values of $\beta_{(\alpha,\beta)} = 19$ and $\beta_{(\alpha,\beta)(\alpha,\gamma)} = 9.6$ were obtained. Fig. 3(b) shows a plot of the quotient of the left-hand side of Eq. (14) versus

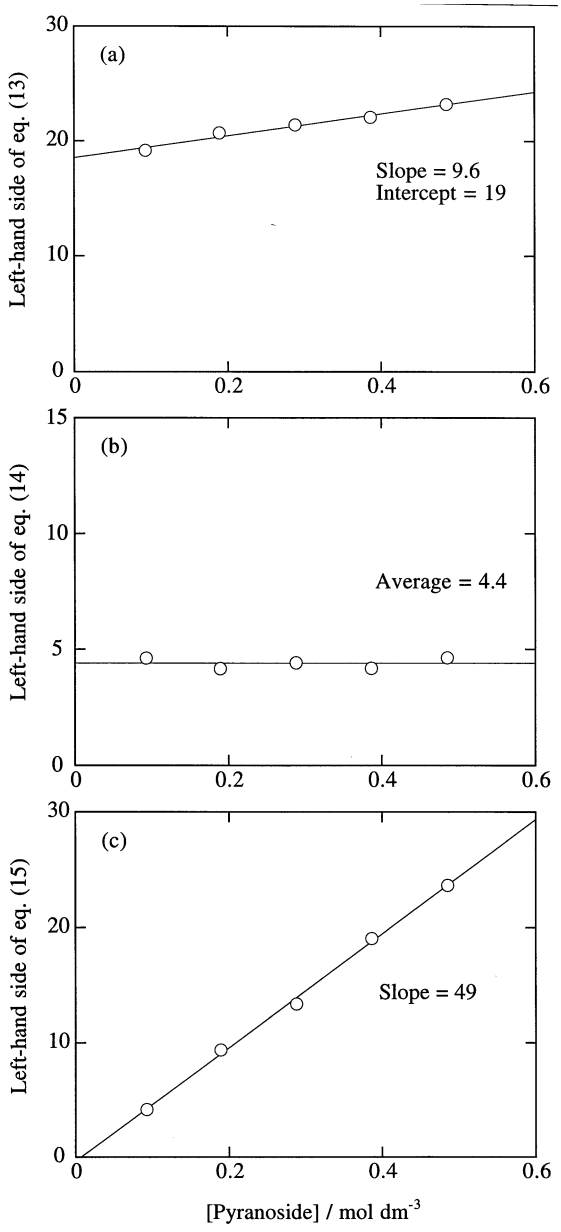


Fig. 3. Determination of formation constants for the α-methyl-D-mannopyranoside system. The quotients on the left-hand side of Eqs. (13), (14) and (15) are plotted as a function of [Pyranoside]. Plots (a), (b) and (c) are for Eqs. (13), (14) and (15), respectively.

[Pyranoside]. A liner relationship was obtained but the slope of the line was indistinguishable from zero. Under conditions of this study the formation of the (α,γ)(α,γ)-bischelate complex was negligible. From the intercept (average) a value of 4.4 was estimated for $\beta_{(\alpha,\gamma)}$.

The equation for the (α,β)(α,β)-bischelate complex is given by Eq. (6) as follows:

$$[(\alpha,\beta)(\alpha,\beta)]/([B(OH)_4^-][\text{Pyranoside}]) = \beta_{(\alpha,\beta)(\alpha,\beta)}[\text{Pyranoside}] \quad (15)$$

A plot of the relevant parameters of Eq. (15) gave a straight line passing through the origin (Fig. 3(c)). From the slope of the line the formation constant of (α,β)(α,β)-bischelate was calculated as $\beta_{(\alpha,\beta)(\alpha,\beta)} = 49$.

We also made the same analysis for the α-methyl-D-glucopyranoside system. The formation constants evaluated for both monomer derivatives are summarized in Table 2. Hydroxyl groups on C-2 and C-3 of α-methyl-D-mannopyranoside arrange in *cis*-configuration, which is quite suitable for the (α,β)-complexation, so that the formation constant of the (α,β)-monochelate complex is high. Since the stability of the (α,γ)-monochelate complex is almost the same between α-methyl-D-mannopyranoside and α-methyl-D-glucopyranoside, borate should mainly bind with hydroxyl groups on C-4 and C-6 positions for both pyranosides.

3.2. Polymer system

^{11}B NMR spectroscopy has provided a powerful tool for the study on the ion binding in polymer solutions [21]. Fig. 4 shows ^{11}B NMR spectra for 0.02 mol dm^{-3} boric acid–borate solutions ($I = 0.1$, NaCl) containing 0.4 or 0.6 monomol dm^{-3} dextrans with various chain lengths. The complexes formed have also shown identical chemical shift values with those for the monomer derivatives. In the case of the longer chain dextran than Dextran T10, the formation of the (α,β)(α,β)-bischelate complex can be negligible. In accordance with a decrease in the chain length of dextran, the signals at −13 and −9 ppm due to the complexes which have (α,β)-chelate bindings increased compared with the signal of (α,γ)-monochelate and/or (α,γ)(α,γ)-bischelate at −18 ppm.

Table 2
Formation constants for the complexation of borate with α-methyl-D-mannopyranoside and α-methyl-D-glucopyranoside ($I = 0.1$ NaCl)

α-Methyl-D-mannopyranoside	$\beta_{(\alpha,\beta)}$	19
	$\beta_{(\alpha,\beta)(\alpha,\gamma)}$	9.6
	$\beta_{(\alpha,\beta)(\alpha,\beta)}$	49
	$\beta_{(\alpha,\gamma)}$	4.4
α-Methyl-D-glucopyranoside	$\beta_{(\alpha,\beta)}$	0.16
	$\beta_{(\alpha,\gamma)}$	3.8
	$\beta_{(\alpha,\gamma)(\alpha,\gamma)}$	1.7

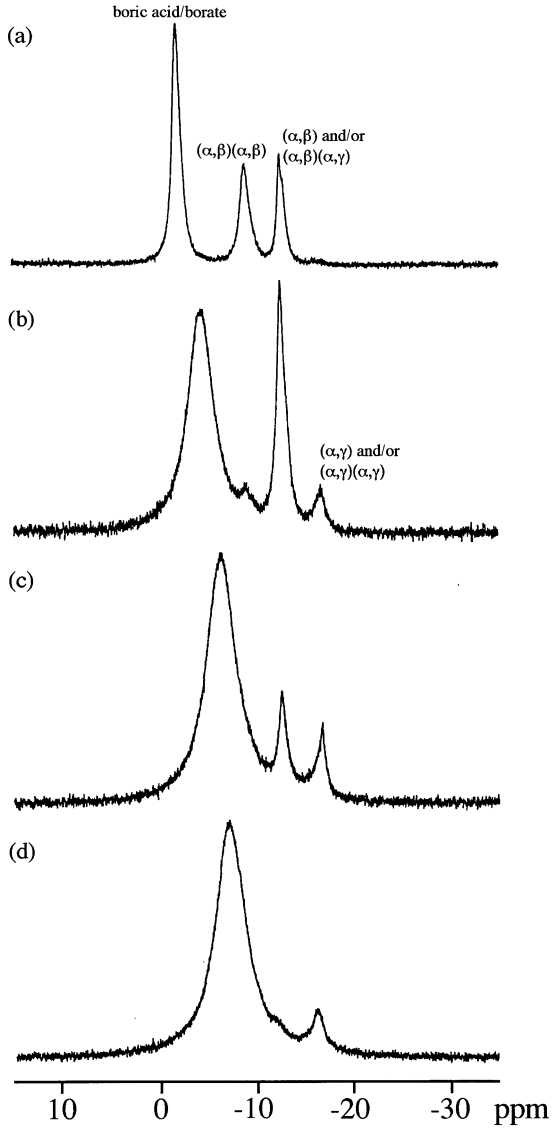


Fig. 4. ^{11}B NMR spectra for 0.02 mol dm^{-3} boric acid–borate solutions ($I = 0.1$, NaCl) containing 0.4 or 0.6 monomol dm^{-3} dextrans with various chain lengths; (a) 0.6 monomol dm^{-3} dextran 3000, pH 7.4; (b) 0.6 monomol dm^{-3} dextran T10, pH 8.3; (c) 0.6 monomol dm^{-3} dextran T40, pH 8.8; (d) 0.4 monomol dm^{-3} dextran T500, pH 9.0.

In the present system, the complexibility of borate with the polysaccharides (in other words, the degree of ion condensation on the polymers) is not so high, therefore, the polyelectrolyte effect can be negligible under the experimental condition. Borate can bind with two glucopyranoside residues on different dextran chains and/or the same chain in the 1:2 complexation. In order to distinguish between the inter-chain and intra-chain bischelate complexes, we must examine the polymer system in more detail. The signal at −9 ppm may be assigned to intra-chain (α,β)(α,β)-bischelate and/or inter-chain (α,β)(α,β)-bischelate, that at −13 ppm to (α,β)-monochelate, intra-chain (α,β)(α,γ)-bischelate and/or inter-chain (α,β)(α,γ)-bischelate, and that around −18 ppm to (α,γ)-monochelate, intra-chain (α,γ)(α,γ)-bischelate and/or inter-chain (α,γ)(α,γ)-bischelate. ^{11}B NMR spectra of the borate-dextran solutions do not allow any direct distinction between the intra- and inter-chain bischelate complexes. Therefore, following equations should be considered for the polymer system.

$$([\text{intra}(\alpha,\ \beta)(\alpha,\ \beta)] + [\text{inter}(\alpha,\ \beta)(\alpha,\ \beta)])/([\text{B(OH)}_4^-] \times [\text{Pyranoside}]) = \beta_{(\alpha,\beta)(\alpha,\beta)\text{intra}} + \beta_{(\alpha,\beta)(\alpha,\beta)\text{inter}}[\text{Pyranoside}] \quad (16)$$

$$([(\alpha,\ \beta)] + [\text{intra}(\alpha,\ \beta)(\alpha,\ \gamma)] + [\text{inter}(\alpha,\ \beta)(\alpha,\ \gamma)])/([\text{B(OH)}_4^-]\ [\text{Pyranoside}]) = \beta_{(\alpha,\beta)} + \beta_{(\alpha,\beta)(\alpha,\gamma)\text{intra}} + \beta_{(\alpha,\beta)(\alpha,\gamma)\text{inter}}[\text{Pyranoside}] \quad (17)$$

$$([(\alpha,\ \gamma)] + [\text{intra}(\alpha,\ \gamma)(\alpha,\ \gamma)] + [\text{inter}(\alpha,\ \gamma)(\alpha,\ \gamma)])/([\text{B(OH)}_4^-][\text{Pyranoside}]) = \beta_{(\alpha,\gamma)} + \beta_{(\alpha,\gamma)(\alpha,\gamma)\text{intra}} + \beta_{(\alpha,\gamma)(\alpha,\gamma)\text{inter}}[\text{Pyranoside}] \quad (18)$$

where [Pyranoside] means the concentration of free pyranoside residue in monomol dm^{-3}, and the formation constants for the intra- and inter-chain bischelate complexations are as follows:

$$\beta_{(\alpha,\beta)(\alpha,\beta)\text{intra}} = [\text{intra}(\alpha,\beta)(\alpha,\beta)]/([\text{B(OH)}_4^-] \times [\text{Pyranoside}]) \quad (19)$$

$$\beta_{(\alpha,\beta)(\alpha,\beta)\text{inter}} = [\text{inter}(\alpha,\ \beta)(\alpha,\ \beta)]/([\text{B(OH)}_4^-] \times [\text{Pyranoside}]^2) \quad (20)$$

$$\beta_{(\alpha,\beta)(\alpha,\gamma)\text{intra}} = [\text{intra}(\alpha,\ \beta)(\alpha,\ \gamma)]/([\text{B(OH)}_4^-] \times [\text{Pyranoside}]) \quad (21)$$

$$\beta_{(\alpha,\beta)(\alpha,\gamma)\text{inter}} = [\text{inter}(\alpha,\ \beta)(\alpha,\ \gamma)]/([\text{B(OH)}_4^-] \times [\text{Pyranoside}]^2) \quad (22)$$

$$\beta_{(\alpha,\gamma)(\alpha,\gamma)\text{intra}} = [\text{intra}(\alpha,\ \gamma)(\alpha,\ \gamma)]/([\text{B(OH)}_4^-] \times [\text{Pyranoside}]) \quad (23)$$

$$\beta_{(\alpha,\gamma)(\alpha,\gamma)\text{inter}} = [\text{inter}(\alpha,\ \gamma)(\alpha,\ \gamma)]/([\text{B(OH)}_4^-] \times [\text{Pyranoside}]^2) \quad (24)$$

Fig. 5 shows plots of the relevant parameters of Eqs. (16)–(18) for Dextran T10. A plot of the left-hand side of Eq. (16) versus the concentration of free glucopyranoside residue [Pyranoside] gave a straight line passing through the origin (Fig. 5(a)), which clearly shows that the formation of the intra-chain (α,β)(α,β)-bischelate complex is negligible. From the slope of the plot in Fig. 5(a), a value of $\beta_{(\alpha,\beta)(\alpha,\beta)\text{inter}} = 0.48$ was obtained. Dextran is a rather rigid polymer owing to the steric and hydrodynamic hindrance [12,13]. Therefore, it should be difficult for dextran to make a loop and to form the intra-chain (α,β)(α,β)-bischelate type cross-link. Based on this fact the formation of intra-chain (α,β)(α,γ)-bischelate and intra-chain (α,γ)(α,γ)-bischelate complexes can also be negligible. A plot of the

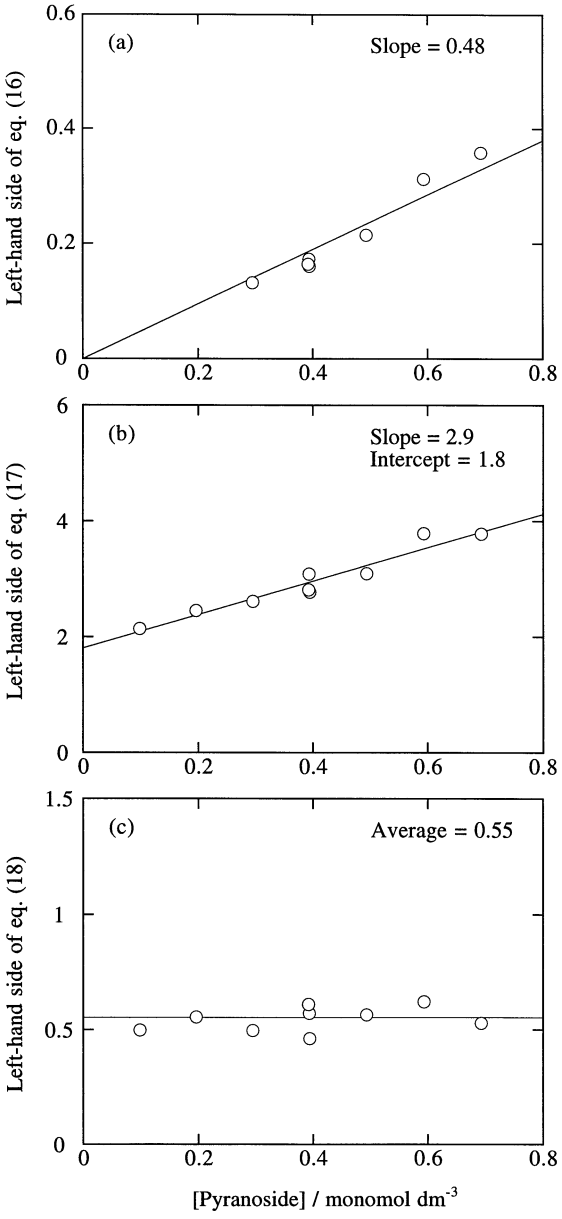


Fig. 5. Determination of formation constants for dextran T10 system. The quotients on the left of Eqs. (16), (17) and (18) are plotted as a function of [Pyranoside]. Plots (a), (b) and (c) are for Eqs. (16), (17) and (18), respectively.

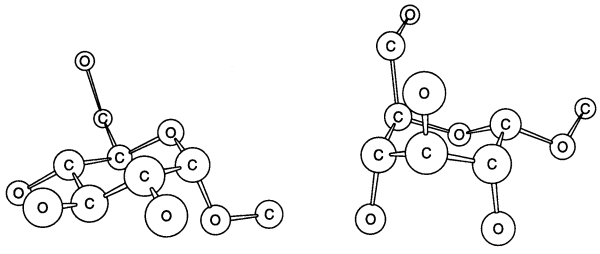
left-hand side of Eq. (17) versus [Pyranoside] gave a good linear relationship (Fig. 5(b)). From the slope and intercept, values of $\beta_{(\alpha,\beta)} = 1.8$ and $\beta_{(\alpha,\beta)(\alpha,\gamma)\text{inter}} = 2.9$ were obtained, where $\beta_{(\alpha,\beta)(\alpha,\gamma)\text{intra}}$ was assumed to be 0 as discussed above. A plot of relevant parameters of Eq. (18) also gave a straight line but the slope of the line was indistinguishable from zero (Fig. 5(c)). Under the conditions of this study the formation of inter-chain (α,γ)(α,γ)-bischelate complex was negligible. The bischelate complexation would be unfavorable because of the bulkiness of the polysaccharides. From the intercept

Table 3
Formation constants of borate complexes with Dextran ($I = 0.1$, NaCl)

	$\beta_{(\alpha,\beta)}$	$\beta_{(\alpha,\beta)(\alpha,\gamma)\text{inter}}$	$\beta_{(\alpha,\beta)(\alpha,\beta)\text{inter}}$	$\beta_{(\alpha,\gamma)}$	$\beta_{(\alpha,\beta)}/\beta_{(\alpha,\gamma)}$
Dextran 3000	19	25	53	1.4	13.5
Dextran T10	1.8	2.9	0.48	0.55	3.3
T40	0.44	0.18		0.40	1.1
T70	0.27	0.09		0.38	0.71
T500	0.05			0.44	0.11

(average) a value of 0.55 was estimated for $\beta_{(\alpha,\gamma)}$, where $\beta_{(\alpha,\gamma)(\alpha,\gamma)\text{intra}}$ was also assumed to be 0. The formation constants for other dextrans with various chain lengths were also evaluated in the same procedure and summarized in Table 3.

The glucopyranoside residue in the middle of the dextran chain has hydroxl groups at C-2, C-3 and C-4 positions. Borate binds with the hydroxyl groups on C-2 and C-3 or C-3 and C-4 to form the (α,β)-chelate type complexes, and on C-2 and C-4 to form the (α,γ)-chelate type complexes. The glucopyranoside has two stable conformers C1 and 1C in solution:



C1 conformer 1C conformer

The C1 is suitable for the (α,β)-complexation and the 1C is suitable for the (α,γ)-complexation. Mono- and oligosaccharides can easily interchange their conformations between C1 and 1C. Therefore, the 1C glucopyranoside changes the conformation to C1 and then bind with borate to form the (α,β)-chelate type complexes. For the dextran, glucopyranoside residues near both ends of the polymer chain may also interchange their conformations, so that the 1C glucopyranosides can probably change the conformation to form the (α,β)-chelate type complexes. However, it is quite difficult to interchange the conformations of glucopyranosides in the middle of the longer polymer chain owing to the hydrodynamic and steric hindrance. Therefore, the 1C glucopyranoside cannot bind with borate to form the (α,β)-chelate type complexes. As can be seen from $\beta_{(\alpha,\beta)}/\beta_{(\alpha,\gamma)}$ values in Table 3 dextran with a shorter chain prefers the (α,β)-complexation to the (α,γ)-complexation, while the stability of the (α,γ)-monochelate complex is almost constant except Dextran 3000, which means that the glucopyranoside residues in the dextran

chain prefer the 1C conformation in the aqueous solution. Fig. 6 shows that $-RT \ln \beta_{(\alpha,\beta)}$ values increase with the increase in the N values. If we could assume the stability of the (α,β)-complex with the glucopyranoside of the C1 conformer which is the active one in the (α,β)-complexation is constant among dextrans with different chain lengths, the difference in the $-RT \ln \beta_{(\alpha,\beta)}$ values among various dextrans corresponds to the difference in the energy to change the conformation from 1C to C1. The increase in the chain length leads to the increase in the energy to change the conformation, that is, the decrease in the apparent stability of the (α,β)-monochelate complex as shown in Fig. 6. The complexation behavior for the long chain dextran is similar to that for Sephadex gel with a low degree of cross-linking [14]. The intrinsic formation constant of a complex formed in dextran or Sephadex gel systems should be expressed in terms of the concentration of C1 or 1C (in monomol dm^{-3}) which is the active conformer in the complexation.

The overall formation constants of 1:1 and 1:2 complexes for D-glucose which is the analogue to the terminal α-anomer of dextran were reported to be $10^{1.90}$ and $10^{4.79}$ [22], respectively. The structure of the other terminal glucopyranoside residue is similar to α-methyl-D-glucopyranoside. Dextran 3000 is the short polymer, which consists of about 19 glucopyranoside residues. The high stability of the (α,β)-monochelate complex should mainly be due to the complexation with adjacent hydroxyl groups in the terminal α-anomer of the polysaccharide. Similarly, the $\beta_{(\alpha,\gamma)}$ value for Dextran 3000 is higher than others, which is also in part due to the contribution of alternative hydroxyl groups in both terminal glucopyranoside residues.

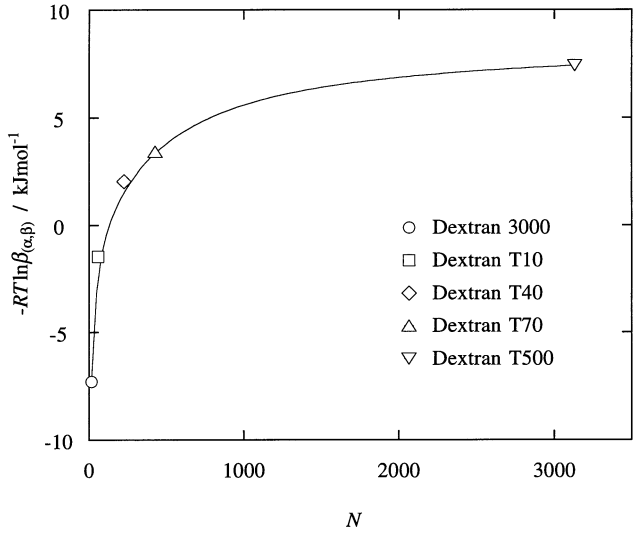


Fig. 6. Dependence of $-RT \ln \beta_{(\alpha,\beta)}$ value on the length of dextran chain. N indicates the average number of glucopyranoside residues in one dextran chain.

4. Conclusions

We have revealed the formation of (α,β)-monochelate, inter-chain (α,β)(α,β)-bischelate, (α,γ)-monochelate and inter-chain (α,β)(α,γ)-bischelate complexes and evaluated their formation constants for the dextran system. The formation of intra-chain bischelate complexes can be negligible for a rather stiff polymer as dextran. The 1C conformer of the glucopyranoside residue should be more stable than the C1 conformer in the dextran chain in aqueous solutions. Because of the steric and hydrodynamic hindrance the glucopyranoside residues in the middle of the polymer chain can hardly change their conformation from 1C to C1 except the residues near both ends of the polymer chain. The stability of the (α,β)-chelate type complex becomes lower with the increase in the chain length of dextran. On the other hand, the formation constants of the (α,γ)-monochelate complex are almost constant among dextrans with different chain lengths except Dextran 3000. Above all, ^{11}B NMR study on the interactions of borate with the linear dextran consisting of pyranoside residues and their monomer derivatives (α-methyl-D-glucopyranoside and α-methyl-D-mannopyranoside) could quantitatively clarify the effect of chain length on the complexation behavior of polysaccharides.

Acknowledgements

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