Novel saccharide-induced conformational changes in a boronic acid-appended poly(L-lysine) as detected by circular dichroism and fluorescence

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Received 14th December 1999, Accepted 15th March 2000

Poly(L-lysine) has been modified with a 4-phenylboronic acid derivative which acts as a sugar-binding site and a dansyl group which acts as a fluorescence probing site. The helix content of this poly(L-lysine) derivative as estimated by CD spectroscopy became maximum at pH 10.3. The helix content was increased from 51 to 79% by complexation of D-fructose with the pendant boronic acid group and the maximum pH shifted from 10.3 to 7.9. Examination of the CD spectra revealed that the conformation changes in the order of β-sheet ⟶ α-helix ⟶ random coil with increasing medium pH. The D-glucose addition induced a similar increase in the helix content and a similar low pH shift of the helix content maximum but the mechanism was somewhat different: one D-glucose was bound to two boronic acid groups to form an intrapolymeric bridge and the resultant CD spectrum was similar to that of the β-turn structure. These saccharide-induced conformational changes were well reflected by plots of fluorescence intensity *vs.* pH. The decrease in the fluorescence intensity in the high pH region (random coil region) was confirmed by fluorescence polarization to be due to the increase in the molecular motion. This is a novel system to control the poly(amino acid) conformation by saccharides and to detect the conformational changes by convenient spectroscopic methods.

Introduction

The conformational changes in polypeptides are based on a subtle balance among several secondary forces such as hydrogen-bonding interactions, electrostatic attraction and repulsion, hydrophobic forces, dipole–dipole interactions, *etc*.[1] It is expected, therefore, that the conformational transitions can be changed by a subtle change in the balance. One of the typical examples is the photocontrol of polypeptide higher-order structures by *cis–trans* photoisomerization of the azobenzene moiety appended in the side-chain.[2–4] It is known that a saccharide family frequently plays crucial roles in determining the higher-order structures of cell membranes and globular proteins.[5] It thus occurred to us that if these higher-order structures can be controlled by saccharides, it would lead to a novel methodology to control their biological functions.[6] Recently, we and others have demonstrated that boronic acids act as a useful '*sugar-interface*' operative in water to recognize saccharides or to harness saccharides as a trigger function.[7–16] We thus expected that if poly(L-lysine) is appropriately modified with a boronic acid group, the helix-coil transition of the resultant polypeptide chain would be controlled by the addition of saccharides.[17] With these objects in mind, we previously synthesized boronic acid-appended poly(L-lysine) (**1**).[17] It was found that when monosaccharides are added to a solution of **1** the helix content (monitored by CD spectroscopy) increases and the pH which gives the maximum helix content shifts to a lower region.[17] In addition, the magnitude of the pH shift was correlated with the affinity of monosaccharides for phenylboronic acid.[17] Here, we newly synthesized **2** bearing a fluorescent dansyl (5-dimethylaminonaphth-1-ylsulfonyl) unit (5 mol%) as well as a 4-phenylboronic acid unit (95 mol%) from poly(L-lysine), expecting that the saccharide-induced conformational changes would be readily detectable by a fluorescence change. Careful examination of the circular dichroism (CD) and fluorescence spectra has revealed that the conformation of **2** changes not only into an α-helix but also into a β-sheet, β-turn or random coil and the changes are correlated with the fluorescence intensity change. Compound **3**, which was synthesized from *N*-α-acetyl-L-lysine methyl ester and dansyl chloride, was used as a monomeric reference for **2** in the fluorescence measurements.

Results and discussion

Influence of added monosaccharides on CD spectra

The typical pH-dependent CD spectra of compound **2** are shown in Fig. 1. The spectral shape is basically similar to that of unmodified poly(L-lysine). Judging from the spectral shape,[1,18,19] one can regard the major structures adopted by **2** as the β-sheet structure with the θ minimum at around 220 nm in the low pH region, the α-helix structure with the θ minimum at around 208 and 222 nm in the neutral to slightly alkaline pH region and the random coil structure at high pH. In the presence of D-fructose a similar CD spectral change appeared although the helix structure became rich at lower pH. As the appended phenylboronic acid residue is rather far from the main chain, it would scarcely affect the transition of the main chain C=O group. Hence, we estimated the helix content using an equation established for poly(L-lysine), *i.e.* $-\{([\theta]_{208} + 4000)/29000\}$.[18] In Fig. 2, $[\theta]_{208}$ and helix content (computed by this equation) are plotted against pH. It is clearly seen that D-fructose which possesses the highest affinity for a boronic acid group among monosaccharides[9–14,20] enhances the helix

DOI: 10.1039/a909822d

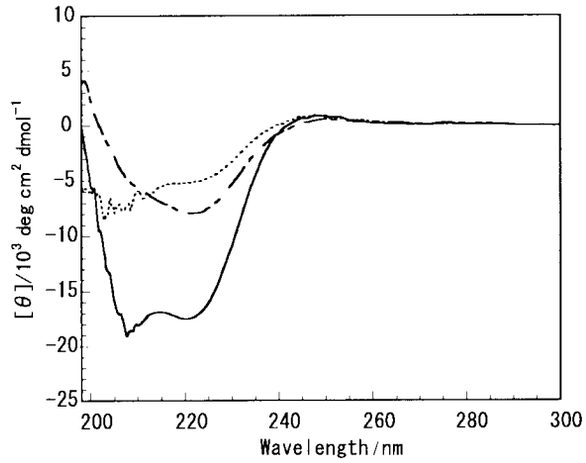
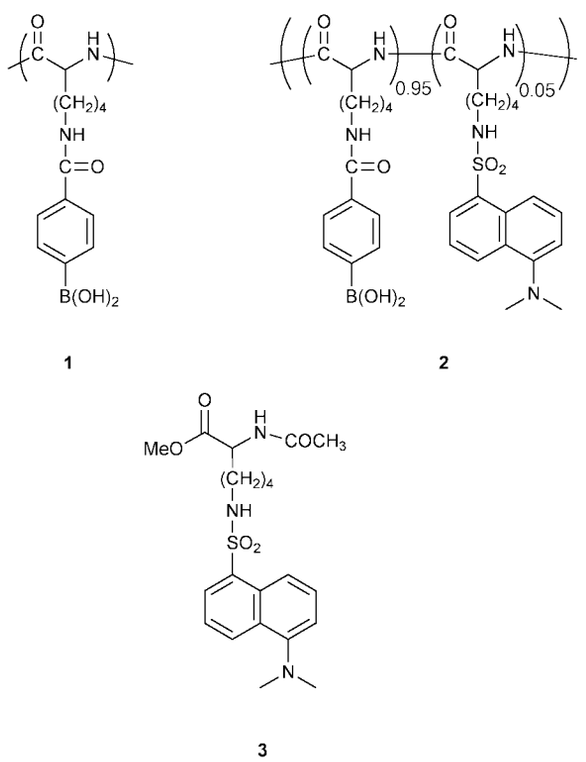


Fig. 1 Typical CD spectra of compound **2** (0.18 monomer unit mmol dm^{-3}) at 25 °C: pH 7.42 – - –, 10.31 ——, 11.60 ----.



content to 79% from 51% and induces a shift of the maximum pH to 7.9 from 10.3. These trends were already found for **1**, and were rationalized by stabilization of the helix structure by the OH···OH hydrogen-bonding interactions among bound D-fructose molecules and by a pK_a shift of the boronic acid–saccharide complexes to lower pH region, respectively.[17]

Very interestingly, we have newly found that the influence of added D-glucose, which was not investigated with compound **1** in detail, is quite unique. As shown in Fig. 3, the CD spectra assignable to the helix structure are also observable at neutral to slightly alkaline pH and the increase in the helix content (85%) and the shift of the maximum to lower pH (8.7) are induced (Fig. 2). The novel finding that the CD sign is inverted to positive at high pH (>10) is interesting. We first considered that this CD spectrum with the positive sign is similar to that assignable to the β-turn structure.[1,18,19,21] It is known that certain monosaccharides can be bound to two boronic acid groups using 1,2-diol and 4,6- or 5,6-diol.[1,16,22,23] To the best of our knowledge, D-glucose is one such monosaccharide that has the high bridge-forming ability.[16,23] It is reasonable to consider, therefore, that the β-turn structure could be stabilized by such

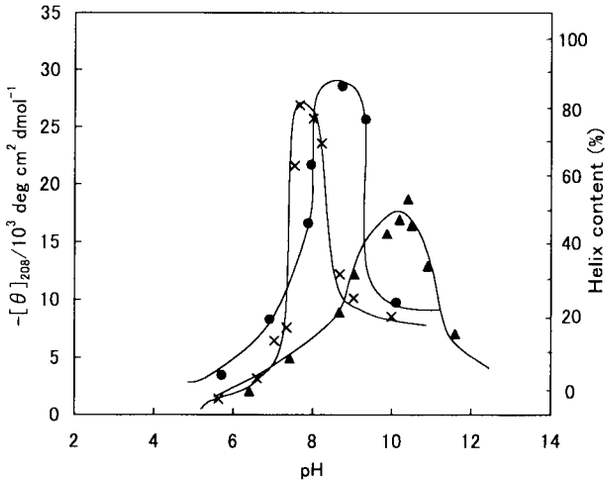


Fig. 2 pH Dependence of $[\theta]_{208}$ and helix content of compound **2** (0.18 monomer unit mmol dm^{-3}) at 25 °C: in the absence (▲), and the presence (53 mmol dm^{-3}) of D-fructose (×) or D-glucose (●).

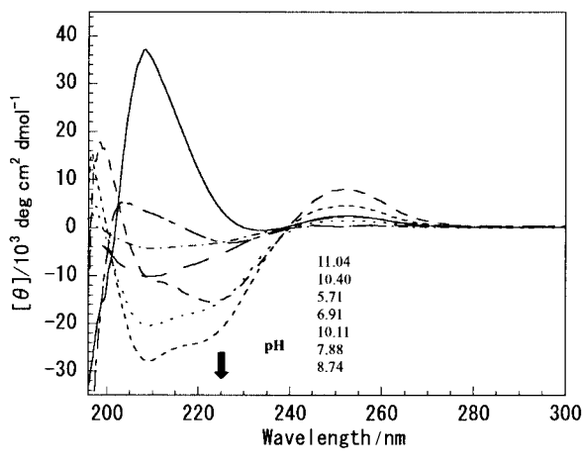


Fig. 3 CD spectra of compound **2** (0.18 monomer unit mmol dm^{-3}) in the presence of D-glucose (53 mmol dm^{-3}) at 25 °C.

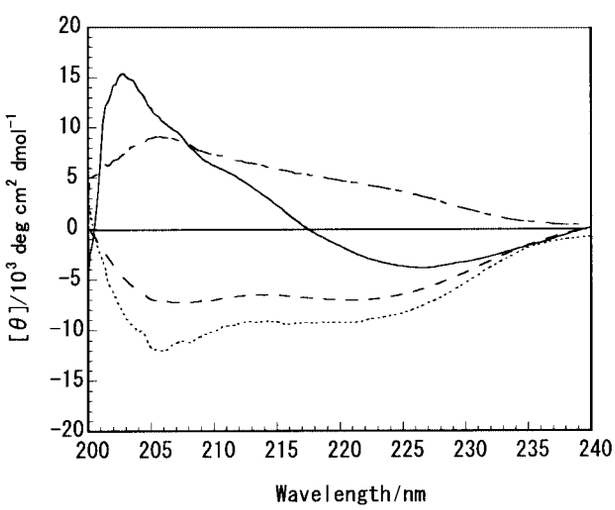


Fig. 4 CD spectra of compound **2** (0.18 monomer unit mmol dm^{-3}) at pH 10.60 and 25 °C in the presence of saccharides (53 mmol dm^{-3}): D-glucose ——, 1-*O*-methyl-α-D-glucopyranoside – – –, D-xylose ----, L-glucose – - –.

an intrapolymeric cross-link. In fact, 1-*O*-methyl α-D-glucopyranoside and D-xylose, in which the formation of the intrapolymeric cross-link is difficult because of the lack of 1-OH and 6-OH, respectively, result in the ordinary CD spectra with the negative sign (Fig. 4). The finding apparently suggests that the positive CD sign observed for D-glucose is related to the

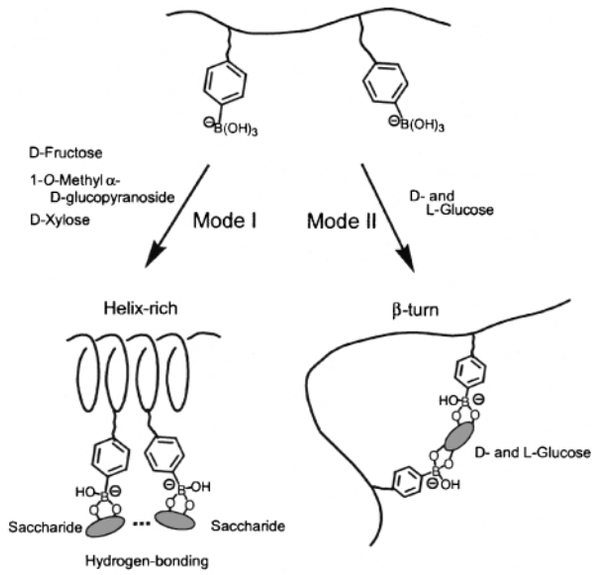


Fig. 5 Complexation modes proposed for the binding of monosaccharides to compound **2**.

bridging effect. We noticed, however, that this CD spectrum with the positive sign is also similar to that observed for the cyclic D-glucose complex with diboronic acid **4**:[7] therein, the **4**·D-glucose complex gives the positive exciton-coupling band whereas the **4**·L-glucose complex gives the negative exciton-coupling band.[7] Hence, this positive band at around 205 nm may be assignable to the first Cotton effect of the exciton-coupling band arising from chiral orientation of two anisyl dipole moments in the D-glucose complex into (*R*) helicity. One can discriminate between these two possible explanations by the CD spectrum obtained in the presence of L-glucose: that is, if it is due to the β-turn structure brought forth by the bridging effect of D-glucose, the positive CD band should appear again even in the presence of L-glucose, whereas if it is due to the exciton-coupling, L-glucose should result in the negative CD band symmetrical to that of D-glucose in this wavelength region.[7] As shown in Fig. 4, L-glucose gave the CD spectrum with the positive sign, which was weaker and broader than that obtained in the presence of D-glucose. This finding supports the view that the positive CD band is due to the formation of the β-turn structure and should not be attributed to chiral orientation of two dipole moments in the 4-boronic acid pendant groups. One may consider, therefore, that the stronger positive CD band for D-glucose results from the synergistic effects of both the β-turn structure and the chiral orientation of the two dipole moments whereas the weaker positive CD band for L-glucose results from the offset of the two opposing CD bands. This conclusion is further confirmed by the finding that L-fructose, which tends to form a 1 : 1 complex with a boronic acid group,[24] gives a helix-rich, negative CD spectrum nearly the same as that obtained in the presence of D-fructose.[25] Thus, the binding modes for monosaccharides are summarized in Fig. 5. In Mode I, the main chain is CD-active because of the helix formation whereas the boronic acid/saccharide complex pendants are CD-silent. In Mode II, not only the main chain but also the saccharide-complexed pendants are CD-active because of the helix formation and the exciton coupling, respectively.

Influence of added monosaccharides on fluorescence spectra

One of the research purposes of the present study is to estimate the applicability of a fluorescence spectroscopic method (which is more convenient than a CD spectroscopic method) to detection of a conformational change in the poly(amino acid) main chain. We chose as a fluorescent probe a dansyl group, the fluorescence spectra of which are known to change sensitively

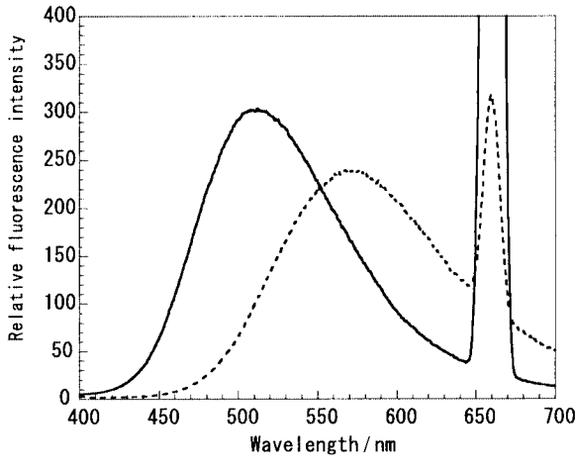


Fig. 6 Fluorescence spectra of compounds **2** (0.18 monomer unit mmol dm^{-3}: ——) and **3** (9.0×10^{-6} mol dm^{-3}: ----) at pH 9.25 and 25 °C: the strong peak at around 660 nm is the twofold overtone wavelength for the excitation wavelength (330 nm).

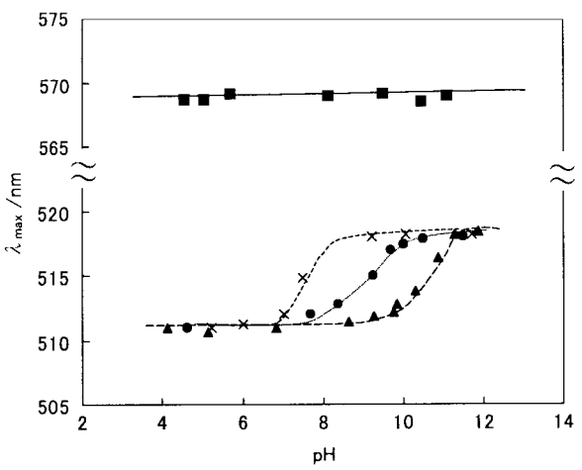
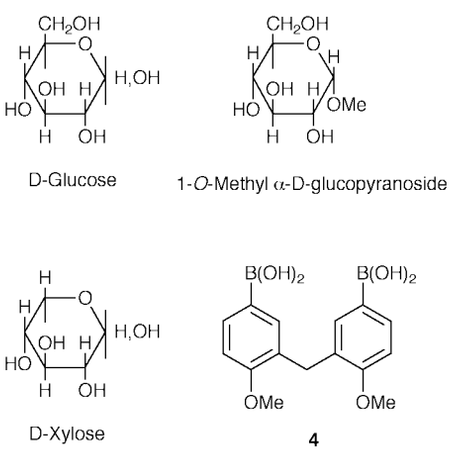


Fig. 7 Plots of emission maximum wavelength (λ_{max}) against pH for compounds **2** (▲), **3** (■) and **2** with D-fructose (×) or D-glucose (●) (53 mmol dm^{-3}).



in response to a change in the environmental medium.[26] Distinctly to characterize the influence of the conformational change in the main chain on the fluorescence spectra, we compared the fluorescence spectra of **2** with those of a monomeric reference compound **3**.

The fluorescence spectra were measured with excitation wavelength at 330 nm which was an isosbestic point in the absorption spectra. The typical fluorescence spectra are shown in Fig. 6. Plots of emission maximum wavelength (λ_{max}) against pH (Fig. 7) reveal two characteristic differences between

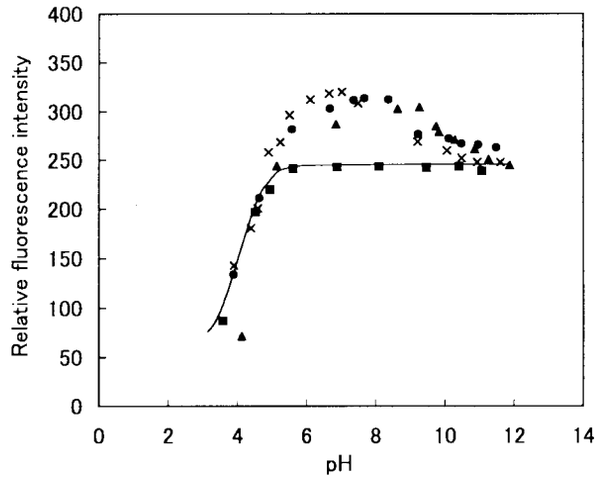


Fig. 8 Plots of maximum fluorescence intensity against pH for compound **2** (▲), **3** (■) and **2** with D-fructose (×) and D-glucose (●): 25 °C, [**2**] = 0.18 monomer unit mmol dm^{-3}, [**3**] = 9.0 × 10^{-6} mol dm^{-3}, [saccharide] = 53 mmol dm^{-3}.

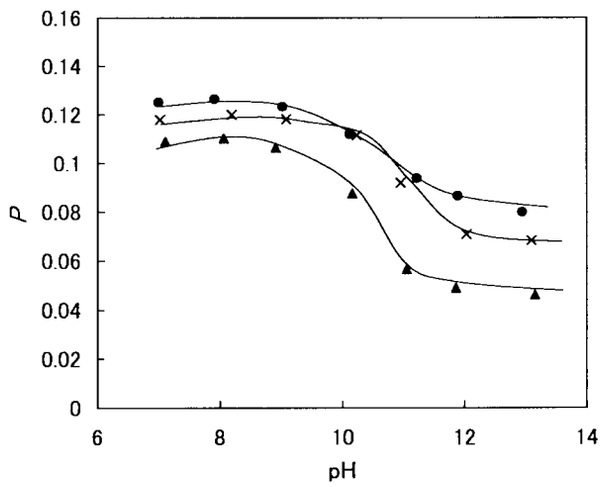


Fig. 9 pH Dependence of fluorescence polarization (*P*) value of compound **2** (0.18 unit mmol dm^{-3}) at 25 °C: in the absence (▲) and the presence (53 mmol dm^{-3}) of D-fructose (×) or D-glucose (●).

compounds **2** and **3**: that is, (i) the λ_{max} for **2** (511–519 nm) appears at much shorter wavelength than that for **3** (568 nm) and (ii) the λ_{max} for **3** is scarcely changed whereas that for **2** shifts to longer wavelength in alkaline pH region (>10). The red shift of the λ_{max} is rationalized such that the dansyl group is surrounded by a more polar environment.[26] One may consider, therefore, that the dansyl group in **2** is fixed in a more apolar environment than that in **3**. Since the pH jump observed for **2** (Fig. 7) is comparable with a conformational change from α-helix to random coil (Fig. 2), one may regard the environment presented by a β-sheet and α-helix as more or less similar to each other whereas that presented by a random coil is more polar.

Fig. 8 shows the fluorescence intensity of the dansyl group in compound **2** or **3** plotted against pH. For **3** the fluorescence intensity increases at pH 3–5 and is saturated to a plateau above pH 5. Therefore, this fluorescence intensity change is attributed to deprotonation of the dimethylamino group ($NHMe_2^+ \longrightarrow NMe_2 + H^+$). The fluorescence intensity increase was also observed for **2** and **2** with D-fructose or D-glucose (Fig. 8), which can again be rationalized by deprotonation of the pendant dimethylamino group. Interestingly, examination of Fig. 8 reveals that in **2** the fluorescence intensity decreases in the alkaline pH region. This bell-shaped pH dependence similar to that observed for plots of $[\theta]_{208}$ (helix content) *vs.* pH (Fig. 2) is not seen for **3**. In fact, the pH where the maximum fluorescence intensity is observed shifts to lower values in the order of none (pH 10.3) > with D-glucose (pH 8.7) > with D-fructose (pH 7.9), as in the order observed in Fig. 2. These results consistently support the view that the fluorescence intensity decrease at pH 8–10, which does not appear for monomeric reference **3**, is attributed to a conformational transition from α-helix to random coil. Presumably, the decrease in the fluorescence quantum yield at pH 8–10 is ascribed either to the increase in the molecular motion in the random-coil polymer main chain or to the fluorescence quenching of the dansyl singlet state by the anionic boronate groups.[12]

To obtain a further insight into the molecular motion we measured the degree of fluorescence polarization (*P*) in the pH 7–12 region. As shown in Fig. 9, the *P* values are distinctly decreased in this pH region, indicating that the dansyl group can obtain higher mobility by a conformational transition from α-helix to other conformations. Of further interest is the order of the *P* values in the high pH region. It is seen from Fig. 9 that the *P* values at pH > 11 are in the order of none < D-fructose < D-glucose: this order implies that the rotational freedom of the pendant group decreases when D-fructose is complexed owing to the hydrogen-bonding interaction among bound D-fructose molecules and further decreases when D-glucose is complexed owing to the intrapolymeric cross-link by D-glucose molecules.

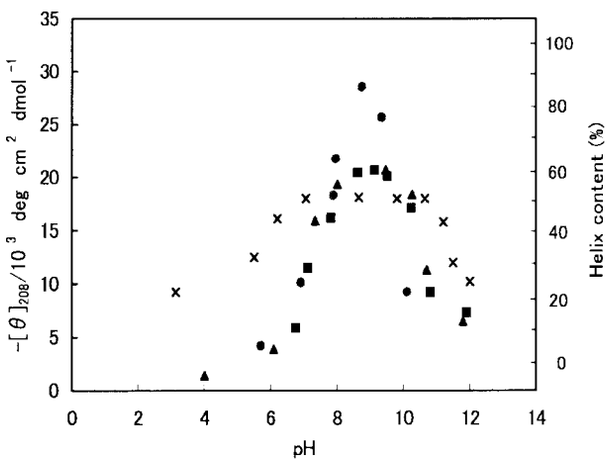


Fig. 10 pH Dependence of $[\theta]_{208}$ and helix content of compound **2** (0.18 monomer unit mmol dm^{-3}) at 25 °C in the presence (53 mmol dm^{-3}) of D-glucose (●), laminaribiose (■), laminaritriose (▲) or laminarihexaose (×): the saccharide concentrations are normalized with respect to the D-glucose unit, *e.g.* [lamimarihexaose] = 1/6·[D-glucose].

Influence of added oligosaccharides on CD and fluorescence spectra

To obtain an insight into the multi-point interaction with compound **2** the CD spectra were measured in the presence of several oligosaccharides. To compare the oligomeric effect with respect to D-glucose (monosaccharide) we selected oligomers composed of the D-glucose units, *viz.* laminaribiose (disaccharide), laminaritriose (trisaccharide) and laminarihexaose (hexasaccharide). The plots of $[\theta]_{208}$ (or helix content) *vs.* pH are shown in Fig. 10. It is seen that with the increase in the D-glucose unit number the main chain of **2** adopts the helix structure in a wider pH range whereas the helix content in the maximum pH region decreases. Presumably, the oligosaccharides can enjoy the multi-point interaction with the boronic acid pendant groups to stabilize the helix structure in a wide pH range, but the interaction mode can be achieved only with some strain in the regular helix structure.[27]

The plots of fluorescence intensity *vs.* pH are shown in Fig. 11. It is seen that the fluorescence intensity is clearly decreased

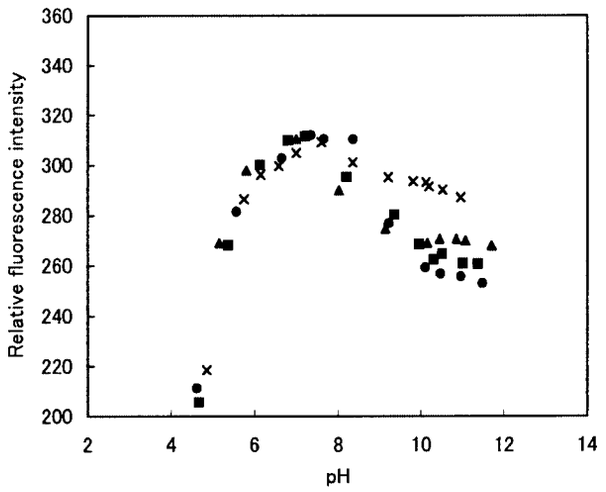


Fig. 11 Plots of maximum fluorescence intensity against pH for compound **2** (0.18 monomer unit mmol dm^{-3}) with D-glucose (●), laminaribiose (■), laminaritriose (▲) or laminarihexaose (×) (53 mmol dm^{-3}): the saccharide concentrations are normalized with respect to the D-glucose unit, *e.g.* [laminarihexaose] = 1/6·[D-glucose].

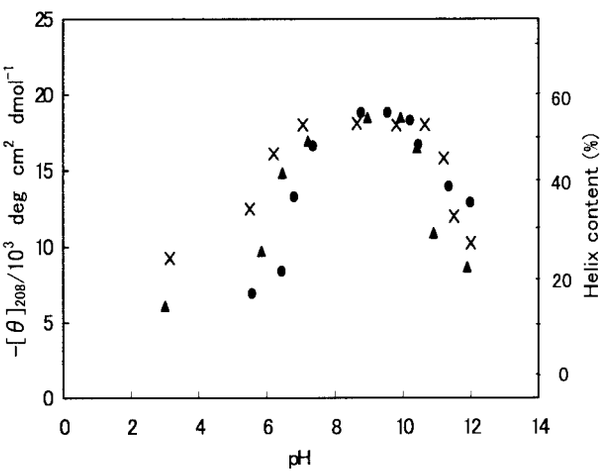


Fig. 12 pH Dependence of $[\theta]_{208}$ and helix content of compound **2** (0.18 monomer unit mmol dm^{-3}) at 25 °C in the presence (53 mmol dm^{-3}) of laminarihexaose (×), cellohexaose (●) or isomaltohexaose (▲): the saccharide concentrations are normalized with respect to the monosaccharide unit.

at pH > 9 and the plots are not much different among D-glucose and oligosaccharides. Careful examination of the plots in the high pH region reveals, however, that the fluorescence intensity decreases in the order laminarihexaose > laminaritriose > laminaribiose > D-glucose: although the difference is small, this order is reproducible beyond the experimental error range (the relative error in the fluorescence intensity is less than 1%). Judging from the relation between the fluorescence intensity and the *P* value (*vide supra*), this tendency implies that the saccharide with the shorter chain length can suppress the molecular motion in the polymer main chain more efficiently.

In Fig. 12 the influence of three hexasaccharides with different structures on the $[\theta]_{208}$ (or helix content) *vs.* pH plots is shown. These saccharides have a trend similar to laminarihexaose: that is, the helix pH region becomes wider whereas the helix content is decreased. We expected that the structural difference in the hexasaccharides would be reflected by a difference in the CD spectral pattern. In fact, however, the CD spectra were more or less similar to one another. The results imply that the flexible hexasaccharides are not suitable as a guest to induce some specific conformational change in the poly(amino acid) main chain even though one can expect the multi-point interaction between the D-glucose units and the boronic acid pendant groups.

Conclusions

The present study has demonstrated that the higher-order conformations of poly(L-lysine) can be controlled by the addition of saccharides if it is appropriately modified with sugar-binding receptors. The CD spectral studies have established that the various higher-order conformational changes can be induced in the poly(L-lysine) main chain by a medium pH change and a structural change in the added saccharides. By using fluorophore-modified compound **2** these conformational changes can readily be monitored by a fluorescence method. We believe that this concept can be extended to other polypeptides and more generally to other naturally occurring high polymers such as proteins, DNA, *etc.* and well imitates sugar-binding behaviours occurring on the surface of proteins, biomembranes, *etc.* Further extension to other poly(α-amino acid)s and other fluorescent dye molecules is currently being investigated in this laboratory.

Experimental

Materials

Hydrobromide salt of poly(L-lysine) (mf 15 000) was purchased from Wako Pure Chemical Industries, Ltd. The preparations of 4-carboxyphenylboronic acid and its acid chloride derivative (**5**) were reported previously.[17]

Poly(L-lysine) derivative with a dansyl group. To a stirred solution of poly(L-lysine) hydrobromide (100 mg, 0.48 unit mmol) and triethylamine (0.33 ml, 2.40 mmol) in methanol (15 ml) at 0 °C was added dropwise a solution of dansyl chloride (9.0 mg, 0.033 mmol) in THF (6 ml) under a nitrogen atmosphere. The reaction mixture was stirred for 12 h at room temperature and then evaporated to dryness. The residue was dissolved in a small amount of methanol and then poured into acetone. The precipitate was filtered off, yielding the required compound: yield (51 mg, 77%). The molar ratio of the dansyl group to the amino group of poly(L-lysine) determined by ^{1}H NMR spectroscopy and elemental analysis was found to be 5 mol%:95 mol% (Found: C, 57.3; H, 9.1; N, 22.5. $(C_6H_{12}N_2O)_{0.95}$ $(C_{18}H_{23}N_3O_3S)_{0.05}$ requires C, 56.69; H, 9.04; N, 20.53%); δ_H (300 MHz; CD_3OD, 25 °C) 1.08–2.14 (6H, m, CH_2), 2.58 (2.3H, bs, CH_2, CH_3 in dansyl), 3.86 (1H, bs, CH), 7.01 (0.05H, d, *J* 7.4, ArH in dansyl), 7.33–7.44 (0.1H, m, ArH in dansyl), 8.06 (0.05H, d, *J* 7.4, ArH in dansyl), 8.30 (0.05H, d, *J* 8.5, ArH in dansyl), 8.55 (0.05H, d, *J* 8.5 Hz, ArH in dansyl).

Poly(L-lysine) derivative with a dansyl group and a phenylboronic acid group (2). 4-Carboxyphenylboronic acid (300 mg, 1.81 mmol) was refluxed in thionyl chloride (6.6 ml, 90.5 mmol) containing a few drops of DMF under a nitrogen atmosphere for 2 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue dissolved in THF (15 ml). This solution was added dropwise to a stirred solution of the poly(L-lysine) derivative with a dansyl group (50 mg, 0.36 monomer unit mmol) and triethylamine (0.75 ml, 5.43 mmol) in methanol (15 ml) at 0 °C under a nitrogen atmosphere. After stirring for 12 h at room temperature, the reaction mixture was evaporated to dryness and the residue dissolved in a small amount of methanol. This was then added to acetone and the precipitate filtered off: yield (75 mg, 74%). The molar ratio of the dansyl group to the phenylboronic acid group determined by ^{1}H NMR spectroscopy and elemental analysis was found to be 5 mol%:95 mol% (Found: C, 57.4; H, 6.5; N, 10.5. $(C_{13}H_{17}BN_2O_4)_{0.95}$ $(C_{18}H_{23}N_3O_3S)_{0.05}$ requires C, 56.76; H, 6.22; N, 10.24%); δ_H (300 MHz; CD_3OD, 25 °C) 1.02–2.09 (6H, m, CH_2), 2.52 (0.3H, bs, CH_3 in dansyl), 3.16 (2H, bs, CH_2), 3.86 (1H, bs, CH), 7.11 (0.05H, d, *J* 7.4, ArH in dansyl), 7.39 (3.9H, bs, ArH and ArH in dansyl), 7.99 (0.05H, d, *J* 7.4, ArH in dansyl), 8.22

(0.05H, d, *J* 8.5, ArH in dansyl), 8.33 (0.05H, d, *J* 8.5 Hz, ArH in dansyl).

***N*-α-Acetyl-*N*-ε-dansyl-L-lysine methyl ester.** To a stirred solution of *N*-α-acetyl-L-lysine methyl ester hydrochloride (954 mg, 4.00 mmol) and triethylamine (2.74 ml, 20.0 mmol) in methanol (10 ml) at 0 °C was added dropwise a solution of dansyl chloride (3.24 g, 12.0 mmol) in THF (20 ml) under a nitrogen atmosphere. After stirring for 12 h at room temperature, the reaction mixture was evaporated to dryness and the residue purified by silica gel column chromatography eluting with ethyl acetate–methanol (7 : 1 v/v) to give *N*-α-acetyl-*N*-ε-dansyl-L-lysine methyl ester as a yellow viscous oil: yield (1.53 g, 88%); $\tilde{\nu}_{max}$/cm^{-1} (neat) 3293 (N–H), 1744, 1661 (C=O) (Found: C, 57.9; H, 6.7; N, 9.7. $C_{21}H_{29}N_3O_5S{\cdot}0.2H_2O$ requires C, 57.44; H, 6.75; N, 9.57%); δ_H (300 MHz; CD_3OD, 25 °C) 1.12–1.51 (6H, m, CH_2), 1.85 (3H, s, CH_3CO), 2.72 (2H, t, *J* 6.6, CH_2), 2.78 (6H, s, CH_3 in dansyl), 3.57 (3H, s, $COOCH_3$), 4.09 (1H, dd, *J* 7.7 and 5.1, CH), 7.18 (1H, d, *J* 7.4, ArH in dansyl), 7.45–7.51 (2H, m, ArH in dansyl), 8.09 (1H, d, *J* 7.4, ArH in dansyl), 8.24 (1H, d, *J* 8.5, ArH in dansyl), 8.46 (1H, d, *J* 8.5 Hz, ArH in dansyl); *m*/*z* 436 (MH^+).

Miscellaneous

^{1}H NMR spectra were measured in CD_3OD with a BRUKER ARX300 apparatus, IR spectra on a SHIMADZU FT-IR 8100M, CD spectra on a JASCO J-720WI spectropolarimeter and fluorescence spectra on a HITACHI F-4500 spectrophotometer. Fluorescence polarization measurements were performed with a Union Giken FS-501A fluorescence polarization spectrometer; emission at 520 nm was monitored upon excitation at 330 nm with a slit width of 3.5 nm for both excitation and emission. The medium pH above 8.5, where the many spectral changes were observed, was adjusted with 4.2 mmol dm^{-3} carbonate buffer, whereas pH below 8.5 was adjusted by adding aqueous HCl solution.

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25 In low molecular-weight diboronic acid derivatives, the CD band appears only when saccharide-containing cyclic complexes (1 : 1 complexes) are formed.[7,8,11,16,23] In contrast, non-cyclic one diboronic acid/two saccharide complexes (1 : 2 complexes) are usually CD-silent.[7,8,11,16,23] Hence, glucose-bridged complexes with **2**, which is CD-active, can be regarded as a sort of saccharide-containing cyclic complexes.
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27 In the presence of these oligosaccharides the positive CD band characteristic of the β-turn structure did not appear even in high pH region: instead, a transition from helix to random coil was induced. Basically, these oligosaccharides possess 1,2-diol and 4,6- or 5,6-diol groups similar to D-glucose at their chain ends and should preserve an ability to bind two boronic acid groups. Therefore, this result may be rationalized by the flexibility of these oligosaccharides that cannot force the main chain to adopt the crooked, energetically unfavourable β-turn structure.