Sugar-induced conformational changes in boronic acid-appended poly(L- and D-lysine)s and sugar-controlled orientation of a cyanine dye on the polymers

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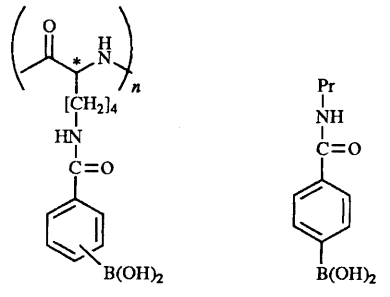
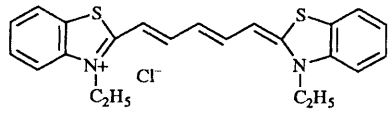
Poly(L- and D-lysine)s have been modified with *para*- or *meta*-phenylboronic acid derivatives which act as sugar-binding sites (L-**1** and D-**1** for the *para*-isomer and L-**2** for the *meta*-isomer). When monosaccharides were added to the L-**1** solution, the helix content (monitored by CD spectroscopy) increased and the pH which gives the maximum helix content shifted to a lower pH region. The magnitude of the pH shift was correlated with the affinity of monosaccharides for phenylboronic acid. The pH dependence of the helix content in the presence of D- or L-fructose was similar although a slight difference was seen at the low pH region. This is a novel attempt towards the control of a polypeptide's conformation by sugars. A cyanine dye (**4**) can be orientated on **1** or **2** in the presence of saccharides. Judging from the CD spectra, the orientation in the presence of **1** is more ordered than that in the presence of **2**. The main driving-force for the association of **4** with **1** or **2** is the electrostatic attraction between cationic **4** and the anionic centre developed on the polymer by complexation of the boronic acid residue with saccharides. The chiral orientation of **4** can be monitored by a CD spectroscopic method: it is mainly governed by the chirality of **1** but not by the chirality of saccharides. This is a novel system to control the dye orientation by the cooperative action of naturally-originated α-amino acids and saccharides.

A helix–coil transition in polypeptides is 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 transition 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 the saccharide family frequently plays a crucial role 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] As an initial and basic study for this kind of research, we here discuss the control of a helix–coil transition of poly(L- and D-lysine)s by saccharides. 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- and D-lysine)s are appropriately modified with boronic acids, the helix–coil transition of the resultant polypeptides would be controlled by the addition of saccharides.[17] In this paper, we report the synthesis of boronic acid-appended poly(L- and D-lysine)s (L-**1**, D-**1** and L-**2**), the influence of added saccharides on the helix–coil transition of **1** and the chiral orientation of a cyanine dye (**4**) along **1** or **2**. Compound **3** was used as a monomeric reference.



1 *para*
2 *meta*

3



4 (NK-1146)

Results and discussion

Synthesis of 1 and 2

3- or 4-Carboxyphenylboronic acid was converted to its acid chloride by treatment with $SOCl_2$. A THF (tetrahydrofuran) solution containing the acid chloride was added dropwise to aqueous NaOH containing poly(L- or D-lysine) (m_f 70 000–150 000) at 5 °C and the mixture was stirred at room temperature for 12 h. The product was recovered as a precipitate by acidifying the solution with aqueous HCl: yield 82% for L-**1**, 50% for D-**1** and 46% for L-**2**. The boronic acid content was estimated by ^1H NMR spectroscopy and by a reaction with sodium picrylsulfonate.[18] Both results showed that the content of the boronic acid residue is higher than 99 unit mol%. Hence, the polymers' structures can be virtually expressed as in **1** or **2**.

Estimation of pK_a values

First, we estimated pK_a values of the boronic acid groups in **1** and **3** at 25 °C by spectrophotometric titration. The pH was

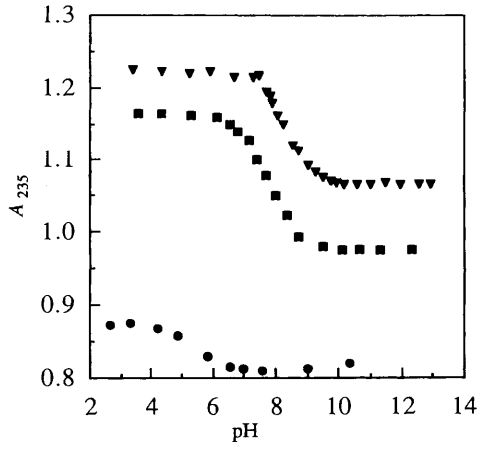


Fig. 1 Spectrophotometric titration of **3** (0.10 mmol dm^{-3}) in the absence (▼) and the presence of *cis*-cyclopentane-1,2-diol (0.010 mol dm^{-3}: ■) and D-fructose (0.10 mol dm^{-3}: ●): 25 °C, μ = 0.010 mol dm^{-3} with NaCl

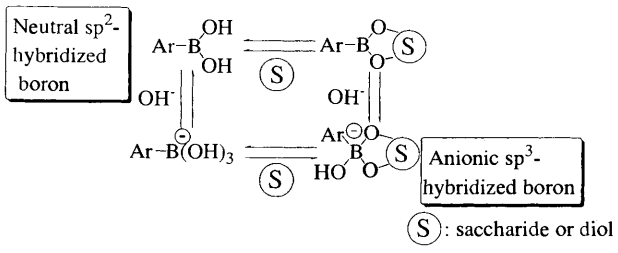


Fig. 2 Acid-dissociation and diol-complexation equilibria of boronic acids

adjusted with aqueous HCl and NaOH. The absorption maxima for neutral species [$ArB(OH)_2$] and anionic species [$ArB^-(OH)_3$] of **3** appeared at 235 and 242 nm, respectively. In Fig. 1 we plot A_{235} against medium pH. From A_{235} *vs.* pH phototitration curves in Fig. 1 the pK_a for **3** was estimated to be 8.34. In the presence of *cis*-cyclopentane-1,2-diol and D-fructose it was lowered to 7.75 and 5.44, respectively. The results are compatible with the previous finding that the pK_a for the boronic acid is lowered when it forms a complex with diols.[10–12,14] The driving force for the pK_a shift can be explained as follows: the acid dissociation and diol complexation equilibria for boronic acids are expressed as in Fig. 2. The boron atom in neutral boronic acids is usually sp^2-hybridized, but when it is involved in a ring structure, the sp^2-hybridized boron is sterically destabilized and tends to be converted to the sp^3-hybridized boron.[12] This situation makes the boron atom in the ring structure more acidic. On the other hand, the pK_a determination for **1** was pretty difficult because of the precipitation at pH < 8. The titration curve for **1** was not saturated even at pH 12. The precipitation also occurred in the presence of D-fructose (0.10 mol dm^{-3}). Only in the presence of *cis*-cyclopentane-1,2-diol (0.010 mol dm^{-3}) could we obtain a regular titration curve, from which the pK_a was estimated to be 10.38. The results indicate that the pK_a of **1** is considerably higher than that of **3**. Polypeptide **1** is classified as an anionic polyelectrolyte. Except at the very early stage of the acid dissociation, the dissociation of the boronic acid group [$ArB(OH)_2 \longrightarrow ArB^-(OH)_3$] must take place under anionic conditions. Hence, the dissociation becomes more and more energetically-unfavourable at a higher pH region. Judging from the pK_a value in the presence of *cis*-cyclopentane-1,2-diol, we presume that the pK_a for **1** is higher by *ca.* 2.6 pK units than that for **3**.

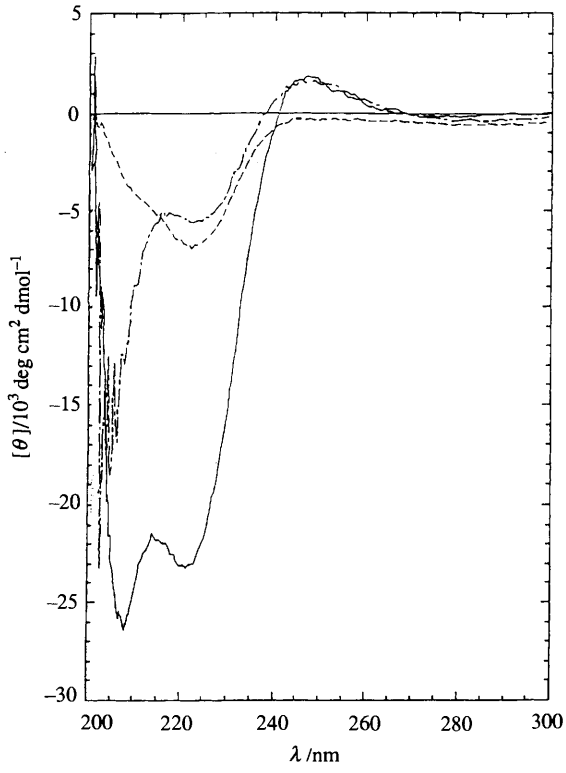


Fig. 3 Typical CD spectra of L-**1** (0.10 monomer unit mmol dm^{-3}) at 25 °C: pH 7.98 – – – –, 9.08 ——, 10.89 — · —

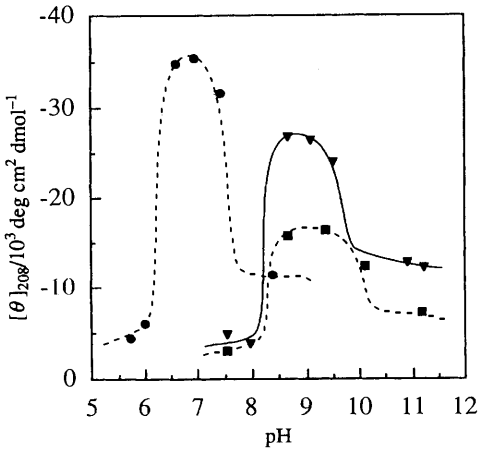


Fig. 4 pH Dependence of $[\theta]_{208}$ of L-**1** (0.10 monomer unit mmol dm^{-3}) at 25 °C: in the absence (▼) and the presence (32 mmol dm^{-3}) of D-fructose (●) or *cis*-cyclopentane-1,2-diol (■)

Influence of added saccharides on the conformational change

The pH-dependent CD change in L-**1** is shown in Fig. 3. A similar spectral change was observed for D-**1** and for those in the presence of *cis*-cyclopentane-1,2-diol or saccharides. The spectral shape is very similar to that of unmodified poly(L-lysine). Furthermore, 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.*, $-\{([\theta]_{208} + 4000)/29\,000\}$.[19] As shown in Fig. 4, the $[\theta]_{208}$ value plotted against pH resulted in a maximum at around pH 9. At the maximum pH the helix content was estimated to be 78%. The $[\theta]_{208}$ decrease at higher pH region is attributed, as in conventional polypeptides,[1] to electrostatic repulsion among negatively charged borate

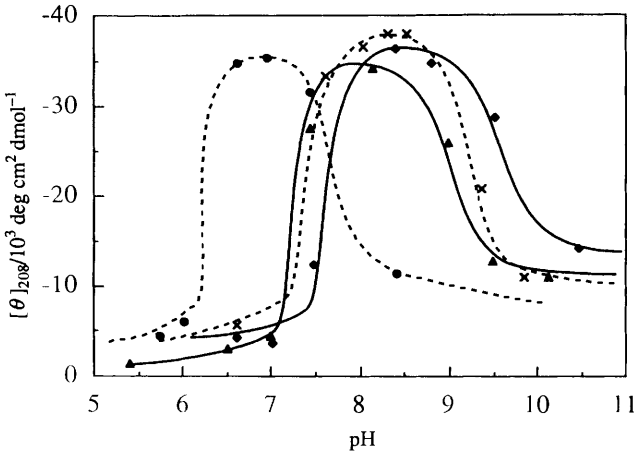


Fig. 5 pH Dependence of $[\theta]_{208}$ of L-**1** (0.10 monomer unit mmol dm^{-3}) at 25 °C in the presence of monosaccharide (32 mmol dm^{-3}): D-fructose (●), D-arabinose (▲), D-galactose (×) and D-glucose (◆)

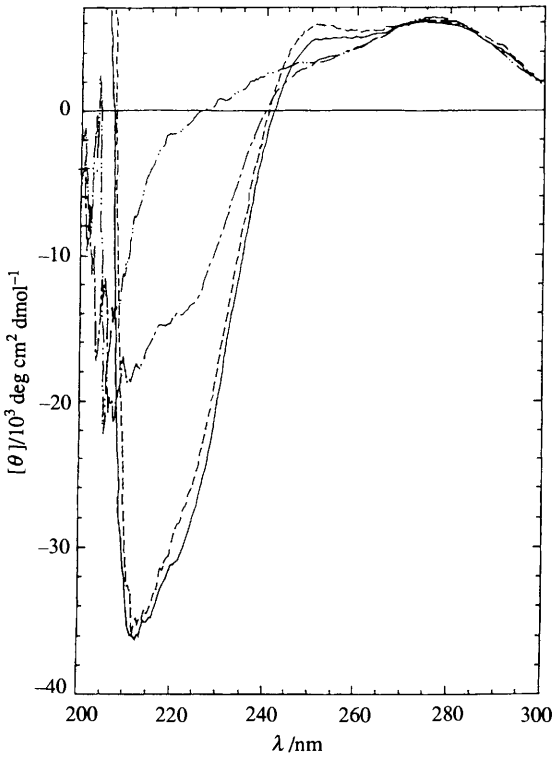


Fig. 6 CD spectra of L-**2** (0.10 monomer unit mmol dm^{-3}) in the presence of D-fructose (32 mmol dm^{-3}) at 25 °C: pH 6.57 – – –, 7.52 ——, 8.57 — · —, 10.44 – · · –

groups. On the other hand, the decrease of $[\theta]_{208}$ at the lower pH region is not well understood at present. L-**1** is barely solubilized at this pH region, so that the helical structure may be disordered by the intermolecular interaction. As shown in Fig. 4, the addition of *cis*-cyclopentane-1,2-diol or D-fructose changed the pH dependence: when *cis*-cyclopentane-1,2-diol was added, the maximum $[\theta]_{208}$ value decreased (helix content 49%) but the maximum pH value did not shift whereas when D-fructose was added, the maximum $[\theta]_{208}$ value increased (helix content *ca.* 100%) and the maximum pH value shifted to 7.0. The basic difference between D-fructose and *cis*-cyclopentane-1,2-diol is that the former boronic acid complex still bears three OH groups whereas the latter boronic acid complex bears no OH group. We consider that the OH···OH hydrogen-bonding interactions among bound D-fructoses stabilize the helical structure and facilitate the creation of anionic charges (*i.e.*, the lower the pK_a because of the facile dissociation of boronic acid groups). Such effects are not expected for *cis*-cyclopentane-1,2-diol.

We found that the maximum $[\theta]_{208}$ value shifts to a lower pH region with increasing monosaccharide concentration. When D-fructose, which has large association constants with phenylboronic acids,[20] was added, the change was already saturated at 32 mmol dm^{-3} whereas when D-glucose, which has relatively small association constants with phenylboronic acids,[20] was added the maximum $[\theta]_{208}$ value continuously shifted to lower pH region and at 1.0 mol dm^{-3} the pH–$[\theta]_{208}$ profile becomes similar to that in the presence of 32 mmol dm^{-3} D-fructose. Hence, we compared the influence of added monosaccharides at 32 mmol dm^{-3} (Fig. 5). It is seen from Fig. 5 that the maximum $[\theta]_{208}$ value shifts to a lower pH region in the order of D-fructose > D-arabinose > D-galactose > D-glucose. This order is consistent with that of the association constants with phenylboronic acids (log K_{ass} values for D-fructose, D-arabinose, D-galactose and D-glucose are 3.64, 2.59, 2.23 and 2.04, respectively).[20] This implies that saccharide having the greater affinity with boronic acid induces the greater maximum pH shift.

L-**1** results in diastereoisomers when the boronic acid residue complexes D- or L-fructose. We considered, therefore, that the helix content may be different between D- and L-fructose. The two pH–$[\theta]_{208}$ profiles are very similar to each other. A slight difference is seen at low pH region where L-fructose can stabilize the α-helical structure more than D-fructose.

The CD spectral shape in L-**2** was similar to L-**1** in the presence of saccharides. It is seen from Fig. 6 that in the presence of D-fructose the CD band at around 220 nm is different from the spectral shape characteristic of the α-helical structure.[1] Judging from the spectral shape,[21] we consider that L-**2** in the presence of D-fructose contains less α-helix.

Chiral orientation of a cyanine dye 4 along the polymer

The foregoing results demonstrate that a negative charge can be generated along polymers **1** and **2** at constant pH only by the addition of saccharides. Ihara *et al.*[22] and others[23,24] previously found that certain ionic dyes are orientated on ionic poly(α-amino acid)s through the interaction between opposite charges. Hence, it occurred to us that cationic dyes would be orientated on **1** or **2** only when the anionic charges are generated by the saccharide addition and the orientation mode would be affected not only by the chirality of **1** or **2** but also by the absolute configuration of added saccharides. If this idea works as expected, it should act as a novel system to arrange dye molecules and also a useful method to detect saccharides in aqueous solution by a spectroscopic method.

We here employed a cationic cyanine dye **4**. Firstly, it was confirmed that the absorption spectrum with λ_{max} 645 nm does not vary at pH 6.4–11.4 (Fig. 7). When L-**1** was added, the absorbance at the λ_{max} decreased with the appearance of a new absorption band at shorter wavelength (λ_{max} 600 nm). This new band is ascribed to aggregated **4** formed on the scarcely-dissociated boronic acid residues because the pK_a of L-**1** is estimated to be higher by one-to-two orders of magnitude than 10.38 (*vide supra*). On the other hand, when D-fructose was added to this solution, the new band further shifted to shorter wavelength (λ_{max} 530 nm) and the 645 nm band was reduced to a weak shoulder. A similar change was also observed for the addition of L-fructose. The results reveal that added fructose generates boronate anions along the polymer and facilitates the aggregation of **4**.

The effects of the D-fructose concentration on the absorption spectra and the ICD spectra are shown in Figs. 8 and 9. In the

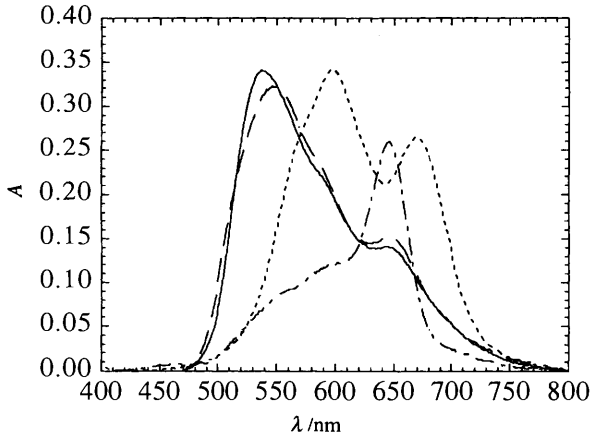


Fig. 7 Absorption spectra of **4** (1.00×10^{-5} mol dm^{-3}) at 25 °C and pH 11.4 (buffered by NaOH): — · — no L-**1**, - - - - L-**1** (1.00×10^{-4} monomer unit mol dm^{-3}), — — L-**1** plus D-fructose (32 mmol), —— L-**1** plus L-fructose (32 mmol)

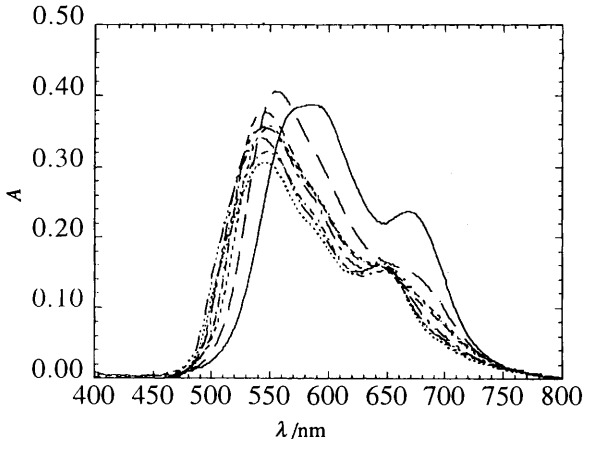


Fig. 8 Absorption spectra of **4** (1.00×10^{-5} mol dm^{-3}) at 25 °C and pH 11.4 as a function of the D-fructose concentration: [L-**1**] = 1.00×10^{-4} monomer unit mol dm^{-3}. [D-fructose] = —— 0, — — 1, - - - 2, - - - - 4, - - - - 8, — — —16, - · - · - 32, — · · · — 67, · · · · 100 mmol dm^{-3}.

absence of D-fructose the perceptible CD band did not appear at the visible region. This implies that although **2** aggregates on L-**1** to give a new absorption maximum at 600 nm, it is not so orientated as to give a CD band. The increase in the D-fructose concentration from 0 to 2.00 mmol dm^{-3} changed the solution colour from light blue to purple. A positive, single CD band appeared at this concentration region. Further increase in the D-fructose concentration did not change the solution colour but in the CD spectral pattern the positive, single CD band was changed to a clear negative exciton-coupling band. These spectral changes suggest that at the low D-fructose concentration the dye molecules can barely be orientated because of the lack of an ample number of boronate anions while at the high D-fructose concentration they can be well orientated because of the presence of an ample number of boronate anions to give an exciton-coupling band: the negative sign is explained by the dipoles in aggregated **4** crossing in an anti-clockwise direction [*i.e.*, (*S*)-chirality]. When the combination of D-**1** and L-fructose which is enantiomeric to the combination of L-**1** and D-fructose was used, the absorption spectra were the same but the CD spectra were symmetrical.

As shown in Fig. 10 the combination of L-**1** and L-fructose also gives a negative exciton-coupling band although the intensity is about half of that for the combination of L-**1** and

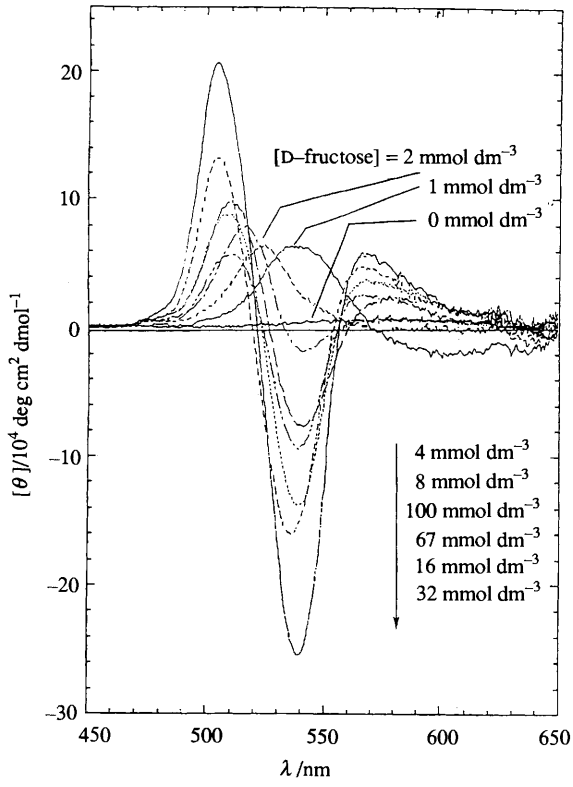


Fig. 9 ICD spectra of **4**. The measurement conditions are similar to those of Fig. 8.

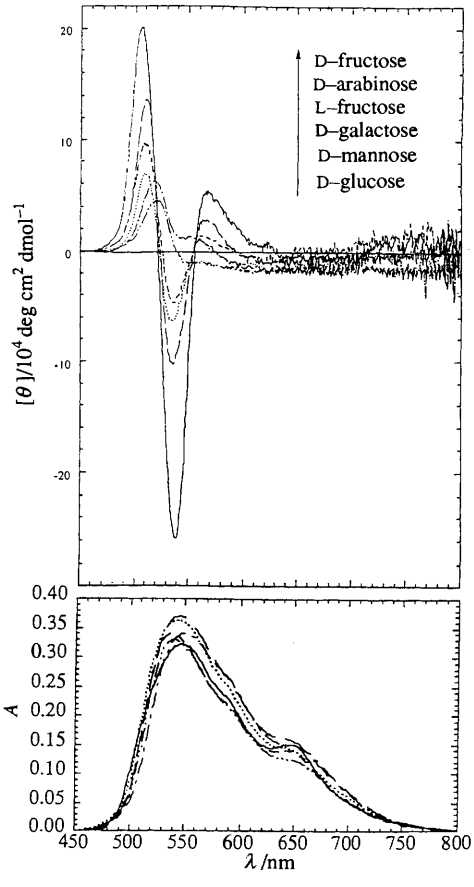


Fig. 10 Absorption and ICD spectra of **4** in the presence of L-**1** and various monosaccharides (32 mmol dm^{-3}): the measurement conditions are similar to those of Fig. 8. —— D-fructose, — — D-arabinose, - - - L-fructose, · · · · D-galactose, — · — D-mannose, - · · - D-glucose

D-fructose. This supports the view that the chirality of **4** is primarily governed by the chirality in the polymer backbone and the chirality of bound saccharides exerts only a secondary influence on the CD spectra. As shown above, the helix content of **1** at the measurement conditions (pH 11.4) is very low and **1** is actually classified into a random conformation. The chiral organization of dyes on poly(α-amino acid)s has been reported by several groups.[22-24] The data show that a helical structure is not necessarily a prerequisite for a chiral organization.[23,24] Presumably, when bulky dyes are bound to a polymer by electrostatic interactions, the polymer becomes too crowded to form a helical structure. However, to accommodate many dye molecules in a limited space around the polymer, the dye molecules are inevitably orientated under the chirality of the polymer backbone.

The absorption and ICD spectra in the presence of various saccharides are illustrated in Fig. 10. Although the absorption spectra are more or less the same, the ICD spectra are markedly different. D-Glucose and D-mannose give a single, positive CD band, similar to that observed for the low D-fructose concentration (Fig. 9). On the other hand, D-arabinose, L-fructose, D-galactose and D-fructose give a negative exciton-coupling band and the intensity increases in this order. As the order of the intensity is parallel to the order of the association constants (except L-fructose)[9-12,14,20] and the sign of the exciton-coupling band is always negative, one can conclude that (*i*) the chirality in **1** primarily governs the chirality in orientated **4**, (*ii*) saccharides are used to generate anionic centres along the polymer chain and (*iii*) chiral discrimination of D *vs.* L operates in the present system, as seen for between D- and L-fructose.

The absorption and CD spectra of **4** in the presence of L-**2** were also investigated. In the absorption spectra the addition of D-fructose caused the shift of the λ_{max} to shorter wavelength. In the CD spectra, on the other hand, the clear exciton-coupling band as observed for L-**1** did not appear. Instead, the L-**2** plus **4** system gave a positive CD band at 525 nm ($[\theta]$ = 60 000 deg cm^2 dmol^{-1} at pH 10.5) in the presence of D-fructose and ($[\theta]$ = 24 000 deg cm^2 dmol^{-1} at pH 10.5) in the presence of L-fructose (the measurement conditions were similar to those recorded in a caption to Fig. 10). The difference implies that the *m*-boronic acid is rather unfavourable to a neat orientation of **4**. When L-fructose was used instead of D-fructose, a similar absorption spectral change was observed but the CD band was weaker than that in the presence of D-fructose. The trend is in line with that obtained from L-**1**.

Conclusions

The present study has demonstrated that the higher-order conformations of poly(L- and D-lysine)s can be controlled by the addition of saccharides if it is appropriately modified with sugar-binding receptors. We believe that this concept is extended to other polypeptides and more generally to other naturally-occurring high polymers such as proteins, DNA, *etc.*, and imitates well the sugar-binding behaviours occurring on the surface of proteins, biomembranes, *etc.*

The chiral organization of dyes has been achieved not only on poly(α-amino acid) chains[22-24] but also on chiral bilayer membranes.[25-27] However, the present system is undoubtedly novel in that cationic dyes are organized on anionic centres developed by saccharide–boronic acid complexation. The diversity of saccharides makes this system more versatile: one can create many sterically different anionic centres by using different saccharides. Further extension to other poly(α-amino acid)s and other dye molecules is currently being investigated in this laboratory.

Experimental

Materials

Hydrobromide salts of poly(L- and D-lysine)s (m_f 70 000–150 000) were purchased from Sigma.

3- and 4-Methylphenylboronic acids (5a and 5b). Compound **5b** was synthesized from 4-bromotoluene (22.4 g, 131 mmol) according to the literature:[28] yield (14.2 g, 80%), mp 247–249 °C (lit.,[28] 245–247 °C); ν_{max}/cm^{-1} [KBr] 1380 (B–O); δ_H([$^2H_6$]DMSO, 25 °C) 7.92 [s, 2 H, B(OH)$_2$], 7.63 (d, *J* 7.8, 2 H, ArH), 7.14 (d, *J* 7.7, 2 H, ArH) and 2.27 (s, 3 H, CH$_3$) [Found: C, 71.3; H, 6.0. C_7H_7BO (calculated as acid anhydride) requires: C, 71.28; H, 5.99%]. It is known that boronic acids are converted to acid anhydrides when they are dried for a long time.[29] Compound **5a** was synthesized from 3-bromotoluene (25.0 g, 146 mmol) according to a similar method: yield (14.1 g, 74%), mp 153–156 °C; ν_{max}/cm^{-1} [KBr] 1330 (B–O); δ_H([$^2H_6$]DMSO, 25 °C) 7.96 [s, 2 H, B(OH)$_2$], 7.58 (m, 2 H, ArH), 7.20 (m, 2 H, ArH) and 2.30 (s, 3 H, CH$_3$) [Found: C, 70.7; H, 5.9. C_7H_7BO (calculated as acid anhydride) requires: C, 71.28; H, 5.99%].

3- and 4-Carboxyphenylboronic acids (6a and 6b). Compound **6b** was synthesized by $KMnO_4$ oxidation of **5b** (6.8 g, 50 mmol) according to the literature:[28] yield (3.0 g, 37%), mp 276–279 °C (lit.,[28] 232–234 °C); ν_{max}/cm^{-1} [KBr] 1300 (B–O), 1670 (C=O) and 3320 (O–H); δ_H([$^2H_6$]DMSO, 25 °C) 12.93 (s, 1 H, CO$_2$H), 8.26 [s, 2 H, B(OH)$_2$], 7.69 (d, *J* 7.8, 2 H, ArH) and 7.14 (d, *J* 7.8, 2 H, ArH) (Found: C, 51.2; H, 4.6. $C_7H_7BO_4$ requires: C, 50.68; H, 4.26%). Compound **6a** was synthesized from **5a** (6.8 g, 50 mmol) according to a similar method: yield (2.8 g, 34%), mp 288–293 °C; ν_{max}/cm^{-1} [KBr] 1300 (B–O), 1670 (C=O) and 3350 (O–H); δ_H([$^2H_6$]DMSO, 25 °C) 12.86 (s, 1 H, CO$_2$H), 8.42 (s, 1 H, ArH), 8.23 [s, 2 H, B(OH)$_2$], 8.00 (m, 2 H, ArH) and 7.47 (t, *J* 7.8, 1 H, ArH) (Found: C, 50.9; H, 4.4. $C_7H_7BO_4$ requires: C, 50.68; H, 4.26).

L-1, D-1 and L-2.† Under a nitrogen stream **6b** (237 mg, 1.43 mmol) was refluxed in thionyl chloride (10 cm^3) containing a few drops of DMF. The reflux was continued for 2 h. The solution was concentrated to dryness *in vacuo*, the residue being dissolved in THF (25 cm^3). Poly(L-lysine) hydrobromide (100 mg, monomer 0.362 unit mmol) was dissolved in an aqueous solution prepared from 1.0 mol dm^{-3} NaOH solution (6 cm^3) and water (10 cm^3). To this aqueous solution cooled at 5 °C the THF solution was added dropwise. After 30 min the solution was warmed up to room temperature. The solution was neutralized (pH 7) with aqueous 1.0 mol dm^{-3} HCl and then concentrated to 10 cm^3. The residual solution was acidified to pH 3, the precipitate thus formed being collected by filtration and washed with water: yield (109 mg, 82%); δ_H(NaOD/D$_2$O, 25 °C) 7.29 (m, 4 H, ArH), 4.00 (br s, 1 H, CH), 2.98 (br s, 2 H, CH$_2$) and 1.10–1.80 (m, 6 H, CH$_2$). D-**1** and L-**2** were synthesized from poly(D-lysine) hydrobromide and **6b** and from poly(L-lysine) hydrobromide and **6a**, respectively: yield (66 mg, 50%) for D-**1** and (61 mg, 46%) for L-**2**; δ_H(NaOD/D$_2$O, 25 °C) 7.75 (s, 1 H, ArH), 7.65 (d, *J* 7.8, 1 H, ArH), 7.35 (d, *J* 7.8, 1 H, ArH), 7.20 (t, *J* 7.8, 1 H, ArH), 4.23 (br s, 1 H, CH), 3.21 (br s, 2 H, CH$_2$) and 1.20–1.85 (m, 6 H, CH$_2$).

4-Propylaminocarbonylphenylboronic acid (3). 4-Propylaminocarbonylphenylboronic acid was synthesized from propylamine (118 mg, 2.0 mmol) and **6b** (300 mg, 1.81 mmol) according to the similar synthetic method used for **1** and **2**: yield (13 mg, 4%), mp 210.1–211.1 °C; ν_{max}/cm^{-1} [KBr] 1530 (N–H), 1620 (C=O) and 2900 (C–H); δ_H(CD$_3$OD, 25 °C) 7.73 (m, 4 H, ArH), 1.62 (m, 4 H, CH$_2$) and 0.97 (t, *J* 7.4, 3 H, CH$_3$).

Integral intensities computed from the ^{1}H·NMR spectra of L-**1**, D-**1** and L-**2** support the view that the ratio can be

† Poly[N^6-(3- or 4-dihydroxyboranylphenylcarbonyl)-D- or -L-lysine]s.

satisfactorily explained by the structures of **1** and **2**. This was further corroborated by the reaction with sodium picrylsulfonate.[18] In this method, the percentage of the unreacted lysine residue could be conveniently estimated by the absorbance of the picrylamine chromophore (λ_{max} 350 nm, ε_{max} 15 000 dm^3 mol^{-1} cm^{-1}).[18] The results showed that the percentage of the unreacted lysine residue is less than 1%.

Miscellaneous

Spectroscopic data were obtained by means of a Bruker 250 MHz FT-NMR (AC-250P) spectrometer for ^1H NMR spectroscopy using tetramethylsilane and sodium 2,2-dimethyl-2-silapentane-5-sulfonate as a reference. IR spectra were recorded on a JASCO infrared spectrophotometer (JASCO A-100). The absorption spectroscopy and circular dichroism were measured by a Shimazu UV–VIS spectrophotometer (UV-160A) and JASCO spectropolarimeter (J-720), respectively.

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