Sugar-controlled association–dissociation equilibria between DNA and boronic acid-appended porphyrin

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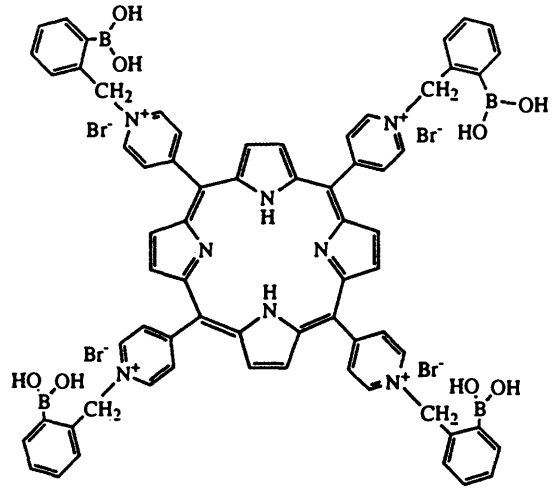
5,10,15,20-Tetrakis{1-[2-(dihydroxyboryl)benzyl]pyridin-1-ium-4-yl}porphyrin tetrabromide 1 has been synthesized to control the DNA-binding properties by physiologically-non-toxic saccharides. At pH 8.0 1 is dicationic and strongly bound to DNA whereas it becomes neutral through saccharide complexation with two boronic acid pendants and the complex is dissociated. Examination with absorption and CD spectroscopy established that poly(dGdC)·poly(dGdC) intercalates 1 whereas poly(dAdT)·poly(dAdT) binds 1 to the outside of the double strand. Since 1 in the presence of calf thymus DNA gave the CD spectrum similar to that in the presence of poly(dAdT)·poly(dAdT), 1 should be bound to the AT region in an outside binding manner. When D-fructose was added, both the absorption and CD spectra changed simply from the 1·DNA complex to 1·D-fructose complex. This indicates that D-fructose can dissociate the 1·DNA complex in a one-step manner. A similar change is observed for D-glucose when the 1 concentration is low, but the dissociation occurred in a two-step manner when the concentration of 1 is high, suggesting the D-glucose-induced reorganization of 1 in DNA. This is a novel method for controlling the DNA-binding properties of porphyrins by saccharides.

Boronic acids can form covalently-bonded complexes with diols (including saccharides) rapidly and reversibly in aqueous solution and therefore are useful as a functional group to design artificial receptors for saccharides.[1-8] It is known that the complexation induces three different changes in the boronic acid function: (i) it becomes more hydrophilic, (ii) the anionic charge (*i.e.* sp^3-hybridized B^-) is developed because of the apparent drop of the pK_a value[1,3-5] and (iii) it becomes chiral. Meanwhile, it is known that positively-charged porphyrins bind oppositely-charged molecules with the aid of electrostatic interactions.[9-14] In particular, the porphyrin–DNA interaction is of great significance from a viewpoint of cancer research and gene technology.[11-14] It thus occurred to us that complexation–decomplexation equilibria in boronic acid-appended porphyrins would be controlled by creation of anionic charges by the binding of saccharides to the boronic moieties. With these systems in mind we synthesized a boronic acid-appended porphyrin **1**. At a pH where two of the four boronic acids are dissociated, they are dicationic overall and should be favourably bound to anionic DNA. On the other hand, the saccharide addition facilitates the dissociation of these two undissociated boronic acids and **1** should become neutral overall. Then, we expected that the porphyrin·DNA complex would be decomplexed. We have already demonstrated that complexation–decomplexation equilibria between **1** and naphthalenedisulfonates or dioxoanthracenedisulfonates can be efficiently controlled by the addition of saccharides.[15] We here report that complexation–decomplexation equilibria between **1** and calf thymus DNA, poly(dAdT)·poly(dAdT) or poly(dGdC)·poly(dGdC) are also controllable by the addition of saccharides although the processes are more complicated than those in the **1**·arenesulfonate system.



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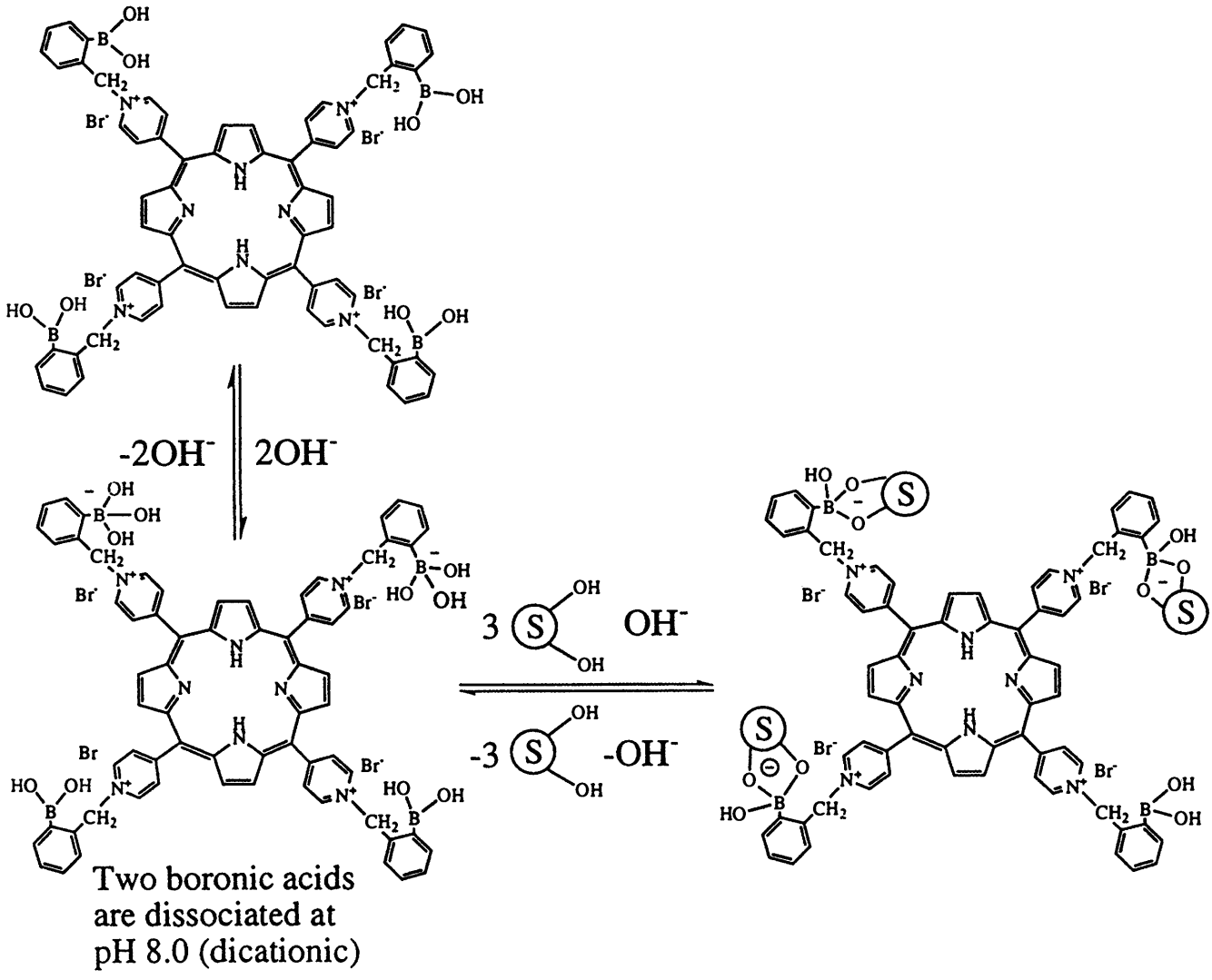
Results and discussion

Porphyrin 1–DNA interactions

A number of investigations on the interaction between porphyrins and DNA have so far been reported.[11-14,16,17] Through these studies it is now possible to classify the binding modes on the basis of absorption spectroscopy and CD spectroscopy. We here employed poly(dAdT)·poly(dAdT) and poly(dGdC)·poly(dGdC) in addition to calf thymus DNA because they show the typical binding modes to positively-charged porphyrins: poly(dAdT)·poly(dAdT) features the outside binding whereas poly(dGdC)·poly(dGdC) tends to intercalate the porphyrins into its minor grooves.[16]

The pK_a values of **1** were determined previously to be 6.94, 7.73, 8.86 and 10.30.[15] We determined the pK_a values of **1** in the presence of D-fructose which shows the highest affinity with monoboronic acids.[1,3-5] The results are summarized in Table 1. We thus adjusted the solution pH to 8.0 with 0.10 mol dm^{-3} phosphate buffer. It is seen from Table 1 that at this pH **1** is dicationic in the absence of D-fructose and changed to a monocationic species in the presence of D-fructose (as shown in Scheme 1). The change in the charge state and the sugar-intensified hydrophilicity should reduce the **1**–DNA affinity.



Scheme 1 denotes saccharide

Table 1 The pK_a values of **1** [a]

D-Fructose/mol dm^{-3}	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
0	6.94	7.73	8.86	10.30
0.1	3.79	4.52	5.30	9.46
0.5	3.33	3.88	4.62	8.13

[a] [**1**] = 1.00 × 10^{-3} mol dm^{-3}, 25 °C, 2.5 vol% MeOH.

The binding mode of cationic porphyrins to DNA can be distinguished by the CD spectral pattern. In the presence of poly(dGdC)·poly(dGdC) the CD spectrum of **1** gave a negative CD band at the Soret band region and the intensity increased with increasing poly(dGdC)·poly(dGdC) concentration (Fig. 1). This indicates that **1** is intercalated into the double strand of poly(dGdC)·poly(dGdC).[13] In the presence of poly(dAdT)·poly(dAdT), on the other hand, **1** gave a positive CD band at a low concentration (< 1.0 × 10^{-6} mol dm^{-3}), which gradually changed into a negative exciton coupling band at higher concentrations (> 1.0 × 10^{-6} mol dm^{-3}) (Fig. 2). This indicates that **1** enjoys the outside binding onto the double strand surface and stacks with each other at its high concentration.[14] Calf thymus DNA should include these different binding modes. As shown in Fig. 3, however, the CD spectrum gave a negative exciton coupling band at the Soret band region. This suggests that **1** is mainly bound to the dAdT region in calf thymus DNA in an outside binding manner and forms the stacking aggregate. This view is also supported by the absorption spectra. The Soret band of **1** is weakened with increasing calf thymus DNA concentration and the λ_{max} shift from 424 to 430 nm. These spectral changes are in line with the formation of the stacking aggregate. The binding constant (K) and the mole number of **1** bound per mole of base pairs (n) were estimated from the Scatchard plot (Fig. 4). The plot consists of two portions but the linear portion at lower concentration region (correlation coefficient 0.98) is meaningful.[11] We obtained K = 1.6 × 10^7 dm^3 mol^{-1} and n = 0.16. These values are slightly greater than those determined for 5,10,15,20-tetrakis(1-methylpyridin-1-ium-4-yl)porphyrin tetraperchlorate: K = 1.1 × 10^7 dm^3 mol^{-1} and n = 0.14.[11]

Effects of added saccharides

As a prelude to the estimation of saccharide addition effects, we carefully checked the absorption and CD spectroscopic changes in **1** in the absence of DNA. Here, we used two 'typical'

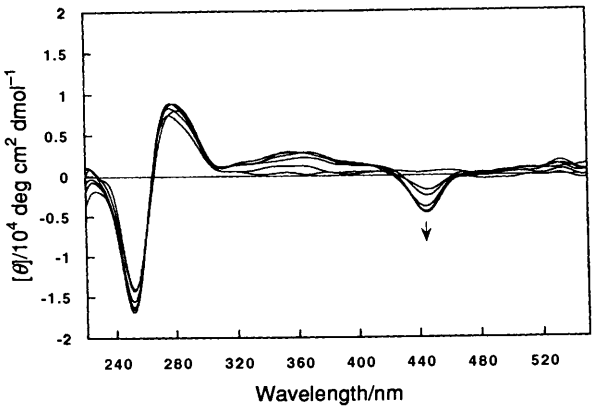


Fig. 1 CD spectral change in **1** (0–8.0 × 10^{-6} mol dm^{-3}) induced by the addition of poly(dGdC)·poly(dGdC) (1.47 × 10^{-5} mol dm^{-3}): 25 °C, water–methanol 300:1 v/v, pH 8.0 with 0.10 mol dm^{-3} phosphate buffer. The ordinate unit is defined as [θ] per base pair mole.

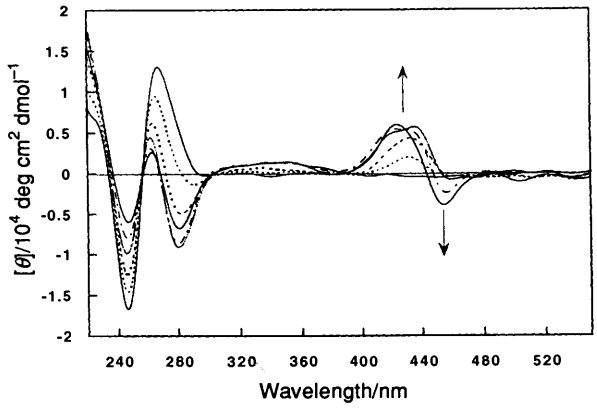


Fig. 2 CD spectral change in **1** (0–8.0 × 10^{-6} mol dm^{-3}) induced by the addition of poly(dAdT)·poly(dAdT) (1.26 × 10^{-5} mol dm^{-3}): the measurement conditions are the same as those in Fig. 1

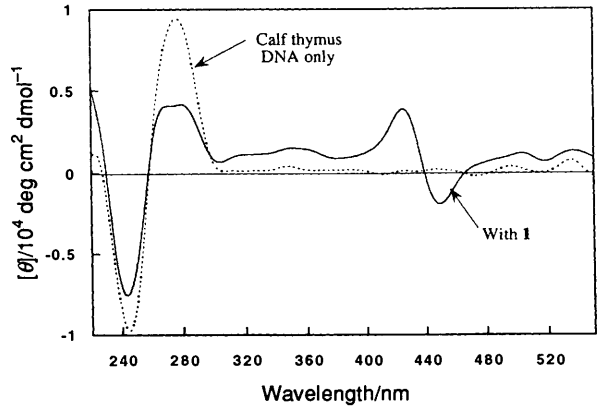


Fig. 3 CD spectral change in **1** (2.5 × 10^{-6} mol dm^{-3}) induced by the addition of calf thymus DNA (1.8 × 10^{-5} mol dm^{-3}): the measurement conditions are the same as those in Fig. 1

monosaccharides, D-glucose and D-fructose: D-glucose has the relatively small association constants for 1:1 D-glucose–boronic acid complexes but tends to form 1:2 complexes which feature the enhanced association constant [18] whereas D-fructose has the largest association constant for 1:1 complexes among monosaccharides.[18] When D-fructose was added to the aqueous solution containing **1**, the absorption spectrum was scarcely changed. On the other hand, when D-glucose was added, the absorbance decreased and the λ_{max} shifted to a shorter wavelength from 424 to 410 nm. A similar D-glucose-induced spectral change was previously found for a boronic acid-appended dye molecule.[19] It was shown that this spectral

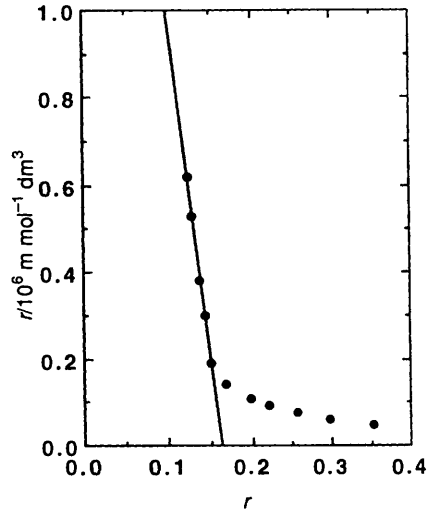


Fig. 4 Scatchard plot for the binding of **1** to calf thymus DNA at pH 8.0

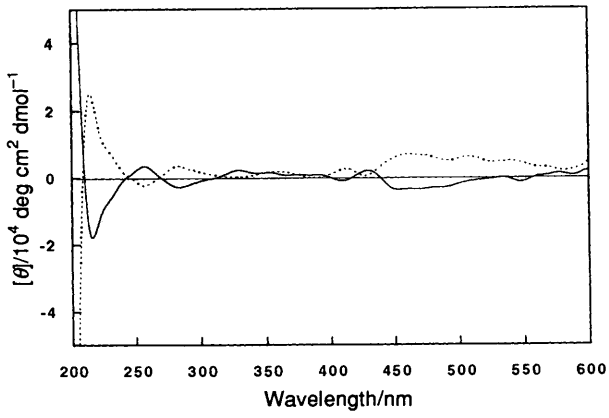


Fig. 5 CD spectra of **1** (8.0 × 10^{-6} mol dm^{-3}) in the presence of D-glucose (——) or L-glucose (- - -). The glucose concentration is 3.0 × 10^{-1} mol dm^{-3} and the measurement conditions are the same as those in Fig. 1, except that in this figure the ordinate unit is defined as [θ] per mol of porphyrin.

change is due to the formation of a 1:2 D-glucose–dye complex, in which the dipole–dipole interaction between the dye molecules induces the shorter-wavelength shift of the λ_{max}.[19] In the 1:2 complex two dye molecules are oriented in the same direction and the complex in aqueous solution is stabilized by the hydrophobic interaction between two face-to-face oriented dye molecules.[19] In the present system, porphyrins *a priori* tend to associate with each other and D-glucose works as a linker to facilitate the intramolecular stacking interaction. The interaction of two glucose-bound porphyrins can be also detected by CD spectroscopy (Fig. 5). Although the CD bands are not very strong, they are reproducible and believable because of the appearance of symmetrical CD spectra given by D- and L-glucose.

The influence of saccharide addition was estimated at two different concentrations of **1** (hereafter shown by R = [**1**]/[DNA base pairs]). When D-fructose was added to the poly(dGdC)·poly(dGdC) solutions containing **1** (R = 0.08 and 0.27), the absorption maximum of **1** shifted to 424 nm with a tight isosbestic point (440 nm). This implies that **1** is pulled out of poly(dGdC)·poly(dGdC) in one step as a **1**·D-fructose complex. The decomplexation is due to the change in the charge of **1** from +2 to 0 (D-fructose complexation changes two neutral boronic acid into two boronate anions) and due to the more hydrophilic nature of the **1**·D-fructose complex than **1**. On the other hand, when D-glucose was added to the R = 0.08 solution, the absorption maximum shifted to 410 nm with an

isosbestic point [422 nm: Fig. 6(*a*)]. This implies that **1** is dissociated from poly(dGdC)·poly(dGdC) in one step as a $(\mathbf{1})_2$·D-glucose complex. Interestingly, when D-glucose was added to the $R = 0.27$ solution, the absorption spectra lost the isosbestic point and changed in a two-step manner [Fig. 6(*b*)]. Conceivably, **1**, discretely bound to poly(dGdC)·poly(dGdC) is reorganized through complexation with D-glucose and finally pulled out of poly(dGdC)·poly(dGdC) as a $(\mathbf{1})_2$·D-glucose complex. This view is also supported by an absorption spectral change. When A_{410} was plotted against added D-glucose concentration, the plot showed the marked decrease at low concentration followed by the gradual increase at high concentration, giving rise to a minimum at 1.0×10^{-3} mol dm^{-3}. These findings for poly(dGdC)·poly(dGdC) are summarized in Scheme 2.

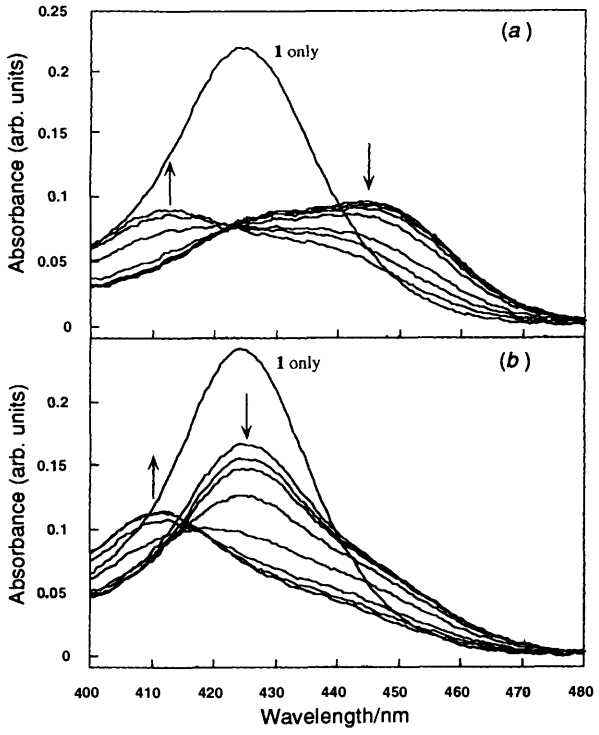
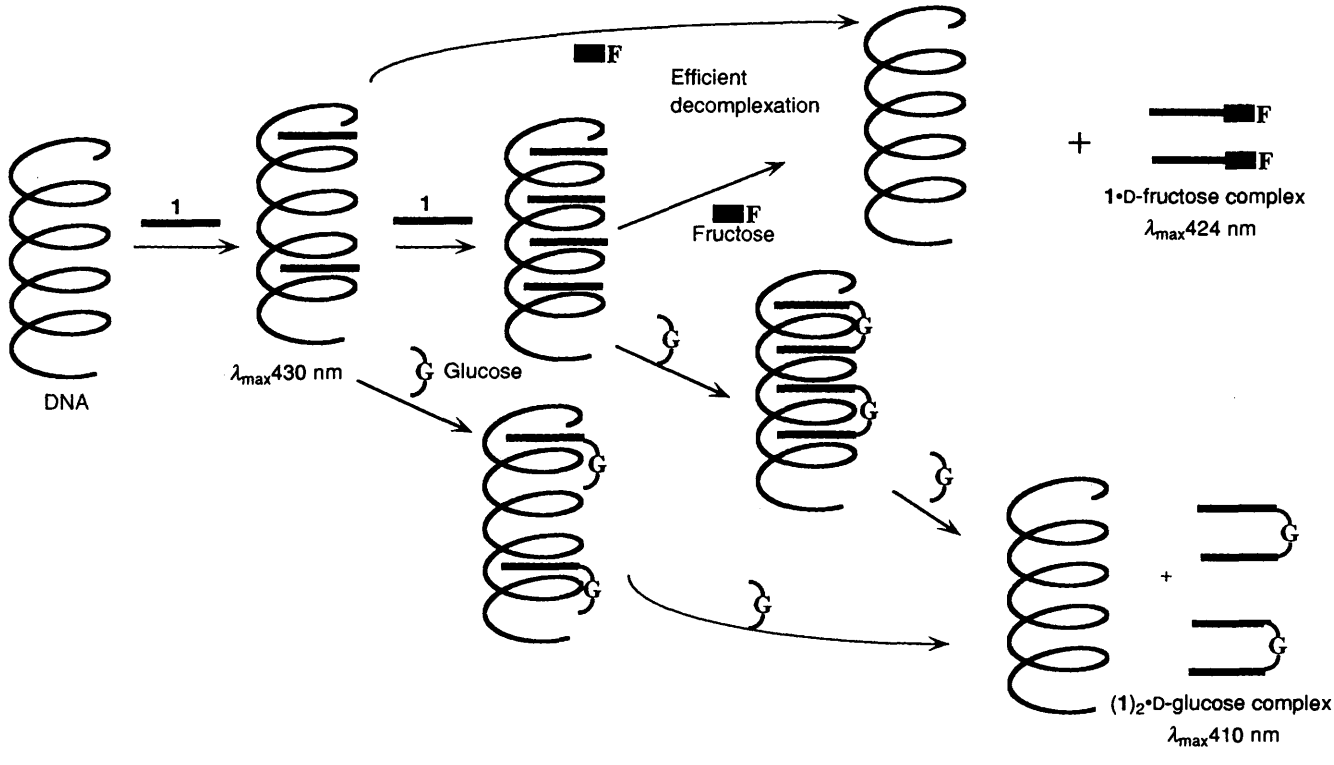


Fig. 6 Absorption spectral change of **1** (2.0×10^{-6} mol dm^{-3}) plus poly(dGdC)·poly(dGdC) (7.4×10^{-6} and 2.5×10^{-5} mol dm^{-3}) induced by D-glucose addition ($0–6 \times 10^{-2}$ mol dm^{-3}): (*a*) $R = 0.08$; (*b*) $R = 0.27$. The measurement conditions are the same as those in Fig. 1.

A sample of poly(dAdT)·poly(dAdT) showed similar spectral changes to those exhibited by poly(dGdC)·poly(dGdC). The spectral change induced by D-fructose addition held a tight isosbestic point (431 nm), indicating that **1** is pulled out of poly(dAdT)·poly(dAdT) in a one-step manner. On the other hand, D-glucose addition gave different spectral changes between $R = 0.06$ [Fig. 7(*a*)] and $R = 0.24$ [Fig. 7(*b*)]: at $R = 0.06$ an isosbestic point appeared at 423 nm, indicating that the dissociation occurs in a one-step manner whereas at $R = 0.24$ it was lost, supporting the occurrence of a two-step dissociation. These findings for poly(dAdT)·poly(dAdT) are summarized as in Scheme 3.

The two-step dissociation of **1** can also be detected by CD spectral changes. For example, **1** in the presence of poly(dAdT)·poly(dAdT) gave a negative exciton coupling bound at $R = 0.24$. When 10^{-4} mol dm^{-3} D-glucose was added, the negative maximum gradually strengthened and shifted to a shorter wavelength [Fig. 8(*a*)]. When the D-glucose concentration was further increased, the exciton coupling band was gradually weakened and finally the CD spectra assignable to the $(\mathbf{1})_2$·D-glucose complex appeared [Fig. 8(*b*)].

Similar spectral changes were also observed for calf thymus DNA. When D-fructose was added, the absorption band at 424 nm increased with increasing D-fructose concentration (Fig. 9). This indicates that **1** bound to calf thymus DNA is pulled out as a **1**·D-fructose complex. A similar spectral change was observed for D-galactose although the efficiency was somewhat inferior to D-fructose. The absorption band at 410 nm which is



Scheme 2 Complexation of **1** with poly(dGdC)·poly(dGdC) and decomplexation induced by D-glucose and D-fructose

assignable to the $(1)_2$•D-glucose complex appeared with the addition of D-glucose. A similar spectral change was also observed for D-xylose although the efficiency was inferior to D-glucose.

Finally, it occurred to us that the calf thymus DNA might possess a chiral recognition ability: that is, the ability to pull

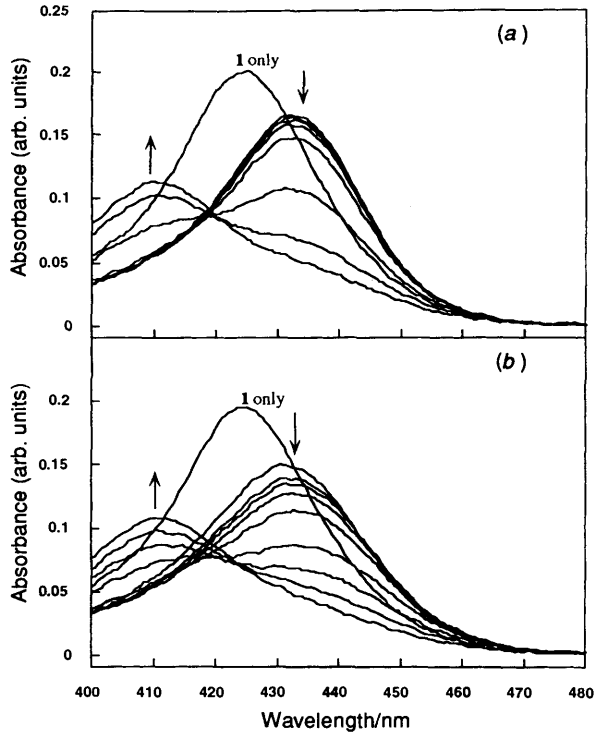


Fig. 7 Absorption spectral change of **1** (2.0×10^{-6} mol dm^{-3}) plus poly(dAdT)•poly(dAdT) (8.3×10^{-6} and 3.3×10^{-5} mol dm^{-3}) induced by D-glucose addition(——): (*a*) = 0.06; (*b*) = 0.24. The measurement conditions are the same as those in Fig. 1.

1 out of calf thymus DNA may be different for D-glucose and L-glucose. When D- or L-glucose was added to the solution containing the **1**•calf thymus DNA complex, the decrease in A_{424} occurred more efficiently with L-glucose than with D-glucose. This implies that the dihedral angle of two porphyrins generated by the D-glucose linker has a higher affinity with the groove of calf thymus DNA than that generated by the L-glucose linker.

Conclusions

We have demonstrated that compound **1** is bound to poly(dGdC)•poly(dGdC), poly(dAdT)•poly(dAdT) and calf thymus DNA in a similar fashion to 5,10,15,20-tetrakis(1-methylpyridin-1-ium-4-yl)porphyrin tetraperchlorate. It has also been shown that by utilizing the saccharide-binding ability of boronic acid moieties and the saccharide-induced creation of anionic charges, one can conveniently control a complexation–decomplexation equilibrium between DNA and **1**. This is a novel method for controlling the DNA-binding properties by physiologically-non-toxic saccharides. Further extension of this work to cover DNA cleavage, foot-printing, *etc.* is currently continued in this laboratory.

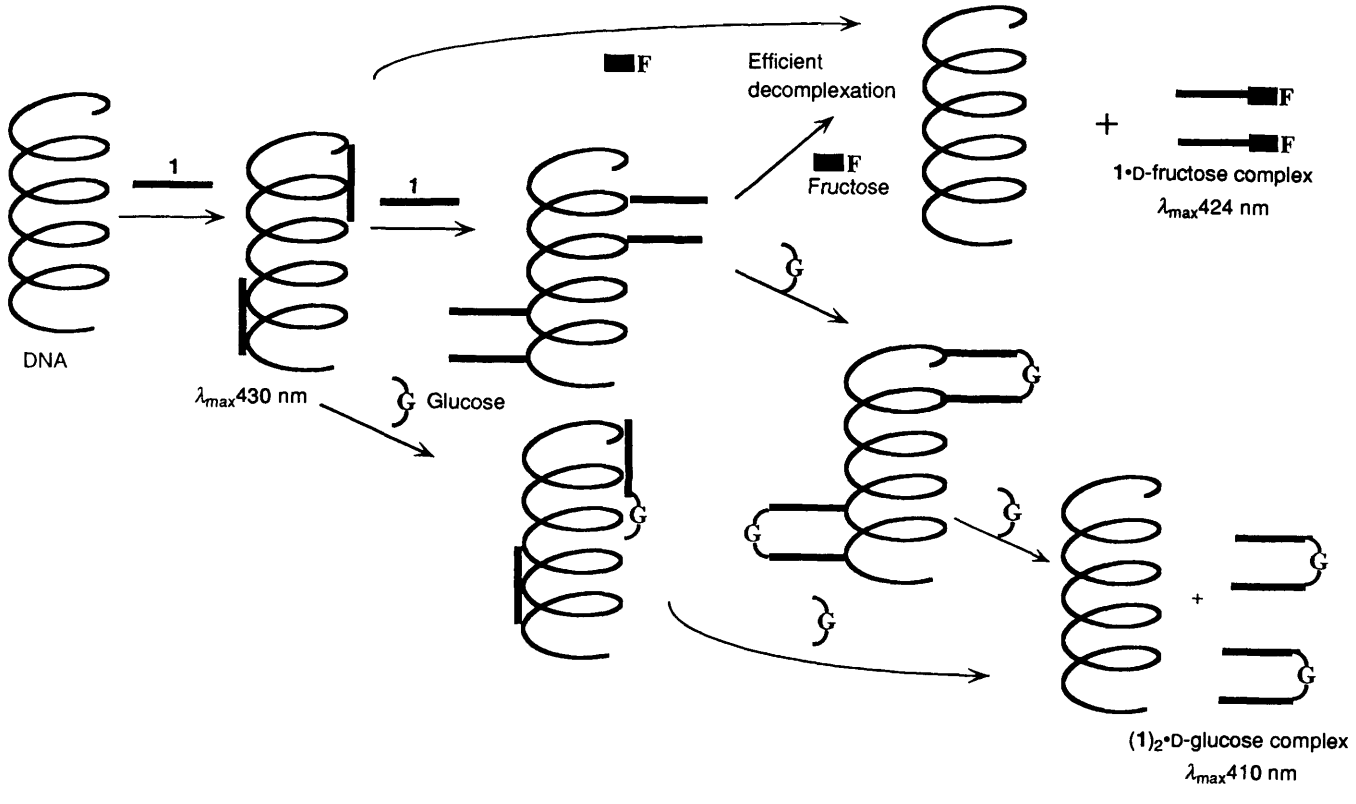
Experimental

Materials

Sonicated calf thymus DNA, poly(dAdT)•poly(dAdT) and poly(dGdC)•poly(dGdC) were purchased from Pharmacia Co. (Inc.).

5,10,15,20-Tetrakis{1-[2-(dihydroxyboryl)benzyl]pyridin-1-ium-4-yl}porphyrin tetrabromide 1

5,10,15,20-Tetra(4-pyridyl)porphyrin (Aldrich Chemical Co. Ltd) (200 mg, 0.32 mmol) and 2-bromomethylphenylboronic acid (700 mg, 3.20 mmol) were dissolved in dry DMF (30 cm^3) and the solution was heated at 80 °C for 24 h. The solution was



Scheme 3 Complexation of **1** with poly(dAdT)•poly(dAdT) and decomplexation induced by D-glucose and D-fructose

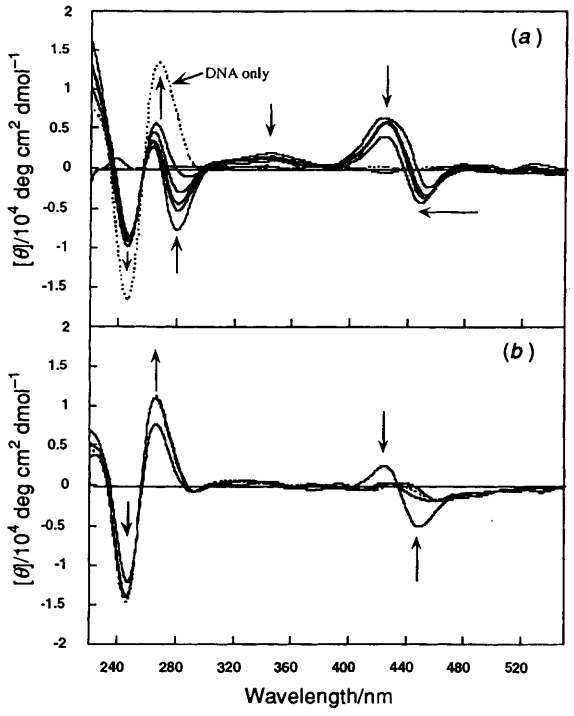


Fig. 8 CD spectral change in **1** (8.0×10^{-6} mol dm^{-3}) in the presence of poly(dAdT)·poly(dAdT) at $R = 0.24$. The D-glucose concentrations are (*a*) 0–1.0×10^{-3} mol dm^{-3} and (*b*) 2.0×10^{-3}–6.0×10^{-2} mol dm^{-3}.

then concentrated *in vacuo* and the oily residue was diluted with acetone. The precipitate thus formed was collected by filtration and dissolved in methanol. The insoluble material was removed by filtration and the filtrate concentrated to dryness. The product was purified by reprecipitation from methanol to acetone, to give a dark purple powder (65%) mp > 350 °C; ν_{max}(KBr disk)/cm^{-1} 3200 (OH), 1340 (B–O); δ_H(250 MHz; CD_3OD; TMS; 27 °C) 6.32 (8 H, s, $ArCH_2$), 7.5–7.9 (16 H, m, ArH), 8.94 and 9.34 (each 8 H, each d, ArH in pyridyl), 9.01 (8 H, br s, β-H) (Found: C, 55.0; H, 4.2; N, 7.2. $C_{68}H_{58}B_4Br_4N_8O_8 \cdot 0.8H_2O$ requires C, 54.72; H, 4.02; N, 7.16%).

Measurements

Measurements were carried out at 25 °C and pH 8.0 with 100 mmol dm^{-3} phosphate buffer. The UV–VIS spectra were recorded at 25 °C on a Shimazu UV-2200 UV–VIS Spectrophotometer. The circular dichroism (CD) spectra were recorded at 25 °C on a Jasco 720 Spectropolarimeter.

pK_a Determination

Determination of pK_a values was carried out in 2.5 vol% MeOH at 25 °C by potentiometric titration. However, it proved difficult to analyse the titration curve for a sample including four dissociation groups. We thus decided to measure the half-titration pH values in the presence of 0.5, 1.5, 2.5 and 3.5 equiv. of NaOH which should correspond to pK_{a1}, pK_{a2}, pK_{a3} and pK_{a4}.

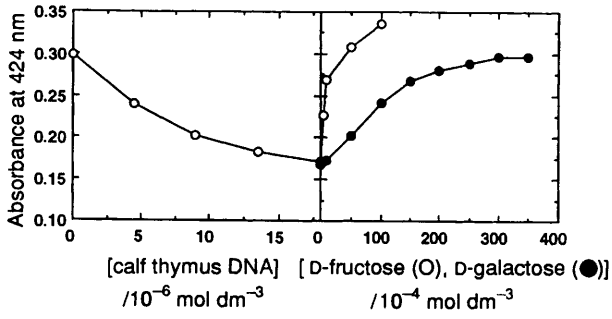


Fig. 9 Plots of A_{424} of **1** (2.0×10^{-6} mol dm^{-3}) against calf thymus DNA concentration (left) and D-fructose or D-galactose concentration in the presence of calf thymus DNA (1.8×10^{-5} mol dm^{-3}) (right)

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