Sugar Sensing Utilizing Aggregation Properties of Boronic-acid-appended Porphyrins and Metalloporphyrins†

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It has been shown that saccharides in water can be sensitively detected by a boronic-acid-appended porphyrin (**1**) and its metal complexes (**2M**$^{2+}$). The idea is based on the phenomenon that absorption and fluorescence spectra of porphyrins change sensitively in response to a shift in the aggregation–deaggregation equilibrium and that the self-association of saccharides with boronic acids in water affects this equilibrium. Among four monosaccharides tested in detail, the spectral change occurs in the order D-fructose > D-arabinose > D-mannose > D-glucose. In **2M**$^{2+}$ the saccharide-binding process is detectable visually through the colour change. The saccharide-induced fluorescence changes are very large because **1** and **2M**$^{2+}$ aggregates are almost non-fluorescent whereas monomeric **1** and **2M**$^{2+}$ generated by saccharide-binding are strongly fluorescent. This is a novel method for sugar sensing, useful in an aqueous system.

The molecular design of artificial receptors which show high affinity and high selectivity comparable to natural systems has recently become a very active area of endeavour. An overview of the past literatures teaches us that hydrogen-bonding interactions are versatilely used for the recognition of guest molecules.[1] We are currently interested in sugar recognition and reading-out of the recognition process.[2–5] Although hydrogen-bonding interactions are also useful for sugar recognition in several systems,[6–8] the effect is exerted only in aprotic organic solvents. Hence, hydrogen-bonding interactions are useless for sugar recognition in water while sugars show significant solubility only in water. How, then, can we *touch* sugars and *recognize* them in water? In an attempt to solve this dilemma we have proposed the use of a boronic acid which self-associatively forms covalent complexes with a variety of sugar molecules in water.[2–5,9–11] Although this strategy is quite different from that employed by nature (using hydrogen-bonding interactions),[1,6–8] this is undoubtedly an expeditious (and probably the best) way to *touch* sugars in water.

Through previous studies we learned that when boronic acid $RB(OH)_2$ forms complexes with sugars, they become more hydrophilic than the starting $RB(OH)_2$. Here, it occurred to us that if $RB(OH)_2$ is appropriately appended to chromophores which tend to aggregate in water, the aggregation–deaggregation equilibrium would be controlled by the concentration, absolute configuration and complex stoichiometry of sugars and the process can be 'read-out' by a colour change: that is, we expected that the boronic acid moiety would act as a 'sugar interface'. With these objects in mind we synthesized a boronic acid-appended porphyrin (**1**) and its metal complexes (**2M**$^{n+}$). Interestingly, we have found that their aggregation properties are influenced by added sugars and the absorption and fluorescence spectra are sensitive to change: that is, *sugars are detectable by a colour change.*

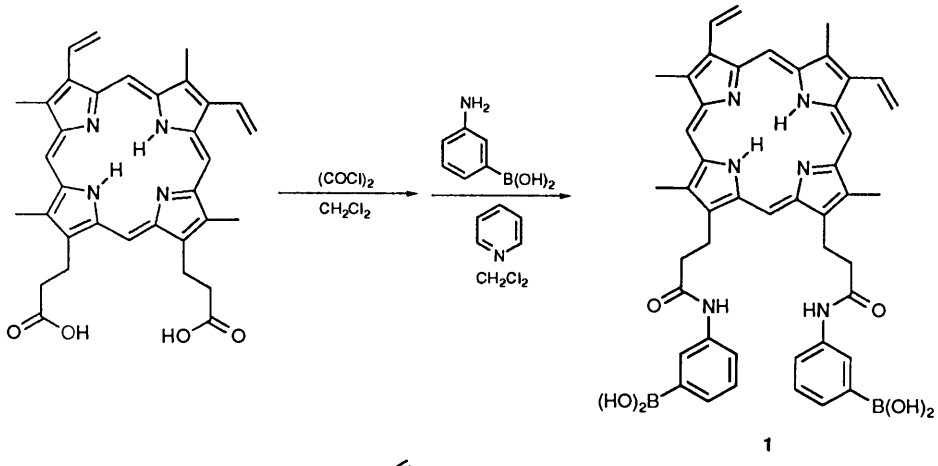
Results and Discussion

Spectral Changes in **1**.—Fig. 1 shows the absorption spectra of **1** in dimethyl sulfoxide (DMSO)–water mixed solvents. The sharp peak (λ_{max} = 405 nm) in DMSO, which is attributed to monomeric **1**, became gradually flattened with increasing water concentration. The broad peak (λ_{max} = 375 nm) is attributable to aggregated **1**. When sodium dodecyl sulfate (SDS: 0.10 mol dm^{-3}) was added to the aqueous solution, the peak became sharp again, indicating that **1** is solubilized discretely in the SDS micelle. The results show that the absorption spectrum of **1** changes in response to a shift of the aggregation–deaggregation equilibrium. Hence, one can expect a similar spectral change if complexation of the appended boronic acids with saccharides induces deaggregation of **1** in water. In general, complexation of boronic acids with saccharides is expressed as in Scheme 1. We measured the absorption spectra of **1** at pH 6.9–11.0 in the absence and the presence of 0.10 mol dm^{-3} D-fructose. The largest spectral change was observed at pH 10.5. We thus employed this pH for the subsequent measurements. Since this pH is higher than the pK_a of boronic acids (*ca.* 9),[10] the change induced by saccharide addition corresponds to the change from $RB^-(OH)_3$ to $R(HO)B^-<\begin{matrix}O\\O\end{matrix}>S$.

In Fig. 2, we show the UV–VIS spectral change induced by added D-fructose. With increasing D-fructose concentration the absorbance gradually increased and the λ_{max} at 375 nm shifted to 380 nm (ε = 8.9 × 10^4 dm^3 mol^{-1} cm^{-1}). According to Inamura and Uchida,[12] protoporphyrin dispersed in basic aqueous solution gives the Soret band at 380 nm (ε = 9.5 × 10^4 dm^3 mol^{-1} cm^{-1}), which is ascribed to the dimer. The coincidence in λ_{max} and ε supports the view that the binding of D-fructose to the boronic acids convert aggregated **1** into dimeric **1**. In Fig. 3, the absorbance at 380 nm is plotted against sugar concentrations. Of the four monosaccharides tested herein, D-fructose showed the largest spectral change. In contrast, the spectrum of protoporphyrin without boronic acids was scarcely changed by the addition of these monosaccharides. The results support the view that deaggregation of **1** is induced by complexation of monosaccharides with the boronic acid moieties which enhances the solubility of **1** in water.

We found that the sugar-binding process can be read-out more sensitively with a fluorescence technique. In the absence of monosaccharides **1** was non-fluorescent because of aggregation (Fig. 4). With increasing monosaccharide concentrations the fluorescence intensity at 632 nm increases conspicuously. Of the four monosaccharides tested herein, D-fructose again showed the largest fluorescence increase (Fig. 5). We have confirmed that even 0.5 mmol dm^{-3} of D-fructose can be detected by this method. In contrast, the fluorescence change in protoporphyrin without boronic acids was hardly induced by the addition of these four monosaccharides.

† Preliminary communication: H. Murakami, T. Nagasaki, I. Hamachi and S. Shinkai, *Tetrahedron Lett.*, 1993, **34**, 6273.



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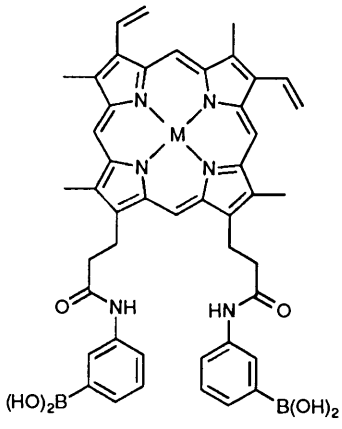
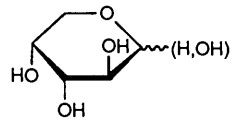
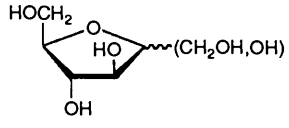


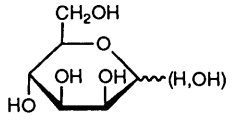
$2M^{2+}$ (M^{2+} = Mg^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+})



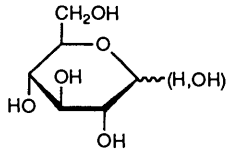
D-Arabinose



D-Fructose



D-Mannose

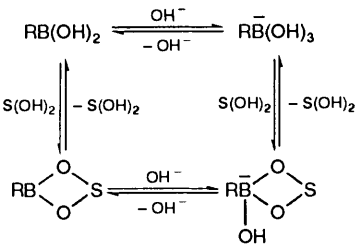


D-Glucose

What is the origin of saccharide-induced spectral changes? The association constants of monosaccharides for boronic acids have been determined.[3,9,10,13] The order of the association constants is exactly in line with the order of the spectral change: *i.e.*, D-fructose > D-arabinose > D-mannose ≥ D-glucose. This implies that complexation of the boronic acids with monosaccharides makes **1** more hydrophilic and the monosaccharide that shows the highest affinity with the boronic acids can induce the deaggregation more effficiently. In other words, the coincidence between the affinity order and the deaggregation order implies that all **1**·monosaccharide complexes (presumably, 1:2 **1**:monosaccharide stoichiometry)* show similar solubility in water.

We also examined the influence of linear-chain saccharides (D-mannitol, D-sorbitol and D-xylitol), disaccharides (D-cellobiose, D-maltose, D-lactose, D-palatinose and D-saccharose) and a trisaccharide (D-raffinose) on the absorption spectra of **1** in water, in the expectation that their complexes would be more hydrophilic than the complexes with four above-mentioned monosaccharides. As shown in Fig. 6, the Soret band increases in the presence of the linear-chain saccharides but the spectral change occurs non-selectively. The magnitude of the spectral change for the linear-chain saccharides appears between D-fructose and D-arabinose. Disaccharides and a trisaccharide (D-raffinose) are mostly ineffective (except D-palatinose). D-Cellobiose and D-maltose are dimers of D-glucose and D-lactose is a dimer composed of D-glucose and D-galactose. As mentioned above, the binding ability of D-glucose is relatively weak. D-Galactose also shows the weak binding ability comparable to D-glucose (data not shown here). Hence, these disaccharides should be bound to the boronic acids to a smaller extent. D-Saccharose and D-raffinose have a D-glucose unit and a furanose unit without a *cis*-diol. These structures also show the weak affinity with the boronic acids. In contrast, D-palatinose displayed a significantly strengthened Soret band

* It is difficult to determine the stoichiometry by the molar ratio method or the continuous variation method because we observe the spectral change including the aggregation–deaggregation process.



Scheme 1 $S(OH)_2$ denotes saccharides

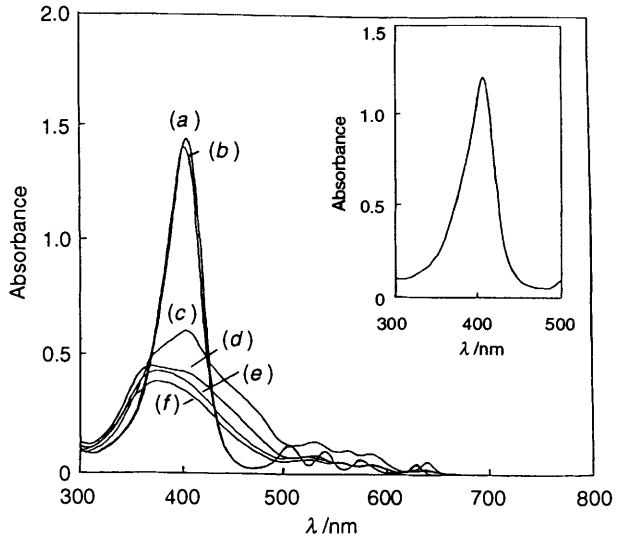


Fig. 1 Absorption spectra of **1** (1.00×10^{-5} mol dm^{-3}) at 25 °C in (*a*) DMSO; (*b*) DMSO–water = 2:1 v/v, (*c*) DMSO–water = 1:1 v/v; (*d*) DMSO–water = 1:2 v/v; (*e*) DMSO–water = 1:30 v/v and (*f*) DMSO–water = 1:300 v/v. The insert spectrum is in DMSO–water = 1:30 v/v in the presence of 0.10 mol dm^{-3} SDS.

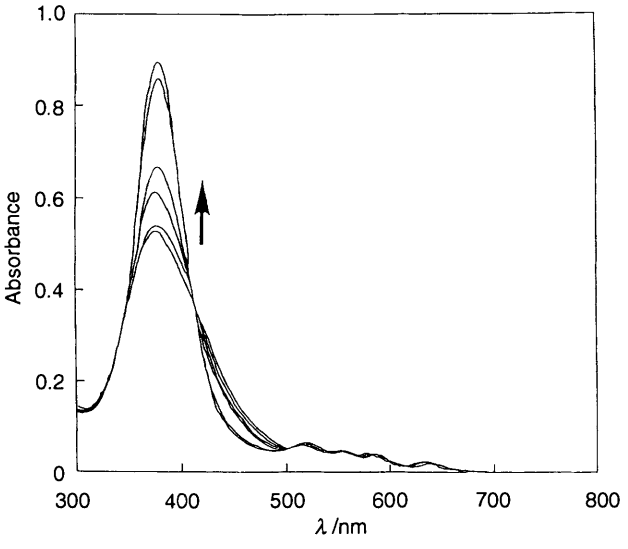


Fig. 2 Spectral change induced by the addition of D-fructose: [**1**] = 1.00×10^{-5} mol dm^{-3}, DMSO–water = 1:30 v/v, pH 10.5 with 0.067 mol dm^{-3} carbonate, 25 °C

(Fig. 6). The affinity with the diboronic acids is ascribed to the furanose unit included in D-palatinose. These results indicate that if the sugar unit which has the large affinity with boronic acids is present as a terminal unit, oligosaccharides can dissociate the aggregate and change the spectrum.

Spectral Change in $2M^{2+}$.—We synthesized metalloporphyrins $2M^{2+}$ (M^{2+} = Mg^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) from **1** and estimated the influence of saccharide addition on their spectral

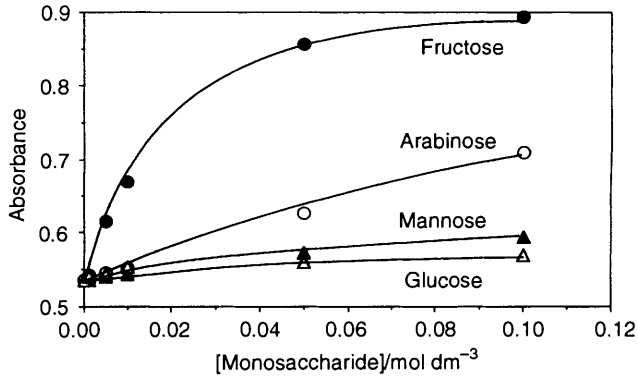


Fig. 3 Absorbance increase at 380 nm plotted against monosaccharide concentration. The measurement conditions are recorded in the caption to Fig. 2.

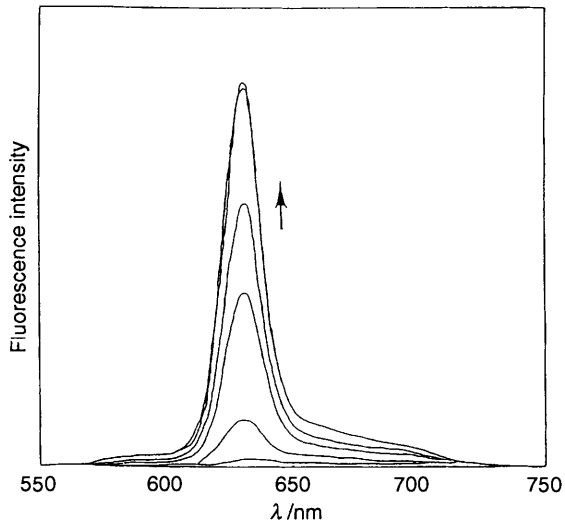


Fig. 4 Fluorescence spectral change induced by the addition of D-fructose: [**1**] = 1.00×10^{-5} mol dm^{-3}, DMSO–water = 1:30 v/v, pH 10.5 with 0.067 mol dm^{-3} carbonate, 25 °C, excitation 415 nm (isosbestic point in Fig. 2). In a preliminary communication (recorded as a footnote on the first page of this article) the wavelength in the abscissa shifts erroneously by 100 nm.

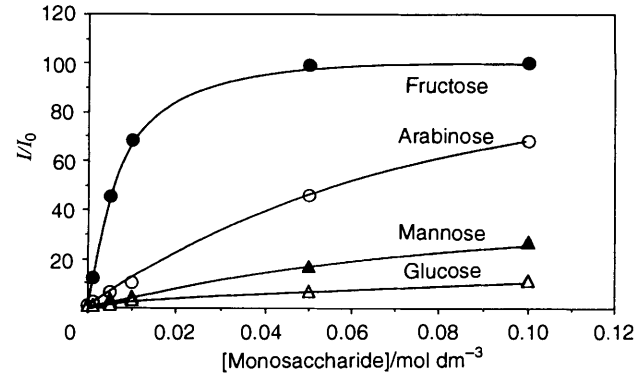
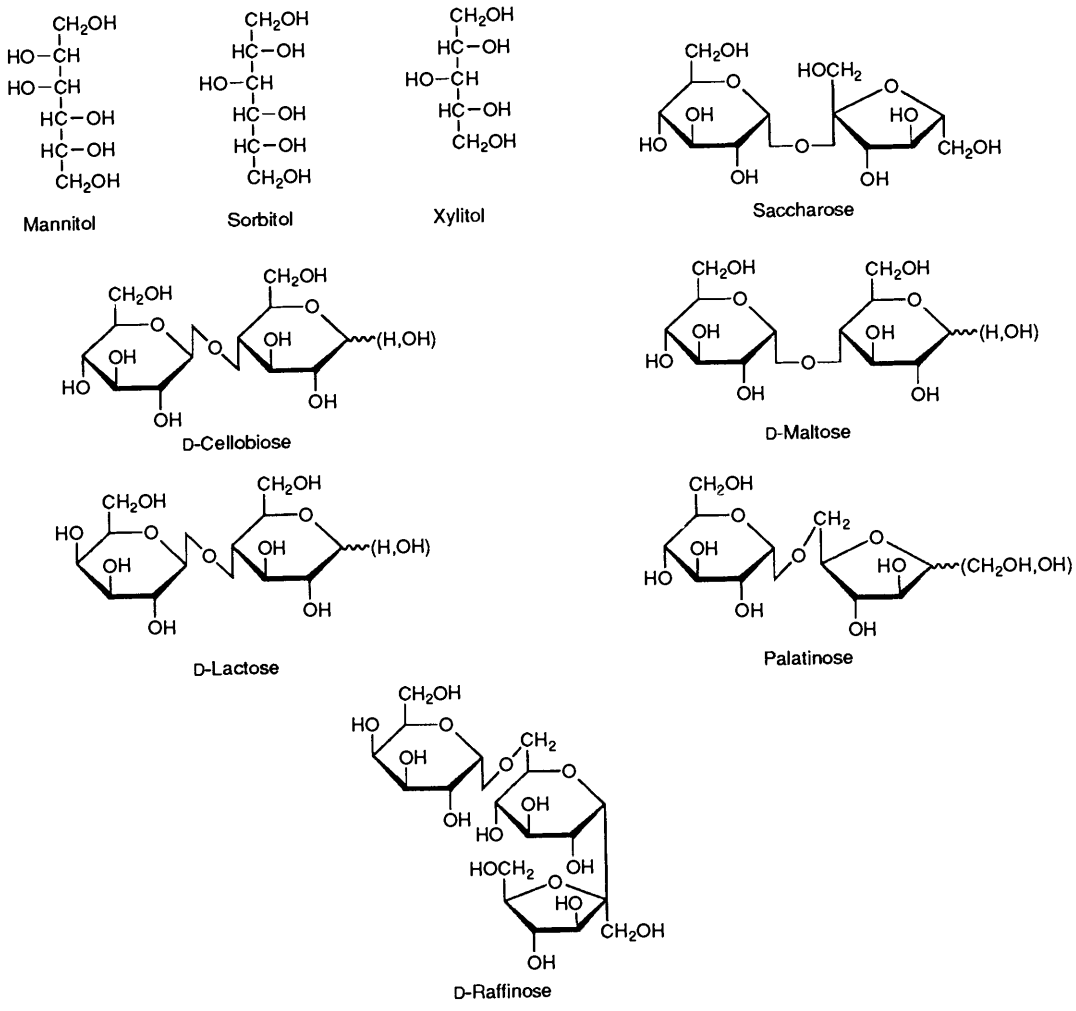


Fig. 5 Fluorescence intensity at 632 nm plotted against monosaccharide concentration. The measurement conditions are recorded in the caption to Fig. 4.

behaviour. Subsequent measurements were made at pH 8.0 where added saccharides induced the largest spectral change. Judging from the pK_a values of boronic acids and their saccharide complexes (*ca.* 9 and 7, respectively),[10] added saccharides should change $RB(OH)_2$ to $R(HO)B^- <^{O}_{O}> S$. The absorption spectra in DMSO and water (pH 8.0) in the presence of SDS micelles are illustrated in Figs. 7–10 and the absorption maxima are summarized in Table 1.



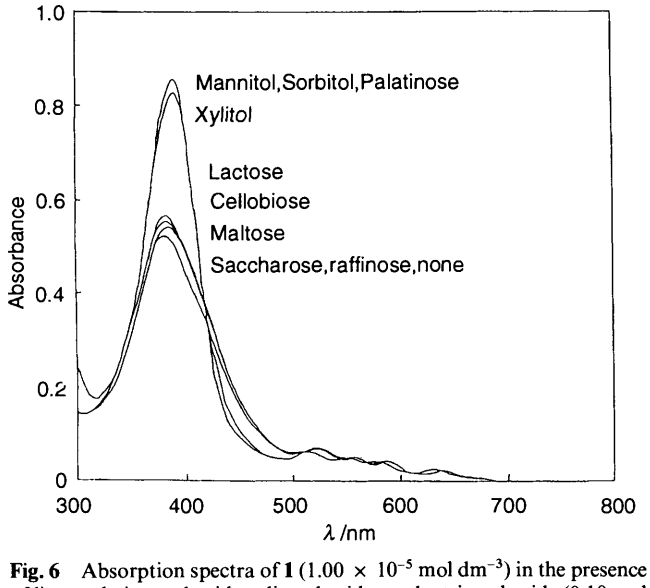


Fig. 6 Absorption spectra of **1** (1.00×10^{-5} mol dm^{-3}) in the presence of linear-chain saccharides, disaccharides and a trisaccharide (0.10 mol dm^{-3}) in water. The measurement conditions are recorded in the caption to Fig. 2.

The absorption spectrum of **2**Mg^{2+} is much broader than those in DMSO and aqueous SDS micelle (Fig. 7). The Soret band (423 nm) shifts to longer wavelength by 5 nm from that in the aqueous SDS micelle. This suggests that **2** Mg^{2+} adopts a J-aggregation mode in water.[14–16] Addition of monosaccharides made the spectral shape sharper. The spectral change occurred again in the order D-fructose > D-arabinose > D-mannose > D-glucose. In particular, the spectrum in the presence of 0.50 mol dm^{-3} D-fructose is very similar to that in the SDS micelle although the Soret band appears at shorter wavelength by 4 nm. Interestingly, the solution colour (yellow) changed to pinkish red in the presence of D-frustose, D-arabinose and D-mannose. One can thus detect the presence of these saccharides visually.

The absorption spectra of **2**Ni^{2+} is shown in Fig. 8. It is seen from Fig. 8(*a*) that the absorption spectrum in the SDS micelle is broader than that in DMSO. This implies that **2**Ni^{2+} is not dispersed as monomers even in the SDS micelle. In aqueous solution the spectrum is very broad and addition of monosaccharides changes the spectra to a smaller extent. The Soret band (387 nm) shifts to shorter wavelength by 11 nm from that in the SDS micelle. These findings indicate that **2**Ni^{2+} adopts a face-to-face orientation and dissociation of this aggregate is energetically difficult. This change in the aggregation mode is similar to that of the Zn^{2+} complex.

As shown in Fig. 9(*a*), **2** Cu^{2+} gives a broad absorption spectrum even in DMSO. The solubility in DMSO is worst among four metalloporphyrins. The results indicate that aggregation of **2**Cu^{2+} occurs very strongly. The absorption spectrum in water is further broadened. The Soret band (393 nm) shifts to shorter wavelength by 10 nm from that in DMSO. In DMSO the Mg^{2+}, Ni^{2+} and Zn^{2+} complexes give a sharp, single peak whereas the Cu^{2+} complex gives a peak with an absorption maximum (403 nm) and a shoulder (393 nm). Since the 393 nm peak is also observed for the Cu^{2+} complex in

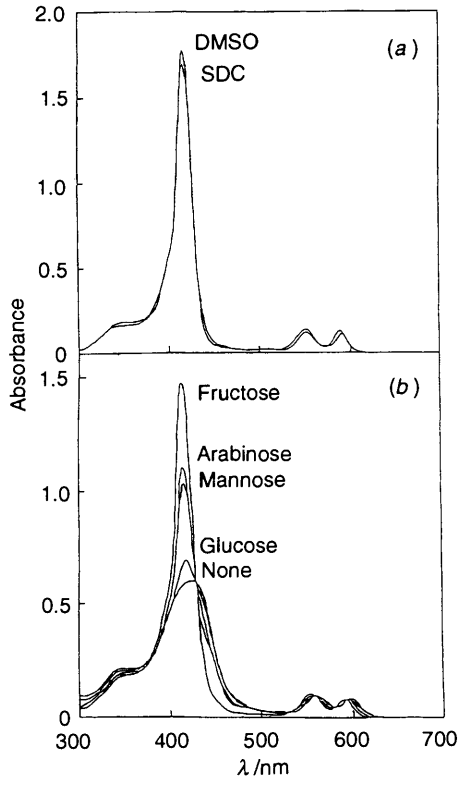


Fig. 7 Absorption spectra of $2Mg^{2+}$ (*a*) in DMSO and aqueous SDS (0.10 mol dm^{-3}) and (*b*) in the presence of monosaccharides (0.50 mol dm^{-3}): $[2Mg^{2+}] = 1.00 \times 10^{-5}$ mol dm^{-3}, DMSO–water = 1:30 v/v, pH 8.0 with 0.067 mol dm^{-3} phosphate, 25 °C

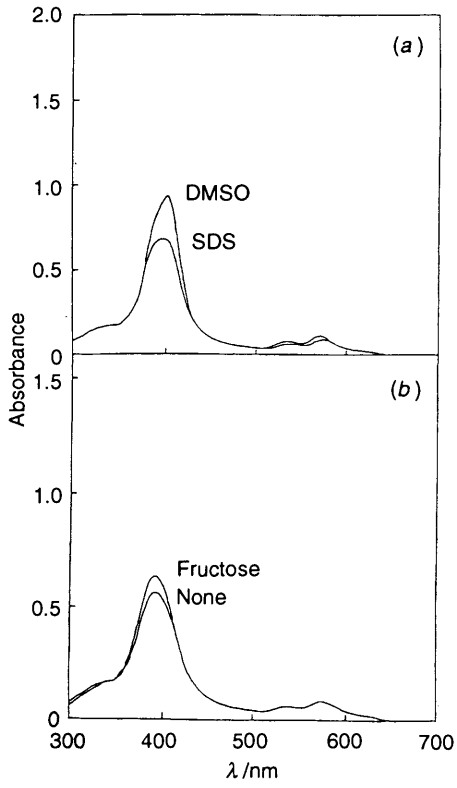


Fig. 9 Absorption spectra of $2Cu^{2+}$ (*a*) in DMSO and aqueous SDS (0.10 mol dm^{-3}) and (*b*) in the presence of fructose (0.50 mol dm^{-3}): $[2Cu^{2+}] = 1.00 \times 10^{-5}$ mol dm^{-3}, DMSO–water = 1:30 v/v, pH 8.0 with 0.067 mol dm^{-3} phosphate, 25 °C

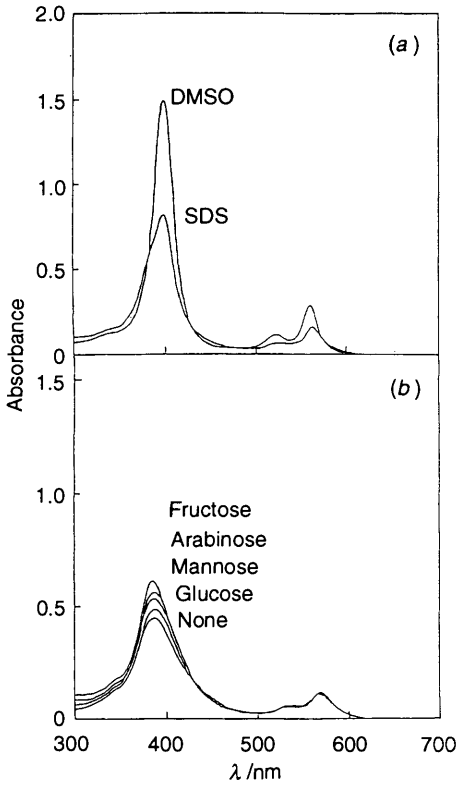


Fig. 8 Absorption spectra of $2Ni^{2+}$ (*a*) in DMSO and aqueous SDS (0.10 mol dm^{-3}) and (*b*) in the presence of monosaccharides (0.50 mol dm^{-3}): $[2Ni^{2+}] = 1.00 \times 10^{-5}$ mol dm^{-3}, DMSO–water = 1:30 v/v, pH 8.0 with 0.067 mol dm^{-3} phosphate, 25 °C

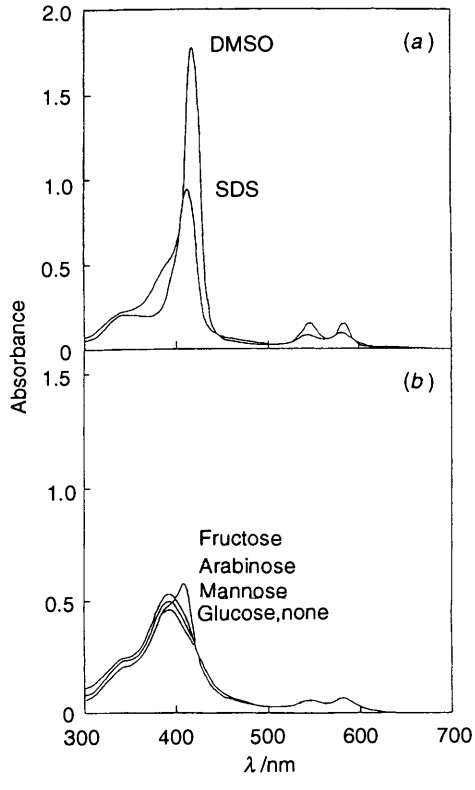


Fig. 10 Absorption spectra of $2Zn^{2+}$ (*a*) in DMSO and aqueous SDS (0.10 mol dm^{-3}) and (*b*) in the presence of monosaccharides (0.50 mol dm^{-3}): $[2Zn^{2+}] = 1.00 \times 10^{-5}$ mol dm^{-3}, DMSO–water = 1:30 v/v, pH 8.0 with 0.067 mol dm^{-3} phosphate, 25 °C

Table 1 Absorption maxima (λ_{max}/nm) and colours of **1** and $\mathbf{2M^{2+}}$ at 25 °C[a]

		Water (pH 8.0)[b]		
Porphyrin	DMSO	0.10 mol dm^{-3} SDS	None	0.50 mol dm^{-3} D-Fructose
1	405	405	375 (yellow)	380 (yellow)
$\mathbf{2Mg^{2+}}$	418	418	423 (yellow)	414 (red)
$\mathbf{2Ni^{2+}}$	398	398[c]	387 (red)	387 (red)
$\mathbf{2Cu^{2+}}$	403[d]	393	393 (yellow)	393 (yellow)
$\mathbf{2Zu^{2+}}$	419	413[e]	394 (yellow)	412 (yellow)

[a] [**1**] or [$\mathbf{2M^{2+}}$] = 1.00 × 10^{-5} mol dm^{-3}. [b] DMSO–water = 1:30 v/v, 0.067 mol dm^{-3} phosphate buffer. [c] A shoulder peak at 387 nm. [d] A shoulder peak at 393 nm. [e] A shoulder peak at 394 nm.

aqueous solution and the Cu^{2+} complex should be more aggregative in aqueous solution than in DMSO, one can assign the 403 nm peak to the monomeric Cu^{2+} complex and the 393 nm peak to the aggregated Cu^{2+} complex. Addition of monosaccharides scarcely changes the spectral shape. The increase in the hydrophilicity induced by the sugar-binding is not enough to disrupt the $\mathbf{2Cu^{2+}} \cdots \mathbf{2Cu^{2+}}$ interaction and disperse them discretely in water.

It is seen from Fig. 10(*b*) that the sugar-induced spectral change in $\mathbf{2Zn^{2+}}$ is quite different from other metalloporphyrins. The Soret band in water (394 nm) shifts to shorter wavelength by 25 nm from that in DMSO and by 19 nm from that in the SDS micelle. This indicates that $\mathbf{2Zn^{2+}}$ aggregate adopts a face-to-face orientation in water. Addition of monosaccharides induced the shift of the λ_{max} to longer wavelength and in particular, addition of D-fructose brought forth a new absorption maximum assignable to monomeric $\mathbf{2Zn^{2+}}$.

The foregoing results indicate that $\mathbf{2Ni^{2+}}$ and $\mathbf{2Cu^{2+}}$ aggregate strongly in water and the increase in the hydrophilicity brought forth by complexation with monosaccharide is not enough to disperse them as monomers whereas $\mathbf{2Mg^{2+}}$ and $\mathbf{2Zn^{2+}}$ are dissociated to monomers though $\mathbf{2Zn^{2+}}$ is more cohesive than $\mathbf{2Mg^{2+}}$. What are the controlling factors for these aggregation properties? The previous studies on metal complexes of tetraphenylporphyrins teach us that in general, Ni^{2+} and Cu^{2+} complexes adopt an 'in-plane' structure whereas Mg^{2+} and Zn^{2+} complexes adopt an 'out-of-plane' structure.[17] In tetraphenylporphyrin complexes with an 'in-plane' structure Ni^{2+} favourably adopts a low spin state and Cu^{2+} has a filled d_{z^2}-orbital, so that they show the poor affinity with an apical ligand. In fact, the absorption spectra of $\mathbf{2Ni^{2+}}$ and $\mathbf{2Cu^{2+}}$ were scarcely affected by added pyridine (0.4 mol dm^{-3}). In contrast, the Mg^{2+} and Zn^{2+} complexes show the moderate affinity with pyridine as an apical ligand, the association constants being 10^2–10^5 dm^3 mol^{-1} for Mg^{2+} and $10^{3.8}$ dm^3 mol^{-1} for Zn^{2+}.[17] In fact, addition of pyridine (0.2 mol dm^{-3}) strengthened the Soret band of $\mathbf{2Zn^{2+}}$ and induced the shift of the λ_{max} from 394 nm to 416 nm. It is now clear from the foregoing consideration that the difference in the aggregation properties can be rationalized in relation to the difference in the porphyrin structure: that is, 'in-plane' complexes, in comparison with 'out-of-plane' complexes, have low affinity for apical ligands and are more cohesive, forming porphyrin stacks.

Since Mg^{2+}- and Zn^{2+}-metalloporphyrins are known to be fluorescent,[18] we investigated the influence of added monosaccharides on their fluorescence spectra (Figs. 11 and 12). As expected from their absorption spectra, the fluorescence intensity was extremely weak. In $\mathbf{2Mg^{2+}}$ the emission maximum is 607 nm, which is the same as that in the presence of the SDS (0.10 mol dm^{-3}) micelle. The result implies that this light is emitted by monomeric $\mathbf{2Mg^{2+}}$: that is a monomer $\rightleftharpoons$ aggregate equilibrium exists in $\mathbf{2Mg^{2+}}$ and the equilibrium is largely inclined to the aggregate. Addition of monosaccharides conspicuously increased the fluorescence intensity (Fig. 11),

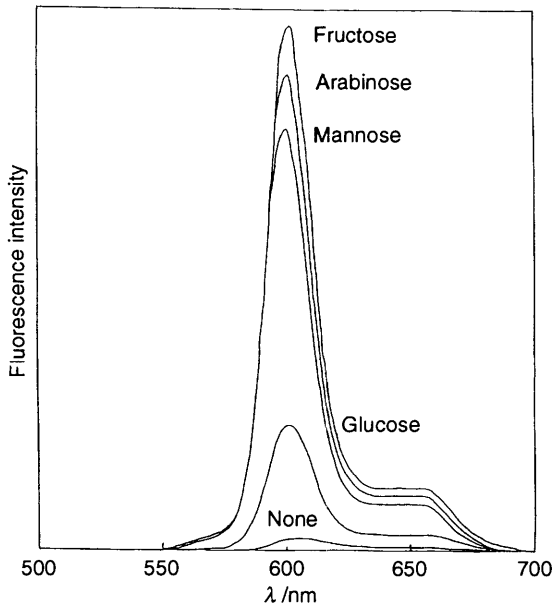


Fig. 11 Fluorescence spectra of $\mathbf{2Mg^{2+}}$ in the absence and the presence of monosaccharides: 25 °C, DMSO–water = 1:30 v/v, pH 8.0 with 0.067 mol dm^{-3} phosphate, [$\mathbf{2Mg^{2+}}$] = 1.00 × 10^{-5} mol dm^{-3}, [monosaccharide] = 0.50 mol dm^{-3}, excitation 427 nm

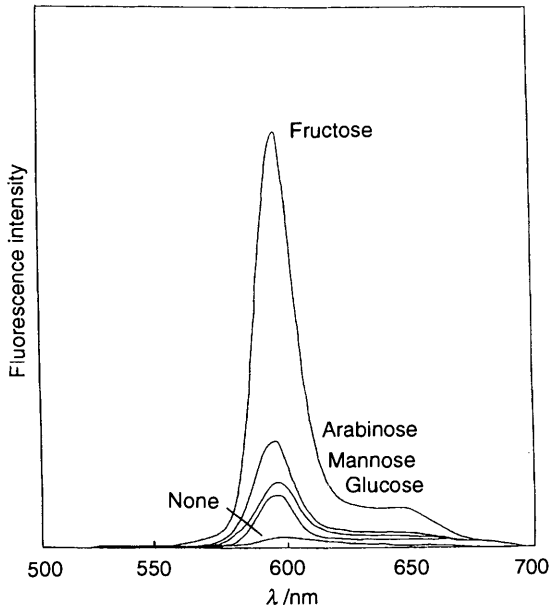


Fig. 12 Fluorescence spectra of $\mathbf{2Zn^{2+}}$ in the absence and the presence of monosaccharides. The measurement conditions are similar to those recorded in the caption to Fig. 11.

indicating that the equilibrium shifts to the monomer side. The fluorescence increase is in the order D-fructose > D-arabinose > D-mannose > D-glucose. A similar trend was observed for

2Zn^{2+}. In this case the emission maximum appears at 597 nm which is the same as that in the SDS (0.10 mol dm^{-3}) micelle. These results show that **2**Mg^{2+} and **2**Zn^{2+} are useful as fluorophores for sensing saccharides in water.

Conclusions

The present study demonstrates that saccharides in water can be sensitively detected spectrophotometrically by utilizing the aggregation properties of **1** and **2**M^{2+}. In some cases the saccharide-binding process is detectable visually through the colour change. The change in the fluorescence spectra is particularly large because **1** and **2**M^{2+} aggregates are almost non-fluorescent because of concentration quenching whereas monomeric **1** and **2**M^{2+} generated by the saccharide-binding are strongly fluorescent. However, the selectivity order obtained so far is always the same. We believe that a selectivity change will be attained by a structural modification of the two boronic acids.

Experimental

Procedure for the Synthesis of Protoporphyrin IX 13^3, 17^3-*Bis*(3-*amidophenylboronic acid*) (**1**).—Protoporphyrin IX (250 mg, 0.44 mmol) was suspended in dry dichloromethane (30 cm^3) and treated with oxalyl chloride (0.5 cm^3) and dimethylformamide (DMF) (0.01 cm^3). The mixture was stirred in the dark for 1 h. The concentration of the reaction mixture under reduced pressure yielded the protoporphyrin bis(acid chloride) as dark green solid, which was used immediately without further purification.

The bis(acid chloride) in dry dichloromethane (10 cm^3) was added dropwise to a solution of dry dichloromethane (7 cm^3) and dry pyridine (3 cm^3) containing 3-aminophenylboronic acid (680 mg, 4.4 mmol). The mixture was stirred in the dark for 10 h. The solvent was removed under reduced pressure and MeOH (50 cm^3) was added to the residue. The precipitate (**1**) was recovered by filtration and reprecipitated from $CHCl_3$–MeOH (280 mg, 82.8%), m.p. > 300 °C; δ_H(250 MHz; [$^2H_6$]DMSO; 298 K; Me_4Si) −3.90 (2 H, s, NH), 3.40, 4.50 (4 H, t, 13^1, 17^1, 13^2, 17^2, CH_2), 3.64, 3.66, 3.72, 3.74 (3 H, s, CH_3), 6.22, 6.45 (2 H, d, *J* 3.8, =CH_2), 7.21, 7.45, 7.77, 7.81 (2 H each, q, d, d, s, ArH), 7.97 (4 H, s, OH), 8.52 (2 H, m, =CH–), 9.99 (2 H, s, amide-H), 10.24, 10.27, 10.29, 10.39 (1 H, s, methine-H) (Found: C, 67.2; H, 5.9; N, 10.0. $C_{46}H_{46}B_2N_6O_6 \cdot H_2O$ requires C, 67.5; H, 5.9; N, 10.3%).

Metallation Procedure.—The Mg^{2+} complex was synthesized as follows. **1** (200 mg, 0.25 mmol) and $MgClO_4$ (200 mg, excess) were suspended in dry pyridine (20 cm^3) and refluxed for 2 h. The reaction was followed by absorption spectroscopy and TLC (SiO_2; $CHCl_3$–MeOH 10:1 v/v). The reaction mixture was cooled to room temperature and aqueous 1 mol dm^{-3} HCl was added (100 cm^3). The precipitate was separated by filtration and washed with MeOH (3 cm^3) to give **2**Mg^{2+} (reddish purple powder, 130 mg, 63%), m.p. > 300 °C (Found: C, 63.6; H, 5.6; N, 9.7. $C_{46}H_{44}B_2N_6O_6Mg \cdot 2.5H_2O$ requires C, 63.7; H, 5.7; N, 9.7%).

Ni^{2+}, Cu^{2+} and Zn^{2+} complexes were synthesized as follows. **1** (200 mg, 0.25 mmol) and $MClO_4$ (M = Ni^{2+}, Cu^{2+} and Zn^{2+}, 200 mg, excess) were suspended in dry DMF (20 cm^3) and stirred for 24 h at room temperature (in the case of $NiCl_2$, the reaction mixture was stirred for 5 h at 50 °C). The reaction was followed by absorption spectroscopy and TLC (SiO_2; $CHCl_3$–MeOH 10:1 v/v). The solvent was removed under reduced pressure and water (30 cm^3) was added to the residue. The precipitate was separated by filtration and washed with MeOH (3 cm^3) to give **2**M^{2+} (M^{2+} = Ni^{2+}, Cu^{2+} and Zn^{2+}). **2**Ni^{2+} (reddish purple powder, 110 mg, 51%), m.p. > 300 °C (Found: C, 63.1; H, 5.1; N, 9.4. $C_{46}H_{44}B_2N_6O_6Ni \cdot H_2O$ requires C, 63.1; H, 5.3; N, 9.6%). **2**Cu^{2+} (reddish purple powder, 135 mg, 63%), m.p. > 300 °C (Found: C, 60.1; H, 4.9; N, 8.9. $C_{46}H_{44}B_2N_6O_6Cu \cdot 3H_2O$ requires C, 60.3; H, 5.5; N, 9.2%). **2**Zn^{2+} (reddish purple powder, 140 mg, 65%), m.p. > 300 °C (Found: C, 57.8; H, 5.1; N, 8.8. $C_{46}H_{44}B_2N_6O_6Zn \cdot 5H_2O$ requires C, 58.0; H, 5.7; N, 8.8%).

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