Sugar-Controlled Aggregate Formation in Boronic Acid-Appended Porphyrin Amphiphiles

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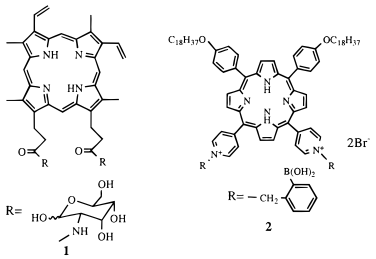
Synthetic amphiphilic compounds can organize in aqueous media as fibers, lamellas, vesicular membranes, etc.[1−10] Oriented molecular aggregates of amphiphilic porphyrins are of particular interest for their photoactive and redox-active functions. As pointed out by Fuhrhop et al.,[11] one may consider the porphyrin macrocycle as a unit for such assemblies as a rigid hydrophobic box with special packing properties. Furthermore, porphyrin-based amphiphiles have an advantage in that their aggregation properties can be conveniently monitored not only by electron micrographic observations but also by spectroscopic methods through the change in their characteristic Soret and Q bands. A few groups have already found that porphyrin-based amphiphiles can form stable oriented aggregates in aqueous media, and electron micrographic observations have proved that in most cases they tend to grow up as fibrous assemblies.[11−14] More recently, Fuhrhop et al.[15,16] demonstrated that a glucosamide group used as a hydrophilic group in an *N*-octylglucosamide acts as the determining factor in helical structure formation. This concept has been amalgamated with porphyrin-based amphiphiles.[11,12] A glycosamide derivative of protoporphyrin IX (**1**) can form stable aggregates in aqueous media: although only fibers (but not the expected helical superstructures such as helices and twisted ribbons) were observable by electron micrographic, it was considered on the basis of their CD-active properties that these fibers should also contain a helical structure.[11,12]

We have recently been interested in the development of new sugar recognition methods useful in aqueous media.[17−19] We have demonstrated the validity of the boronic acid function as a saccharide receptor site in saccharide recognition of rigid matrices.[17−20] It occurred to us that boronic acid-appended, amphiphilic porphyrins may create superstructures upon simply mixing the porphyrins with saccharides in aqueous media and that the resultant superstructure may reflect the absolute configuration of the added saccharides.[21] With these objectives in mind, we synthesized a new amphiphilic porphyrin, **2**. Two pyridyl groups and two octadecyl groups are introduced to balance hydrophilicity with lipophilicity, placed at the 5,10- and 15,20-positions, respectively, to separately create the hydrophilic and the lipophilic sites in **2**. The boronic acid groups were introduced ortho to the pyridylmethyl groups to facilitate the formation of anionic boronate ester complexes with saccharides owing to the electrostatic interaction.[19d] Spectroscopic, light-scattering, DSC, and electron micrographic studies have established that **2** can form stable fibrous aggregates in aqueous media only in the presence of saccharides, which are possibly "twisted", reflecting the absolute configuration of added saccharides.



Compound **2** was synthesized from 4-(octadecyloxy)benzaldehyde, 4-pyridinecarboxaldehyde, and pyrrole via 5,10-dipyridyl-15,20-bis[(4-(octadecyloxy)phenyl]porphyrin. The product (mp 214−215 °C) was identified by IR and ^{1}H NMR spectroscopy and elemental analysis.

The absorption spectra of **2** (1.00×10^{-5} M) were measured in water−methanol mixtures at 25 °C. The extinction coefficient at the Soret band (λ_{max} 429 nm, $\epsilon = 1.71 \times 10^5$ M^{-1} cm^{-1} in 100% methanol) decreased with increasing water concentration and reached a constant value above 50 vol % water ($\epsilon = 1.26 \times 10^5$ M^{-1} cm^{-1}). The results suggest that **2** aggregates in mixed solvents containing over 50 vol % water. The extinction coefficient at the Soret band (0.3 vol % methanol) decreased in 0.1 M carbonate buffer (pH 10.0, λ_{max} 432 nm, $\epsilon = 0.85 \times 10^5$ M^{-1} cm^{-1}) or in the presence of D-fructose (1.00×10^{-2} M, λ_{max} 432 nm, $\epsilon = 0.79 \times 10^5$ M^{-1} cm^{-1}). This change can be attributed to the aggregation induced by charge neutralization by CO_3^{2-} added or B^- developed upon saccharide addition. The Q bands decreased simultaneously. This view is also supported by the light-scattering experiments. Although **2** (1.00×10^{-4} M) was not dissolved homogeneously in aqueous solution (0.3 vol % methanol) adjusted to pH 10.0 with 0.10 M carbonate buffer,[22] sonication with monosaccharides (5.00×10^{-2} M D-arabinose, D-fructose, D-fucose, D-glucose, and D-threitol) resulted in homogeneous solutions. The average particle size (diameter) determined by a light-scattering method was 203−324 nm (the smallest particle with 203 nm for D-fucose and the largest particle with 324 nm for D-glucose). As the molecular size of **2** is ∼4.2 nm, these particles should consist of high molecular weight aggregates of **2**.

Further evidence for oriented aggregate formation was obtained from DSC measurements in the presence of D- and L-fructose.[23] In the first scan, no endothermic peak was observed. From the second scan onward, an endothermic peak reproducibly appeared at 39 °C for both fructoses. The ΔH values (64 kJ mol^{-1} for D-fructose and 63 kJ mol^{-1} for L-fructose) were

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(21) We first tested this idea with boronic acid-appended protoporphyrin IX.[19a,b] Although spectroscopic evidence for the formation of molecular aggregates was obtained, they were not so stable as to be detected by electron micrography.[19a,b]
(22) Because of the similar solubility problem, we could not measure DSC and TEM in the absence of saccharides.

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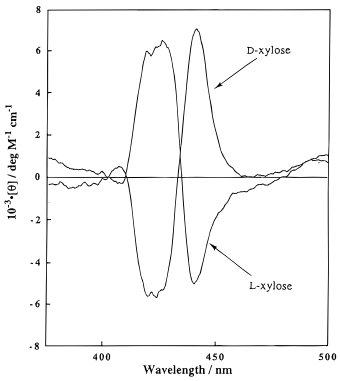

Figure 1. CD spectra of **2** (2.00×10^{-5} M) in the presence of D- or L-xylose (1.00×10^{-2} M): 25 °C, 0.3 vol % methanol, pH 10.0 with 0.10 M carbonate buffer.

Table 1. Correlation between the Absolute Configuration of Monosaccharides and the Sign of Exciton Coupling Bands

mono-saccharide	direction of 2-OH	λ_{max} and λ_{min} ([θ]) of Cotton effects[a]	
		first	second
D-fructose	up	453 (-6.9×10^3)	426 ($+1.0 \times 10^4$)
L-fructose	down	453 ($+5.7 \times 10^3$)	425 (-5.7×10^3)
D-xylose	down	454 ($+7.1 \times 10^3$)	427 (-5.7×10^3)
L-xylose	up	454 (-5.0×10^3)	427 (-6.4×10^3)
D-glucose	down	443 ($+9.2 \times 10^3$)	410 (-4.0×10^3)
L-glucose	up	443 (-5.1×10^3)	410 ($+3.4 \times 10^3$)
D-arabinose	up	454 (-4.6×10^3)	422 ($+8.1 \times 10^3$)
D-ribose	down	457 ($+5.8 \times 10^3$)	432 (-2.1×10^4)
D-fucose	down	457 ($+8.5 \times 10^3$)	430 (-1.6×10^4)

[a] 0.3 vol % methanol, pH 10.0, with 0.10 M carbonate buffer, [**2**] = 2.00×10^{-5} M, [monosaccharide] = 1.00×10^{-2} M.

comparable with those for oriented aggregates of amphiphiles containing aromatic nuclei.[3] Hence, this peak is attributable to the gel−liquid crystal phase transition temperature.

The molecular orientation in porphyrin-based aggregates is conveniently monitored by CD spectroscopy.[11,12,19] In the absence of saccharides, **2** was CD-silent. As shown in Figure 1, **2** gave a positive exciton coupling band [with the first positive Cotton effect at λ_{min} 454 nm ([θ]$_{min}$ $+7.1 \times 10^3$ deg M^{-1} cm^{-1}) and the second negative Cotton effect at λ_{max} 427 nm ([θ]$_{max}$ -5.7×10^3 deg M^{-1} cm^{-1})] in the presence of D-xylose and a negative exciton coupling band in the presence of L-xylose. We measured the CD intensity of **2** (2.00×10^{-5} M) as a function of xylose concentration: the CD intensity increased with increasing xylose concentration and became almost saturated around [xylose]/[**2**] = 300. Conceivably, all boronic acid groups are converted to the xylose complexes above this concentration ([xylose]/[**2**] = 300−1000). We also measured linear dichroism (LD) under the same measurement conditions, but a perceptible response was not obtained. The results clearly indicate that two enantiomers of fructose induce the chiral orientation in the **2** aggregate in opposite directions. Similar exciton coupling bands were also observed for other monosaccharides (Table 1). Careful examination of Table 1 reveals that there exists a consistent correlation between the absolute configuration of monosaccharides and the sign of exciton coupling bands: without exception (at lease for the six monosaccharides tested herein), positive-sign monosaccharides possess a downward-directed 2-OH group (relative to the furanose or pyranose ring), whereas negative-sign monosaccharides possess an upward-directed 2-OH group. This correlation suggests that the boronic acid groups in **2** form a cyclic boronate ester with the 1,2-diol group of monosaccharides[24] which eventually governs the chiral orientation in the porphyrin−porphyrin stacking aggregates.

When the CD spectra were measured as a function of medium temperature, the [θ] values were unaffected below 39 °C, whereas they decreased with the temperature rise above 39 °C.

(23) [**2**] = 1.00×10^{-3} M and [fructose] = 0.50 M in water−methanol, 15:1 v/v (pH 10.0 with 0.10 M carbonate buffer). The methanol concentration is higher than the standard concentration (i.e., water−methanol, 300:1 v/v) in order to carry out the measurement at the higher **2** concentration.

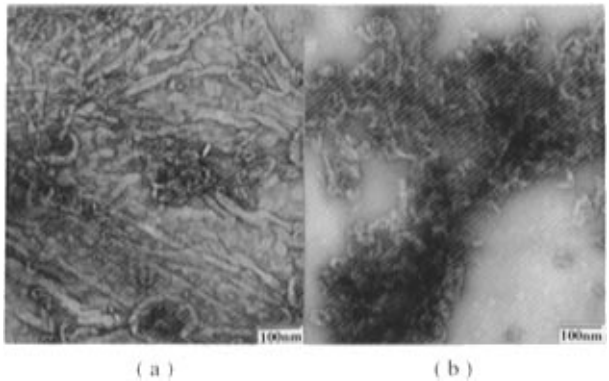

Figure 2. Electron micrographs (TEM) of **2** in the presence of (a) D-fructose and (b) D-ribose. For the sample preparation method, see text. Compound **2** gave a picture similar to (a) in the presence of D-glucose and similar to (b) in the presence of D-fucose.

The results indicate that the chirally-oriented superstructures with the large [θ] values are immobilized in the gel phase, whereas they are disordered by thermal molecular motion in the liquid crystal phase.

Finally, we observed the aggregation mode of **2**−monosaccharide complexes with TEM, expecting that the absolute configuration of monosaccharides composing the hydrophilic moiety of amphiphiles is reflected in the aggregation morphology.[8] The sample solutions for TEM observation were prepared as follows: an aqueous solution (water−methanol, 15:1 v/v; pH 10.0 with 0.10 M carbonate buffer) containing **2** (1.00×10^{-3} M) and monosaccharide (5.00×10^{-1} M) was sonicated with a probe-type sonicator (S&M Model VC-50) for 30 s[25] and then mixed with an aqueous solution containing 2 wt % uranyl acetate.[26] As shown in Figure 2, **2** gave well-developed fibrous (or tubular[26]) aggregates in the presence of D-fructose or D-glucose. Judging from the CPK molecular model of **2** with an extended conformation for two octadecyl groups, the long axis of this molecule is ∼4.2 nm. Since the diameter of the fibrous aggregates is ∼10 nm, they should be constructed from radial orientation of **2** with hydrophobic octadecyl groups inside and hydrophilic boronic acid groups outside.[26] On the other hand, **2** gave less-developed coagulated fibrous aggregates in the presence of D-ribose or D-fucose (Figure 2).

In conclusion, the present study demonstrated that the morphology of orientated aggregates in aqueous media can be controlled by adding saccharides. This concept well imitates the morphological functions of certain cell membranes, the surfaces of which are covered by saccharides. In this system, the boronic acid group plays a crucial role in composing the hydrophilic moiety of the amphiphile: saccharides are bound to the boronic acid sites and induce chiral orientation to the aggregate, which is determined by the absolute configuration of saccharides. Undoubtedly, this is a novel method for the control of aggregate morphology by physiologically-nontoxic saccharides. The results suggest further potential applications and extensions of the present system to the saccharide control of porphyrin-mediated photoreactions and redox reactions, selective absorption of guests onto the aggregate surface, selective membrane transport, etc.

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(25) In Furhop's porphyrin system it took 3 months for **1** to grow as fibrous aggregates. In our system, 30 s sonication reproducibly resulted in the oriented aggregates.

(26) Since the addition of uranyl acetate before sonication changes the solution pH and affects the boronic acid−monosaccharide complexation equilibrium, this method cannot be used. However, even though uranyl acetate was added after sonication, a shadowy string was observed in the center of a fibrous aggregate (see Figure 2). This suggests that the aggregates are tubular hollow fibers.