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Sensitive Detection of Saccharides by an Amphiphilic Phenylboronic Acid at the Air-Water Interface in the Presence of Quaternized Amines

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The detection of mono- and disaccharides by monolayers of *m*-hexadecyloxyphenylboronic acid at the air-water interface becomes very sensitive if the boronic acid is located in a cationic environment provided by a water-soluble quaternized polymeric amine or a quaternized amphiphilic amine.

In this work we continue our studies on sugar recognition by boronic acids,[1-4)]a phenomenon which was first observed on boronic acids in polymer matrices.5) As described previously,1) monolayers of *m*-hexadecyloxyphenylboronic acid **1** are influenced upon covalently binding mono- and disaccharides. However, the concentration limit above which sugars influence the monolayer of the monoboronic acid is high compared with the very sensitive spectroscopic detection of mono- and disaccharides by diboronic acids.2-4) The efficient complexation with boronic acids requires the boron atom to be sp^3-hybridized (-B(OH)$_3^-$) which is achieved at high pH. In this work we report how to facilitate sp^3-hybridization by interaction of the boronic acid monolayer with quaternized amines. This allows for the first time a very sensitive detection of sugars at the air-water interface on the basis of covalent bonding. 6)

The synthesis of compound **1** was described previously.1) Poly(4-vinylpyridine) was quaternized with methyl iodide in acetonitrile/ethanol. The poly(4-vinyl-N-methylpyridinium) iodide, abbreviated PVI, was washed with acetonitrile and recrystallized from water. The degree of quaternization was 100% as determined by ^{1}H-NMR. The average molecular weight as measured by static light scattering was 2500 before quaternization. The concentration of PVI in the subphase was 0.01 wt%. Saccharides (Fluka, for microbiology) and dimethyldioctadecylammonium bromide (Sogo Pharmaceutical Co.) were used without further purification. Water was ion exchanged and bidistilled (Barnstead). A modified FSD-20 type film balance on an air-suspended table in a clean-air zone was used for measuring π-A isotherms at 293 ± 0.1 K and a barrier speed of 36 cm^2/min. The maximum molecular area was 0.81 nm^2 for **1** and benzene as spreading solvent was allowed to evaporate within 10 minutes. UV-reflection spectra during compression were measured with a MCPD-110 type spectrometer (Otsuka Electronics) equipped with a photodiode-array detector, a deuterium lamp and optical fibers. UV-absorption spectra of **1** in MeOH/H$_2$O were measured with a UV-2200 type spectrometer (Shimadzu). All solutions were freshly prepared. The stoichiometry of the complex between **1** and D-glucose at the air-water interface was determined by using an M-2500 mass spectrometer (Hitachi) in SIMS$^-$-mode. A build-up film of 80 layers of **1** on a subphase containing 11 mM D-glucose at pH 11 (carbonate buffer) was prepared by vertical dipping of a steel plate with a modified surface. The initial transfer ratio of 1.0 in the up-stroke mode decreased subsequently. A peak at a m/z-value of 532 corresponds to the double-charged, hydrated complex consisting of two sp^3-hybridized molecules of **1** bridged by one sugar molecule (Scheme 1). This is the same composition as determined by ^{1}H-NMR in homogeneous solution.1)

Figure 1 depicts typical π-A isotherms of **1** at pH 10.0 (0.04 M carbonate buffer) when the subphase contains D-fructose at various concentrations and the polycation PVI. The area occupied by one boronic acid anion in the absence of sugars (*A'*) is smaller when the subphase contains PVI, which reduces electrostatic repulsion, compared with the area in the absence of PVI. Upon the addition of sugars to the subphase the molecular area (*A''*) and the compressibility of the monolayer increases. The influence of PVI on the hybridization state of the boron atom can be seen in the UV-spectra of **1**, depicted in Fig. 2. The absorption peaks below 250 nm decrease with increasing amounts of PVI in the neutral solution. The same spectral change can be achieved by increasing the pH. This proves that the polycation facilitates sp^3-hybridization to anionic $-B(OH)_3^-$. In case of the monolayer, a similar spectral change is observed in the UV-reflection spectra (not depicted) of compound **1** upon adding PVI, though an absorption peak from PVI below 255 nm overlaps the spectrum of **1**.

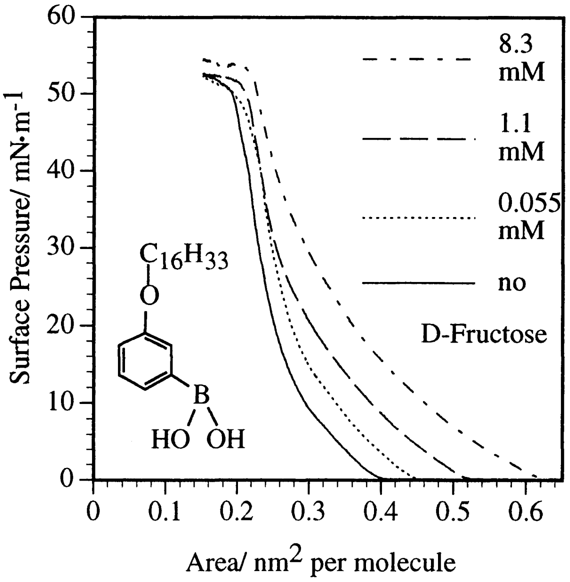


Fig. 1. Typical pressure-area isotherms of monoboronic acid **1** at pH 10.0 and 293 K on a subphase containing 0.01 wt% PVI and D-fructose.

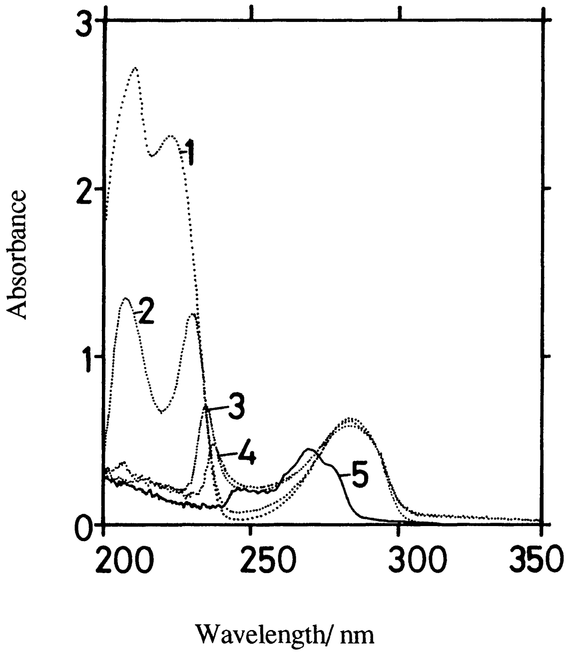
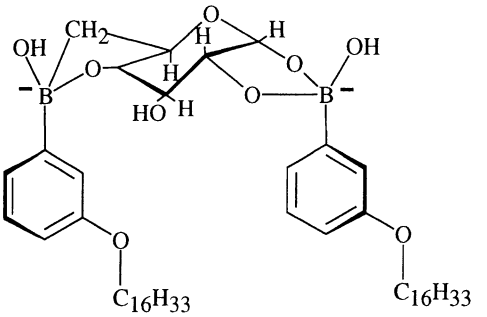


Fig. 2. UV-spectra of **1** in water/methanol at pH 7 (curve 1) and after adding increasing amounts of PVI at the same pH (curves 2-4). The absorption peaks below 250 nm subsequently decrease. After adding carbonate buffer (pH 11, reference curve 5) there are also no peaks below 250 nm.



Scheme 1.

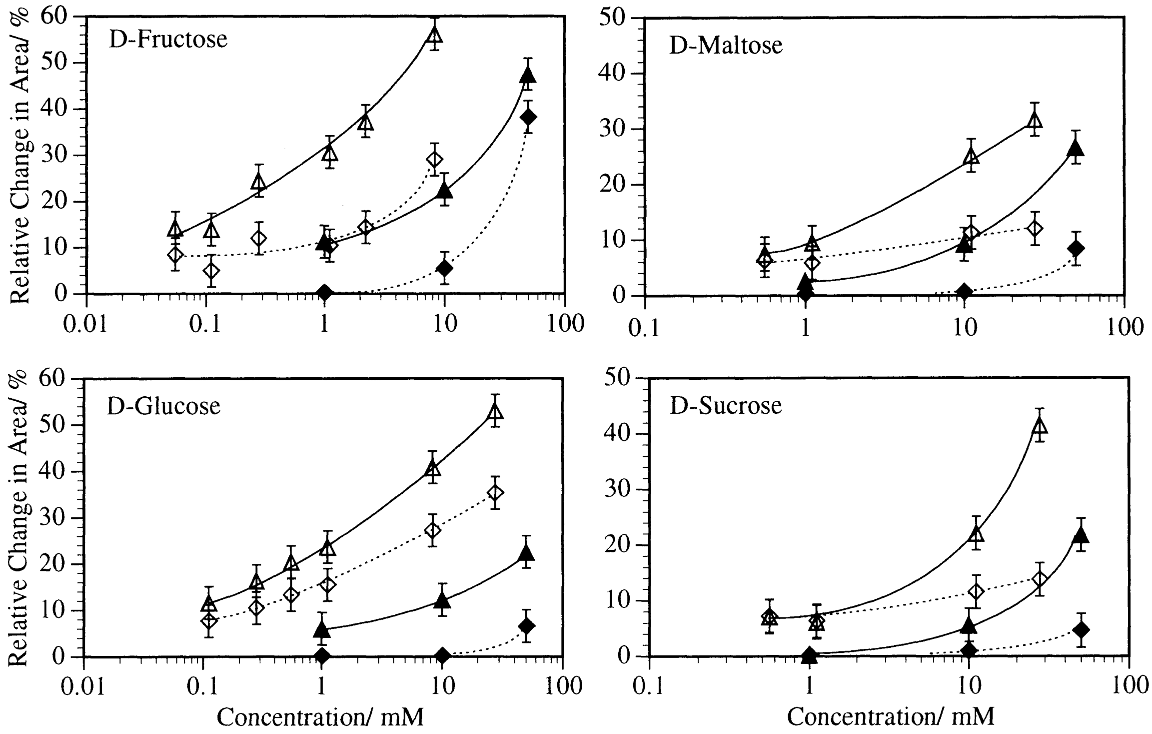


Fig. 3. Relative change (A''-A')/A' of the area occupied by one molecule of **1** at two different surface pressures at pH 10.0 and 293 K in the absence (closed symbols) and presence (open symbols) of 0.01 wt% PVI and various amounts of mono- and disaccharides in the subphase.

▲ and △: 10 mN/m
◆ and ◇: 30 mN/m

The relative change in the molecular area (A''-A')/A' in the absence and presence of PVI at two different surface pressures is depicted for D-fructose, D-glucose, D-maltose and D-sucrose in Fig. 3. The changes are considerably larger in the presence of PVI and the detection limits are shifted towards a lower sugar concentration. For example, 0.05 mM of D-fructose or 0.1 mM D-glucose can be unequivocally detected. This effect results from the cationic environment of the boronic acid provided by PVI.

The limiting area per molecule A^0 at zero-pressure is plotted over the concentration of D-fructose or D-glucose in the absence and presence of PVI in Fig. 4. The area is smaller in the presence of the quaternized cation due to reduced repulsion between the boronic acid head groups. The limiting area extrapolated from the condensed monolayer is less affected by monosaccharides compared with the molecular area of the expanded film and increases significantly only at higher sugar concentration. In the presence of PVI this effect is observed already at lower concentrations. Despite this shift there are no principle differences in the plot of A^0 vs. the sugar concentration and hence no differences in the reaction mechanism.

The before-mentioned results show the possibility to improve the sensitivity at one and the same pH-value. Vice versa, by interaction of the monoboronic acid with a quaternized amphiphilic amine, e.g. dimethyldioctadecylammonium bromide (DMDOA), sugar recognition of the monolayer takes place even at pH 6.5 (on pure water). Compound **1** itself does not form a stable monolayer at this pH. However, at a molar ratio of 2:1 between **1** and DMDOA the monolayer is stable and the shape of the pressure-area isotherms reproducibly changes upon the addition of D-fructose to the neutral subphase.

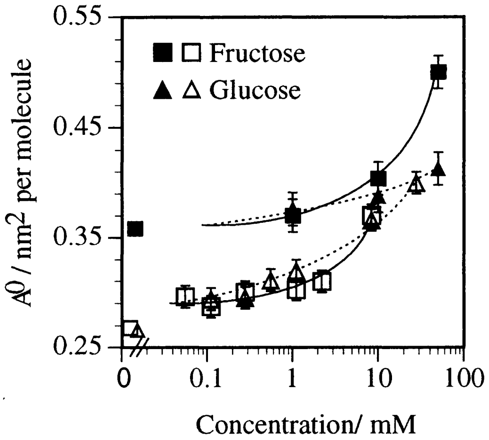


Fig. 4. Limiting area A^0 in the absence (closed symbols) and presence (open symbols) of 0.01 wt% PVI as a function of the concentration of monosaccharides in the subphase at pH 10.0 and 293 K.

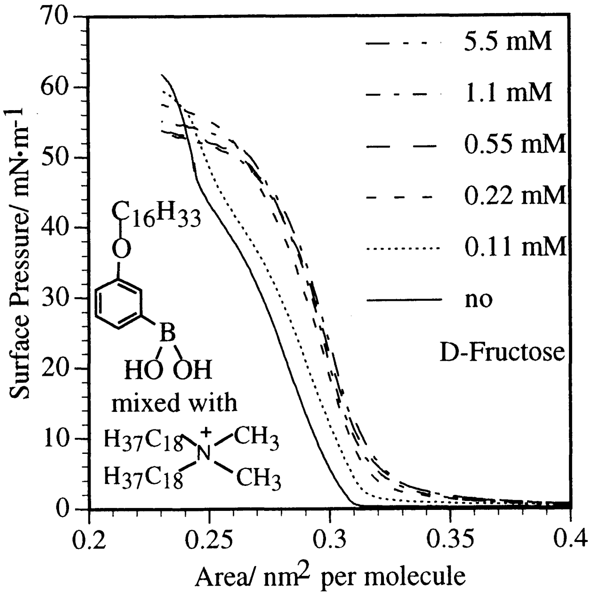


Fig. 5. Pressure-area isotherms of monolayers of **1** containing 33 mol-% dimethyldioctadecylammonium bromide on water at 293 K in the presence of various amounts of D-fructose.

In Fig. 5, π-A isotherms are depicted for various concentrations of D-fructose. The molecular area was calculated by using the average molecular weight (MW 440) of the mixture. Interestingly, the monolayer switches between two stages: A smaller molecular area in the absence of sugar and a larger but constant molecular area above 0.22 mM up to 5.5 mM at least. These stages represent the monolayer of DMDOA mixed with uncomplexed and complexed **1**, respectively. The complexation is accomplished at 0.22 mM D-fructose. In case of D-glucose the concentration limit for complete expansion of the monolayer is 1 mM.

In conclusion, it was shown that the sugar recognition by monolayers of a monoboronic acid becomes very sensitive and is achieved even at neutral pH when the boronic acid is placed in the cationic environment of quaternized amines.

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References

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