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Note

Concentration dependence of hydrophobicity of monosaccharides estimated by fluorescence of pyrene

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It is well known that polysaccharides such as starch and cyclodextrins have hydrophobic characteristics and can incorporate hydrophobic compounds into their hydrophobic cavities. It is generally considered that their hydrophobic sites are composed of the CH and CH_2 groups of the glucose unit. We have shown by using the fluorescence of 8-anilino-1-naphthalenesulfonic acid that hemicelluloses extracted from the wood cell wall also have hydrophobic characteristics [1], because it is considered that hydrophobicity of hemicellulose influences lignification in the wood cell wall [2]. We have reported that the hydrophobicity of hemicellulose depends on many factors, including the chemical composition of saccharide residues, the linkage type between residues, and the degrees of polymerization and branching. However, details of these factors have not been clarified. The present work deals with the differences of the hydrophobicities of monosaccharides themselves.

Monosaccharides have some hydrophobic character, but it is weak. Janado and Yano found that various saccharides are partitioned to the polystyrene gel phase from the aqueous phase [3]. Further, they showed that the solubilities of various hydrophobic hydrocarbons increase with the monosaccharide concentration, and they called this effect

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"cosolvent effects of saccharides" [4]. The effect was quantified by the association constants between saccharides and hydrocarbons.

In the present work, the hydrophobicity of the monosaccharides, D-glucose (Glc), D-galactose (Gal), D-mannose (Man), D-xylose (Xyl), and L-arabinose (Ara), was investigated by fluorescence spectroscopy. These monosaccharides are the main residues of wood hemicelluloses. For this experiment, pyrene was used as the probe of the hydrophobicity of the saccharide environment. The fluorescence emission spectrum of pyrene has four peaks located at approximately 372, 378, 382, and 392 nm. The intensity ratio of the 1st and the 3rd peaks decreases with an increasing hydrophobicity around the pyrene molecules. For example, the value is 1.87 in water, compared to about 1.1 in surfactant micelles, and 0.58 in cyclohexane [5]. Therefore, this fluorescence intensity ratio can be used to measure the hydrophobicity of a solution.

In this work, the solubility of pyrene in various concentrations of saccharides was first measured. Then, the association constants between pyrene and saccharide were determined from the solubility data using Janado's equation [4]. Finally, the hydrophobicities of the saccharides were measured by the intensity ratio of fluorescence radiated from the associated pyrene. The amount of associated pyrene was calculated from association constants.

Consequently, the saccharides investigated in this work were classified into two different groups, depending on the measured concentration dependence of the hydrophobicity. The first group includes Glc and Xyl, and the second contains Gal, Man, and Ara. In the first group, the hydrophobicity of the pyrene surroundings increases slightly with the saccharide concentration. In contrast, for the saccharides in the second group, the hydrophobicity decreases with increasing concentration. The reason for the different behaviors observed for the two groups is proposed to be due to their different molecular structures.

Solubility of pyrene in monosaccharide solutions.—The solubility of pyrene in various concentrations of saccharides was measured by the following method. A solution of 100 μL of pyrene solution in ethanol (10 mmol/L) was put in a test tube and dried in a vacuum. Then, 20 mL of monosaccharide solution (ranging in concentration from dilute to concentrated) was added to the test tube, and the solution was stirred for 24 h at 25 °C. The solution was centrifuged at 3000 rpm. Finally, the absorbances of the pyrene in water (A^0) and in saccharide solution (A) were measured at 335 nm by an ultraviolet spectrometer (Perkin–Elmer, Lambda 14) operating at 25 °C. All monosaccharides and the pyrene were purchased from Sigma Co., Ltd. Water for all measurements was obtained by filtration through a Millipore filter with a pore size of 0.22 μm.

Fig. 1 shows the solubility change of pyrene expressed as the absorbance ratio (A/A^0) plotted against the saccharide concentration. Solubilities increase with increasing saccharide concentration until about 30–40 wt% for all saccharides, but decrease above this concentration. The increasing solubility of pyrene with saccharide concentration indicates that the saccharides have the ability to solubilize pyrene. The decreasing solubility observed at higher concentrations is, of course, caused by the lower water content.

To avoid the effect of varying water content, the solubility change of pyrene was expressed as the relative solubility (C/C^0), where C and C^0 are the solubilities in the

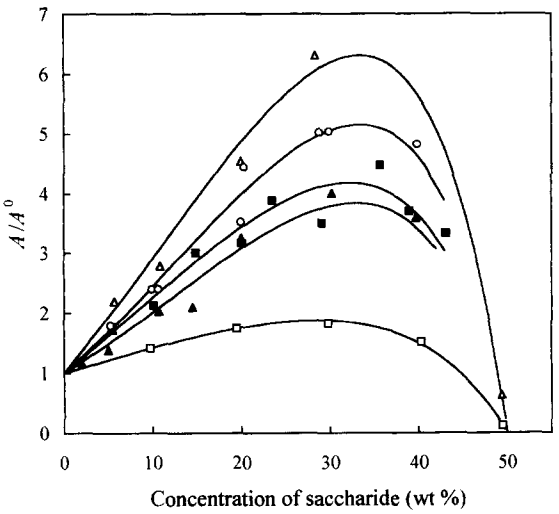

Fig. 1. Solubility change of pyrene in various monosaccharide solutions at 25 °C. The solubility is expressed by the absorbance ratio at 335 nm. The symbols □, ▲, △, ■ and ○ denote D-glucose, D-galactose, D-mannose, D-xylose, and L-arabinose, respectively.

aqueous part of the solution and in water, respectively. C/C^0 is calculated from A/A^0 data and the saccharide concentration and is given by

$$\frac{C}{C^0} = \frac{A/(1000 - mM)}{A^0/1000} \tag{1}$$

where M is molecular weight of saccharide, and m is molality of saccharide in mol/kg water. Fig. 2 shows the relationship between C/C^0 and m. The values of C/C^0 increase with concentration from unity at $m = 0$ for all saccharides. This behavior indicates that all saccharides have the ability to solubilize pyrene, just like surfactants. This ability of saccharides has already been observed for other hydrophobic molecules [4,6].

Association constants of pyrene to monosaccharides.—To discuss the solubilising ability, an association system was introduced in accordance with the method by Janado and Yano [4]. The apparent association constant (K) between pyrene (P) and saccharide (S) can be written as

$$K = \frac{[\mathrm{PS}_\nu]}{[\mathrm{P}] \cdot [\mathrm{S}]^\nu} \tag{2}$$

where [P], [S], and [PS_ν] are equilibrium molar concentrations of the species, and ν is the average number of bound saccharide molecules per pyrene molecule. The following equation is used to describe the relationship between C, C^0, and m:

$$\ln\left(\frac{C}{C^0} - 1\right) = \ln K + \nu \ln m \tag{3}$$

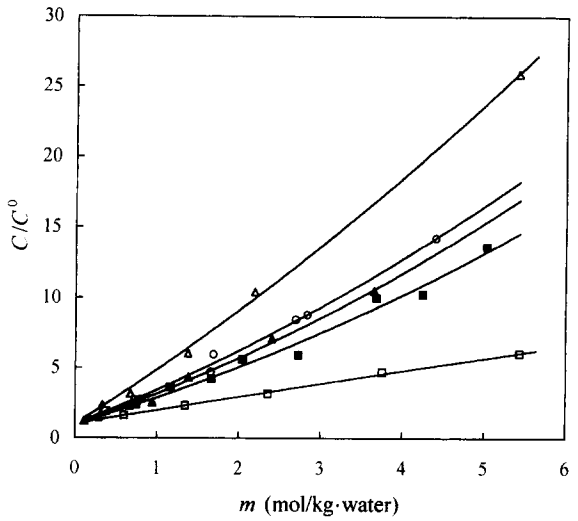

Fig. 2. Relative solubility of pyrene (C/C^0) versus molality of monosaccharide (m). The relative solubility means the solubility in the water part of the solution, and is calculated by eq. (1). The symbols □, ▲, △, ■, and ○ denote D-glucose, D-galactose, D-mannose, D-xylose, and L-arabinose, respectively.

because $[P] = C^0$, $[PS_\nu] = C - C^0$, and $[S] \approx m$ at $[S] \gg [P]$ and $[S] \gg [PS_\nu]$. $[P] = C^0$ because the solubility in the solvent part of a solution is equal to that in a solvent not containing saccharide. The values of K and ν can be estimated by the intercept and the slope of the $\ln(C/C^0 - 1)$ versus $\ln m$ curves, respectively.

Fig. 3 shows the plots of $\ln(C/C^0 - 1)$ versus $\ln m$. For all saccharides, nice linear relations were observed between these parameters. The values of K and ν for the different saccharides are listed in Table 1. From the K values, it was found that the association of pyrene and saccharide increases in the order Glc < Xyl ≤ Gal ≤ Ara < Man. The value of ν was approximately unity for all saccharides, indicating that the pyrene and the saccharides approximately form 1:1 complexes. The linear relation observed from dilute to concentrated solutions suggests that the association constants are independent of the concentration. In other words, the fraction of associated pyrene, $[PS_\nu]/[P]$, strongly depends on the saccharide concentration, $[S]^\nu$, according to eq. (2).

Hydrophobicity of the surroundings of pyrene solubilized by monosaccharides.—The hydrophobicities of saccharide solutions were estimated by the fluorescence spectrum of dissolved pyrene. The emission spectrum of pyrene was measured by a fluorescence spectrometer (Perkin–Elmer, LC-50). The excitation wavelength was fixed at 335 nm, and the emission wavelength was scanned from 350 to 400 nm at a rate of 100 nm/min. The slits of excitation and emission were set to 10 and 2.5 nm, respectively. A narrower emission slit is generally better for measuring emission spectra of pyrene, but some other researchers have used a 0.2 nm emission slit. However, a 2.5 nm emission slit was used as this was the smallest available for the instrument used in my studies.

Fig. 4 shows the observed intensity ratio of 1st and 3rd fluorescence peaks of pyrene ($B_{obs} = I_1/I_3$) plotted against the logarithmic saccharide concentration. The value of

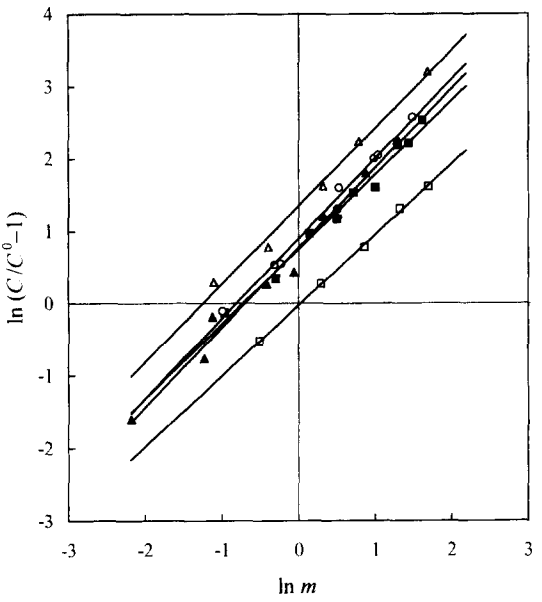

Fig. 3. Logarithmic plot of relative solubility against the molality of monosaccharide according to Janado's equation [eq. (3)]. The symbols □, ▲, △, ■, and ○ denote D-glucose, D-galactose, D-mannose, D-xylose, and L-arabinose, respectively.

B_{obs} generally decreases with increasing saccharide concentration from the initial value in water ($B_{water} = 1.86$). However, for Gal, Man, and Ara, it begins to increase at comparatively high concentration. The decrease of B_{obs} in the low-concentration range indicates that the surrounding of pyrene becomes increasingly hydrophobic. It suggests that monosaccharides have hydrophobic sites like starch and other polymeric saccharides. The observation that B_{obs} starts to increase at comparatively high concentrations of Gal, Man, and Ara will be discussed later.

The value of B_{obs} can be considered to be a result of a combination of the values of associated and free pyrene molecules according to

$$B_{obs} = B_{ass} \cdot x + B_{water} \cdot (1 - x) \tag{4}$$

Table 1
Association parameters between pyrene and monosaccharides at 25 °C according to eq. (3)

Monosaccharides	K	ν
D-Glucose	0.98	0.98
D-Galactose	2.17	1.01
D-Mannose	3.86	1.08
D-Xylose	2.12	1.03
L-Arabinose	2.46	1.10

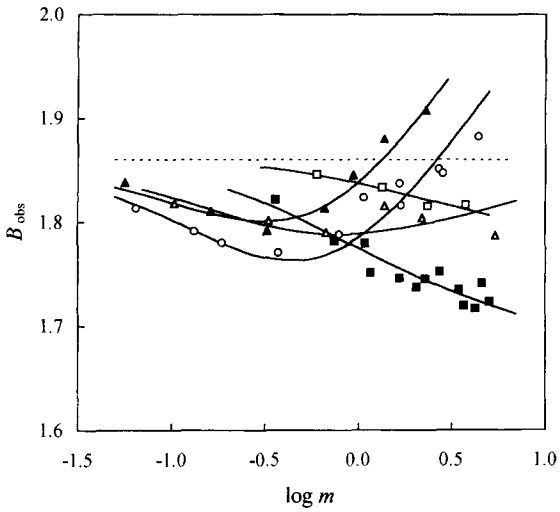

Fig. 4. Observed intensity ratio of the 1st and the 3rd peaks of fluorescence of pyrene ($B_{obs} = I_1 / I_3$) against the logarithmic molality of the saccharides (m). The symbols □, ▲, △, ■, and ○ denote D-glucose, D-galactose, D-mannose, D-xylose, and L-arabinose, respectively. The dashed line shows the intensity ratio in water ($B_{water} = 1.86$).

where B_{ass} is the intensity ratio of fluorescence radiated from associated pyrene, and x is the molar fraction of associated pyrene to the total amount of pyrene. The value of x is calculated from association parameters evaluated in previous section by the expression

$$x = \frac{[PS_\nu]}{[P] + [PS_\nu]} = \frac{K \cdot m^\nu}{K \cdot m^\nu + 1} \tag{5}$$

Fig. 5 shows the dependence of B_{ass}, calculated by eqs (4) and (5), on the saccharide concentration. For all saccharides, B_{ass} shows an approximately linear dependence on log m, i.e., the logarithmic saccharide concentration.

The linear relation between B_{ass} and log m can be represented by the experimental formula

$$B_{ass} = B'_{ass} + B''_{ass} \cdot \log m \tag{6}$$

where B'_{ass} and B''_{ass} are two empirical values. The estimated values of these are listed in Table 2. Because a low B_{ass} indicates high hydrophobicity, and vice versa, the hydrophobicities of saccharides increase in the order Glc < Xyl < Man < Gal < Ara at low concentrations, and Gal < Ara < Man ≤ Glc < Xyl at higher concentration. B_{ass} of Gal and Ara are particularly large at high concentrations, indeed greater than B_{water}. This means that the associated pyrene under these circumstances is presented with more hydrophilic surroundings than that of water itself. This hydrophilicity results in the increase of B_{obs} observed at higher m, see Fig. 4. The curves in Fig. 4 represent the values calculated by eq. (6) with the estimated values of B'_{ass} and B''_{ass}.

From the relationship between B_{ass} and m, the different saccharides can be classified into two groups. In the first group, which includes Glc and Xyl, the values of B''_{ass} are

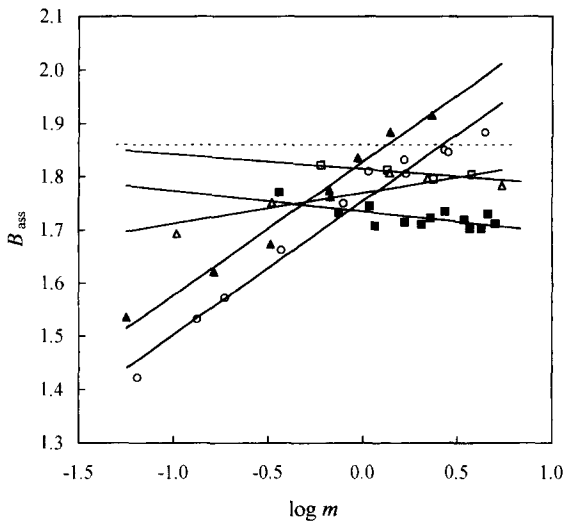

Fig. 5. Intensity ratio of fluorescence radiated from associated pyrene (B_{ass}) against the logarithmic molality of the saccharides (m). The symbols □, ▲, △, ■, and ○ denote D-glucose, D-galactose, D-mannose, D-xylose, and L-arabinose, respectively. The dashed line shows the intensity ratio in water.

negative, and those of B_{ass} decreased slightly with increasing saccharide concentration. In the second group, including Gal, Man and Ara, the values of B''_{ass} are instead positive, and those of B_{ass} increase with increasing saccharide concentration.

Plausible cause for the different characteristics.—It is believed that the difference in behavior observed between these two groups is related to the different molecular structures of the saccharides. Glc and Xyl molecules belonging to the first group have hydrophobic surface areas approximately equally distributed on the α- and β-axial sides. In contrast, Gal, Man, and Ara molecules have hydrophobic surface areas predominantly distributed on the β-axial side.

For Glc and Xyl, the hydrophobicity was weak for dilute solutions, but it increased with increasing concentration. In dilute solutions of Glc and Xyl, it is believed that either the α-axial or the β-axial side interacts with pyrene because of the 1:1 stoichiometric complex. Because the effective surface area at dilute concentration is considered to be half of the total hydrophobic surface area, the hydrophobic characters of Glc and

Table 2
Hydrophobicity change of associated pyrene according to eq. (6)

Monosaccharides	B'_{ass}	B''_{ass}
D-Glucose	1.81	−0.028
D-Galactose	1.83	0.251
D-Mannose	1.77	0.058
D-Xylose	1.73	−0.039
L-Arabinose	1.75	0.251

Xyl are less obvious. However, in highly concentrated solutions of Glc and Xyl, the pyrene molecule may be sandwiched between two saccharide molecules. Then, it is considered that the total of hydrophobic surface area affects the hydrophobicity at high concentration. Therefore, the hydrophobicity around pyrene would grow with increasing saccharide concentration.

On the other hand, Gal, Man, and Ara have large β-axial hydrophobic surface areas. Considering that almost all of the hydrophobic surface area is effective, the hydrophobicities become comparatively strong also in dilute solutions. However, in highly concentrated solutions of Gal, Man, or Ara, the comparatively hydrophilic surface at the α-axial side may lead to decreasing hydrophobicity at high concentration. Consequently, the hydrophobicity experienced by the pyrene molecules would decrease with an increase of concentration in Gal, Man, and Ara solutions.

At these high saccharide concentrations, the possibility of self-association of saccharides exists. If self-association of saccharides themselves occurs at high concentration, the interaction of pyrene to associated saccharides must be considered. However, the details are not clear by present experiments, and it is thought that the effect is minor because of $\nu \approx 1$ for all saccharides.

Incidentally, no relation was observed between hydrophobicity and the association constant, meaning that these parameters indicate different characteristics that are caused by different phenomena.

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