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Anomeric Configuration, Glycosidic Linkage, and the Solution Conformational Entropy of O-Linked Disaccharides

André M. Striegel*

Contribution from Solutia Inc., 730 Worcester Street, Springfield, Massachusetts 01151

Received December 9, 2002; E-mail: amstri@solutia.com

Abstract: Oligosaccharides perform a large number of biological roles, as dictated by their chemical structure and spatial arrangement. While conformational entropies are usually determined in vacuo by computer modeling, molecular recognition processes normally take place in solution. Here I show results of experiments using size-exclusion chromatography (SEC), an entropically driven solution technique. These clearly differentiate the individual contributions of the α and β anomeric configurations and of the (1 → 4) and (1 → 6) glycosidic linkages to the solution conformational entropy of O-linked disaccharides. I also distinguish between the members of the epimeric disaccharide pair isomaltose−melibiose and trace the difference to that between their constituent monosaccharides, α-glucose and α-galactose.

Introduction

A large number of molecular recognition processes feature disaccharides and higher homologues in both supporting and starring roles.[1] The biological roles of disaccharides range from nutrition to being essential components of plant cell walls to moderating the biosynthesis, structure, and transport functions of glycoproteins.[1a,b] Individually, anomeric configuration and glycosidic linkage in the carbohydrate region have been found to influence, sometimes terminally, docking and binding of enzymes and bacterial toxins.[2] DNA ligands ("aptamers") have been designed that bind selectively to cellobiose with little or no affinity for lactose, maltose, or gentiobiose,[3] while for certain glycolipids in cell membranes the orientation, conformation, and motion of the disaccharide headgroup relative to the bilayer surface are contingent upon the glucose−galactose carbohydrate moiety.[1c]

To date, most studies of the conformation and conformational entropy of disaccharides have been performed with computer modeling, generally dealing with the gas phase.[4] Here I compare the solution conformational entropies of various O-linked disaccharides using size-exclusion chromatography (SEC), demonstrating the individual influence of anomeric configuration and of glycosidic linkage on the flexibility of the carbohydrates in solution. SEC is an entropically driven technique which has thus far been used for separation of oligosaccharides with different degrees of polymerization.[5] Previous attempts at distinguishing between isomers of mono- and disaccharides by SEC have been unsuccessful.[5b,6] Here I show how such separation was accomplished with the use of high-resolution oligomeric columns, and employing *N*,*N*′-dimethyl acetamide with LiCl (DMAc/LiCl) as both the solvent and the chromatographic mobile phase. DMAc/LiCl has been used before in the study of maltooligosaccharides.[7] It is a highly favored medium in which to effect dissolution, characterization, and derivatizations of difficult to dissolve biopolymers such as cellulose, amylose, amylopectin, etc.,[8] and was seen to work extremely well in the present application to the constituent repeat units of these and other polysaccharides.

Experimental Section

Materials. Disaccharides and galactose were purchased from Sigma-Aldrich, and glucose was purchased from Fischer. All carbohydrates are D(+) and sold to at least 99% purity by the manufacturer, except for galactobiose, which was a mixture of 91% β and 8% α anomers. Carbohydrates were used as received, without further purification.

Size-Exclusion Chromatography (SEC). Unfiltered sample solutions (injection volume = 150 μL, concentration = 2.5 mg/mL in

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Table 1. $-\Delta S$ of Disaccharides in DMAc/0.5% LiCl (80 °C), As Determined by Size-Exclusion Chromatography

disaccharide	anomeric configuration and glycosidic linkage	$-\Delta S$ (J mol^{-1} K^{-1})
maltose	α-(1 → 4)	20.448 ± 0.002
isomaltose	α-(1 → 6)	20.663 ± 0.007
melibiose	α-(1 → 6)[a]	20.602 ± 0.015
trehalose	β,β-(1 → 1)	21.008 ± 0.010
cellobiose	β-(1 → 4)	20.507 ± 0.014
gentiobiose	β-(1 → 6)[b]	21.037 ± 0.022

[a] Melibiose is a galactopyranosyl−glucopyranose; all other disaccharides in the table are glucopyranosyl−glucopyranoses. [b] Mixture of 91% β anomer, 8% α anomer, as reported by the manufacturer.

DMAc/0.5% LiCl; preparation of the solvent has been described in ref 8d−f) were analyzed with an SEC system using DMAc/0.5% LiCl as mobile phase at 0.500 mL/min flow rate. Separation occurred over a column bank consisting of four analytical PLgel 5 μm 50 Å SEC columns, purchased from Polymer Laboratories. Detection was performed with an Optilab DSP interferometric differential refractive index (DRI) detector, from Wyatt Technology Corp. Column and detector temperatures were 80.0 ± 0.1 °C. The interconnecting tubing between the column bank and the detector was wrapped with heating tape to prevent heat loss during transfer. For all chromatographic determinations, results are averages of quintuplicate injections. Minor flow rate fluctuations for the saccharide measurements were corrected by comparing the retention time of the solvent peak in each injection (including individual maltose injections) to the average value of this peak for all maltose injections. Data acquisition was performed using Wyatt's ASTRA for Windows software (V. 4.73.04).

Calculation of $-\Delta S$ of Mono- and Disaccharides.[9] Calculation of the standard conformational entropy difference between the mobile and stationary phases for the disaccharides and monosaccharides in solution was based on the retention times of the peak maxima (V_R), as measured by SEC, as well as on the solute distribution coefficient (K_{SEC}). These two parameters are related via

$$K_{SEC} = (V_R - V_o)/V_i$$

where V_o is the void volume of the columns (measured with 21 000 g/mol narrow polydispersity linear polystyrene, from Polymer Laboratories), and V_i is the internal pore volume (measured with Toluene, from Fischer). As all saccharides are neutral and, moreover, the LiCl in the mobile phase should screen any electrostatic interactions between the analyte and the stationary phase, the separation can be safely assumed to proceed by a strict size-exclusion mechanism. Consequently,

$$\Delta S = R \ln K_{SEC}$$

Here I have used $R = 8.31451$ J mol^{-1} K^{-1}.[10] The use of the negative sign (i.e., of $-\Delta S$) stems from the fact that solute permeation in SEC is associated with a decrease in conformational entropy.

Results and Discussion

Results from our experiments are shown in Table 1, along with relevant structural information for the disaccharides considered here. The standard entropy difference between the phases, $-\Delta S$, corresponds to the difference between the conformational entropy of the disaccharides in the flowing mobile phase outside the pores of the column packing as compared to that of the disaccharides in the stagnant mobile phase inside the pores. The negative sign of ΔS is associated with a decrease in entropy due to the more limited mobility of the carbohydrates inside the pores.[9] Values were calculated as described in the Experimental Section. Here I merely note that a larger entropy difference between the phases corresponds to a larger conformational entropy in solution. It becomes immediately obvious from these data that the present method can discriminate, with statistical significance, between the various sugars studied. From the table, a number of comparisons may be effected and conclusions drawn. First, the β anomers are seen to have a higher flexibility, vis-à-vis their α counterparts, whether in the (1 → 4) or (1 → 6) conformations, as demonstrated by $-\Delta S_{cellobiose} > -\Delta S_{maltose}$ for the (1 → 4) case and $-\Delta S_{gentiobiose} > -\Delta S_{isomaltose}$ for the (1 → 6) case. The excess entropy imparted by the equatorial linkage in the β sugars is further highlighted by β,β-trehalose (with two such linkages), which has a $-\Delta S$ comparable to that of gentiobiose. It should be noted that the particular gentiobiose sample used was a mixture of 91% β, 8% α anomers. As such, a higher value than that reported in the table for the $-\Delta S$ of this disaccharide is expected for the pure β form. Conformational analysis has shown that increasing the number of axial linkages to the glycosidic oxygen results in a smaller amount of accessible conformations.[11]

Previous experiments with polysaccharides[12] have shown that under identical solvent/temperature conditions, dextran, which possesses α-(1 → 6) linkages, adopts a less restricted structure in solution than does amylose, which has an α-(1 → 4) backbone. Pullulan, with possesses both types of linkage in the form of maltotriose (α-(1 → 4)) units connected end-wise via α-(1 → 6) linkages, ranked between amylose and dextran. Here I observe that the (1 → 6)-linked disaccharides possess a higher conformational entropy than their (1 → 4)-linked structural isomers. The $\Delta\Delta S$ is more than twice as large for the β anomers than for the α, 0.531 J mol^{-1} K^{-1} between gentiobiose and cellobiose, 0.215 J mol^{-1} K^{-1} between maltose and isomaltose. Because of the excellent hydrogen-bond-accepting ability of DMAc/LiCl,[8c,13] the H-bonds present in the crystal lattices of cellobiose[8a,14] and gentiobiose[4b,15] are not expected to exist in the solutions studied here. Figure 1 illustrates the difference in the elution profiles of maltose (α-(1 → 4)) and isomaltose (α-(1 → 6)), and also between the latter and its β analogue, gentiobiose. While recent attempts at distinguishing between maltose and isomaltose using size-exclusion chromatography were unsuccessful,[6] here I clearly see a difference. The large conformational freedom of the β-(1 → 6) linkage[4b,11] is reflected in gentiobiose having the largest value for $-\Delta S$ of the disaccharides included in this study.

Thus far, comparisons have been between glucopyranosyl−glucopyranose compounds, that is, "glucose−glucose" disaccharides. In comparing isomaltose to melibiose, I contrast two α-(1 → 6) disaccharides, one glucopyranosyl−glucopyranose (isomaltose) and one galactopyranosyl−glucopyranose (meli-

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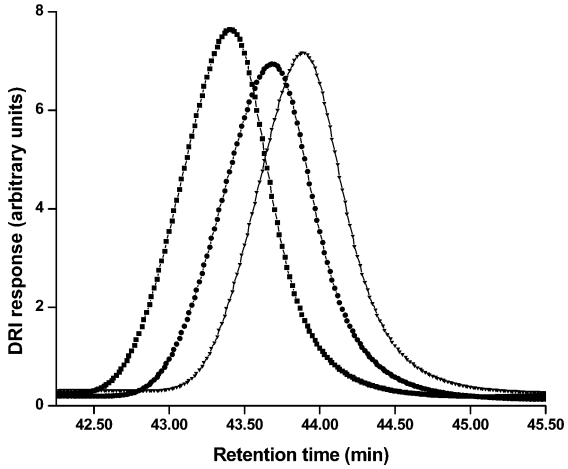

Figure 1. SEC/DRI elution profiles of maltose (▼), isomaltose (●), and gentiobiose (■) (DMAc/0.5% LiCl, 80 °C).

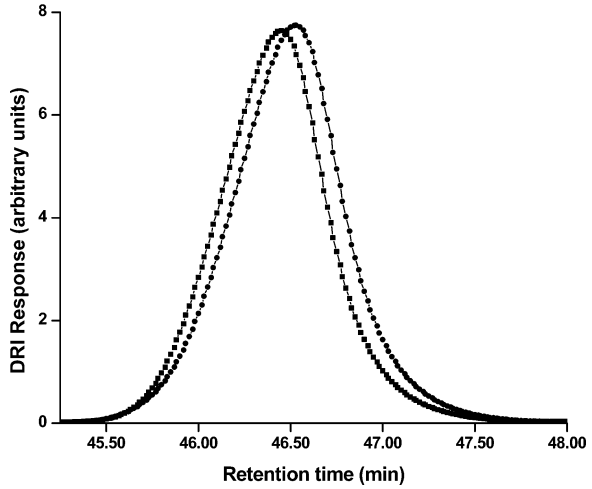

Figure 2. SEC/DRI elution profiles of α-glucose (■) and α-galactose (●) (DMAc/0.5% LiCl, 80 °C).

biose). These diastereomers differ only in the position of the hydroxyl group at *C*4 (i.e., they are epimers), equatorial in the case of isomaltose, axial in melibiose. Isomaltose, which possesses all-equatorial hydroxyl groups, is seen to possess the larger conformational entropy of the two, with a $\Delta\Delta S$ of 0.062 J mol^{-1} K^{-1}. It is interesting to note that this is almost the same difference as that between maltose and cellobiose, where $\Delta\Delta S = 0.059$ J mol^{-1} K^{-1}.

The conformational properties of oligo- and polysaccharides are generally considered to be determined by the relative conformations of the monosaccharide residue pairs that are linked together glycosidically to each other, as well as by the conformations of the individual monosaccharide residues.[14,16] To address the latter point with respect to isomaltose and melibiose, I also studied their constituent monosaccharides, α-glucose and α-galactose, individually. Previous attempts at discerning between these monosaccharides using SEC were not successful.[5b] Here, I was readily able to distinguish one from the other, as seen in Figure 2. For glucose, $-\Delta S = 17.859 \pm 0.014$ J mol^{-1} K^{-1}, while for galactose $-\Delta S = 17.702 \pm 0.009$ J mol^{-1} K^{-1}. The larger conformational entropy of the former is due to the same axial−equatorial difference at *C*4 as that in the melibiose−isomaltose epimers. Thus, the difference between the disaccharides is a reflection of that between their constituent units.

Conclusions

In comparing the relative rankings of the conformational entropies of gentiobiose, trehalose, isomaltose, melibiose, cellobiose, and maltose, I draw the following conclusions. The β configuration is more entropically favored than the α for identical glycosidic linkages. The (1 → 6) linkage is highly favored, such that even in an α configuration it possesses a greater entropy than a β-(1 → 4) linkage (i.e., $-\Delta S_{\text{isomaltose}} > -\Delta S_{\text{cellobiose}}$). Two β configurations, however, afford disaccharides an even greater flexibility than an α-(1 → 6) linkage. This last conclusion, obviously based on the $-\Delta S$ of β,β-trehalose, derives from the fact that it is highly unlikely that the large conformational entropy of this disaccharide is related solely to the (1 → 1) linkage. It is supported by results from molecular modeling of disaccharide analogues, where it has been found that the diequatorial pseudodisaccharide analogue of β,β-trehalose is the most flexible, as compared to other (1 → 1), (1 → 2), (1 → 3), (1 → 4), and (3 → 3) linkages, whether they be in diaxial, diequatorial, or axial−equatorial conformations.[17] Last, given identical glycosidic linkages and anomeric configurations, the conformational entropy of a disaccharide in solution will be directly proportional to the number of hydroxyl groups in the equatorial position.

The experimental method presented here can be applied to the study of other disaccharides, for example, O-, S-, and N-linked disaccharides with different constituent units than those studied here. It can also be used to determine the conformational entropy of structural isomers and diastereomers of higher oligosaccharide homologues, both in aqueous and in organic media and over a range of temperatures. It should thus find favor with researchers in areas as varied as plant physiology, biomolecular recognition and mimicry, drug design, food and cereal science, molecular modeling, and analytical chemistry.

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