Influence of Anomeric Configuration, Degree of Polymerization, Hydrogen Bonding, and Linearity versus Cyclicity on the Solution Conformational Entropy of Oligosaccharides

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ABSTRACT: In their various incarnations oligosaccharides have roles that are oftentimes conformationally dependent, where this dependency can be dictated by the anomeric configuration, glycosidic linkage, and/or hydrogen bonding (H-bonding) of the sugar molecules. Examples of this phenomenon are linkage- and configuration-selective aptameric binding to oligosaccharides and taste responses that appear to depend on the H-bonding-controlled shape of sugars. Differences in the behavior and use of oligosaccharides as a function of degree of polymerization are also well-known, a classic example being the case of cyclodextrins (CDs) and the different uses and properties of α-, *β*-, and *γ*-CD. Here, we have measured the solution conformational entropy of three homologous series of oligosaccharides, linear malto- and cellooligosaccharides and cyclodextrins, using high-resolution oligomeric size-exclusion chromatography (SEC), an entropically controlled separation method. We measured the change in Δ*S* of the malto- and cello- series as a function of degree of polymerization (DP) and compared the effects of α vs *β* anomeric configuration at each DP. By comparing select maltooligosaccharides with CDs, we also measured the effect of linearity vs cyclicity on Δ*S*. Additionally, by performing all of these studies under both hydrogen-bond-accepting and non-hydrogen-bond-accepting conditions, we were able to isolate the effects of H-bonding on the Δ*S* of the malto- and cellooligosaccharides and cyclodextrins as well.

Introduction

The roles of oligosaccharides are as varied as they are fundamental. They provide nutritional and flavoring support, constitute essential building blocks of plant cell walls, and are involved in processes such as fertilization and parasite infection.[1] Physiologically, oligosaccharides promote mineral absorption, improve gastrointestinal conditions, and have demonstrated immunomodulatory effects.[2] While there is a large number of classes of oligosaccharides (malto-, isomalto-, cello-, fructo-, and xylooligosaccharides, to name a few), many oligosaccharide functions are highly class-specific. Maltooligosaccharides, for example, are heavily used in the food and pharmaceutical industries[3a] and have shown to correlate, via their hygroscopicity, the humectant properties of corn starch hydrolysates with the latter's carbohydrate composition,[3b] while cellooligosaccharides have shown promise as prebiotics candidates[3c] and are widely used as model compounds in the study of cellulose structure.[3d,e] The use of cyclodextrins in separation science is widespread and growing,[3f] as is their potential in the area of drug delivery.[3g]

Similar to the case with glycosidic linkage, anomeric configuration has been shown to influence properties such as cryptobiological cell protection[4a] as well as enzymatic and bacterial binding and docking,[4b,c] with certain DNA aptamers showing selectivity toward cellobiose but not toward other O-linked disaccharides.[4d] Bacteria and parasites often possess surface carbohydrates that initiate infection by interaction with appropriate protein surface glycosylation sites in the host organism ("site-directed presentation").[5] While the exact role of the flexibility of cell surface oligosaccharides in glycolipids and glycoproteins is not yet entirely clear, the importance of this flexibility for the structural targeting of recognition events is commonly accepted.[6] In all these cases, the role of the conformational entropy of the oligosaccharides is vital. As stated by Carver in studying oligosaccharides as ligands: "[T]he primary source of the entropic barrier was the loss of conformational degrees of freedom of the *ligand* on binding... [T]he dominant effect for oligosaccharide binding is the loss of degrees of freedom in the conformational flexibility of the ligand."[5]

The role of hydrogen bonding is likewise essential in carbohydrates, not only at the polymeric level (e.g., inter- and intramolecular H-bonding are largely responsible for the insolubility of cellulose in most common solvents)[7] but also at the oligomeric level, where intramolecular H-bonding in maltose is believed responsible for this disaccharide's accession to the appropriate receptor site on the tongue and, consequently, for the generation of the sweet response.[8]

Investigations of the conformational entropy of oligosaccharides have usually proceeded by computer modeling.[3d,9] Furthermore, while entropically controlled molecular recognition phenomena normally occur in solution, the computer modeling studies have predominantly (though not exclusively) taken place in vacuo. Previously, our group introduced the application of size-exclusion chromatography (SEC), an entropically controlled separation method, to measurement of the solution conformational entropy (Δ*S*) of select O-linked disaccharides and their monosaccharide constituents.[10] For the case of glucopyranosyl−glucopyranose pairs, we quantitated the difference in Δ*S* between α and *β* anomeric configurations for the (1→4) and (1→6) glycosidic linkages as well as the difference between these glycosidic linkages for each type of anomeric configuration. We were also able to ascribe the entropic difference between glucopyranosyl−glucopyranose and galactopyranosyl−glucopyranose disaccharides to the epimerically generated entropic difference in their monosaccharide constituents.

Here, by comparing three homologous series, linear malto- and cellooligosaccharides and cyclodextrins, we have been able to measure the dependence of Δ*S* on degree of polymerization (DP) for each series. We have also been able to compare the difference in Δ*S* as a function of anomeric configuration for

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Table 1. $-\Delta S$ of Oligosaccharides in DMAc and DMAc/0.5% LiCl (50 °C), As Determined by Size-Exclusion Chromatography

oligosaccharide	$-\Delta S$ (J mol^{-1} K^{-1}) DMAc	$-\Delta S$ (J mol^{-1} K^{-1}) DMAc/0.5% LiCl	$\lvert\Delta S\rvert_{\text{H-bond}}$ (J mol^{-1} K^{-1})
α-glucose (M_1)	8.893 ± 0.022	12.944 ± 0.026	3.961 ± 0.034
maltose (M_2)	10.905 ± 0.035	15.433 ± 0.043	4.528 ± 0.055
maltotriose (M_3)	12.244 ± 0.056	16.937 ± 0.030	4.693 ± 0.064
maltotetraose (M_4)	13.310 ± 0.074	18.238 ± 0.099	4.928 ± 0.124
maltopentaose (M_5)	14.091 ± 0.030	19.243 ± 0.092	5.152 ± 0.097
maltohexaose (M_6)	14.741 ± 0.070	20.105 ± 0.141	5.364 ± 0.157
maltoheptaose (M_7)	15.173 ± 0.061	20.959 ± 0.099	5.786 ± 0.116
cellobiose (C_2)	10.975 ± 0.024	15.607 ± 0.011	4.632 ± 0.026
cellotriose (C_3)	12.525 ± 0.051	17.382 ± 0.055	4.857 ± 0.075
cellotetraose (C_4)	13.901 ± 0.036	18.831 ± 0.047	4.930 ± 0.059
cellopentaose (C_5)	15.076 ± 0.063	20.157 ± 0.068	5.081 ± 0.093
α-cyclodextrin (α-CD)	13.754 ± 0.064	18.265 ± 0.103	4.511 ± 0.121
β-cyclodextrin (*β*-CD)	14.347 ± 0.071	19.171 ± 0.059	4.824 ± 0.092
γ-cyclodextrin (*γ*-CD)	14.937 ± 0.112	20.233 ± 0.212	5.296 ± 0.239

each DP and have contrasted the effects of linearity vs cyclicity on ΔS of α-(1→4)-linked oligosaccharides of various DP. Additionally, by conducting experiments both in the non-H-bond-accepting solvent *N*,*N*-dimethylacetamide (DMAc) and in the superb H-bond-acceptor DMAc/LiCl,[11] we have quantitated the effects of H-bonding in all of the aforementioned cases. It is this investigation into the effects of H-bonding (which would have been quite difficult to conduct under aqueous conditions of analysis) that, in our opinion, has proven the most interesting aspect of the present study. Not only does this study allow qualitative comparisons but it also provides quantitative experimental data on how the titular parameters affect the solution behavior of three important series of O-linked oligosaccharides.

Experimental Section

Materials. Oligosaccharides and glucose were purchased from Sigma-Aldrich. All carbohydrates are D(+) and sold to at least 95% purity by the manufacturer. Carbohydrates were used as received, without further purification. Toluene and LiCl were purchased from Fisher and polystyrene standard from Pressure Chemical. LiCl was dried in a vacuum oven at 165 °C for 18 h and then stored in a desiccator. Preparation of DMAc/0.5% LiCl, as well as its application in the analysis of a variety of natural and synthetic polymers, has been described in detail in ref 12.

Size-Exclusion Chromatography (SEC). Unfiltered sample injections (injection volume = 100 μL, concentration = 2.5 mg/mL in DMAc and also in DMAC/0.5% LiCl) were analyzed with an SEC system using degassed DMAc and DMAc/0.5% LiCl as mobile phases at 0.500 mL/min flow rate. Separation occurred over a column bank consisting of four analytical PLgel 5 μm particle size, 50 Å pore size SEC columns, purchased from Polymer Laboratories. Detection was performed with a Waters 410 differential refractive index (DRI) detector from Waters Corp. Column and detector temperatures were maintained at 50.0 ± 0.1 °C. The injection compartment temperature was 40.0 ± 0.1 °C, the highest temperature allowed by the instrumentation used, a Waters 2695 separations module. The interconnecting tubing between the column bank and the detector was wrapped with insulating tape to prevent heat loss during transfer. For all chromatographic determinations, results are the averages of six injections, three each from two separate sample solution vials. Minor flow rate fluctuations for the saccharide measurements were corrected by comparing the retention time of a toluene marker peak in each injection (including individual maltose injections) to the average value of this peak for all maltose injections. Data acquisition was performed using Data Apex's Clarity software (V. 2.4.0.195).

Calculation of $-\Delta S$ of Oligosaccharides.[10,13] Calculation of the standard conformational entropy difference between the mobile and stationary phases for the oligosaccharides 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

$$K_{SEC} = (V_R - V_0)/(V_i - V_0)$$

where V_0 is the void volume of the columns (measured with 30 000 g/mol narrow polydispersity linear polystyrene) and V_i is the total column volume (measured with toluene). The internal pore volume of the system is defined as the difference between V_i and V_0. As all oligosaccharides are neutral, as there is no evidence of analyte interaction with the column stationary phase, and as elution of larger oligosaccharides always preceded that of smaller sugars, the separation can be safely assumed to proceed by a strict size-exclusion mechanism. Moreover, we observe a difference of one part per thousand or less in the values of K_{SEC} for α-glucose, maltose, and cellobiose when measured at 50 °C vs at 80 °C. This last fact strongly supports the conclusion that separation is predominantly entropic in nature (characteristic of "ideal" SEC behavior), as enthalpic interactions with the column packing material would lead to temperature-dependent values of the distribution coefficient.[13a] Consequently, we can write

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

Here, we have used $R = 8.31451$ J mol^{-1} K^{-1}. The standard entropy difference, $-\Delta S$, denotes the difference between the conformational entropy of the oligosaccharides in the flowing mobile phase outside the pores of the column packing vs the entropy of the oligosaccharides in the stagnant mobile phase inside the pores. 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 due to the more limited analyte mobility inside the pores.

Results and Discussion

Results from our experiments in both DMAc and DMAc/LiCl are shown in Table 1 and Figure 1. Maltooligosaccharides are linear α-(1→4)-linked glucopyranosyl-glucopyranose oligomers, whereas the linkage in the cellooligosaccharides is *β*-(1→4). α-, *β*-, and *γ*-cyclodextrins (CDs) are the cyclic equivalents of maltohexaose, maltoheptaose, and maltooctaose, respectively. Here, we examined maltooligosaccharides with DP 1 through 7 (M_1-M_7), cellooligosaccharides with DP 2 through 5 (C_2-C_5), and α-, *β*-, and *γ*-CDs. This allowed for a number of interesting comparisons, described below.

Influence of DP. First, we note that for all three homologous series $-\Delta S$ increases as a function of increasing DP. This is true regardless of the presence or absence of H-bonding. From a qualitative standpoint this is not particularly surprising, as the conformational phase space occupied by flexible molecules increases with added repeat units. Here, though, we have been able to quantitate those changes. In Figure 2 we see evidence of this in the earlier elution of maltohexaose as compared to maltopentaose, the former being more highly excluded from the pores of the column packing than the latter.

Influence of Anomeric Configuration. Second, we observe that the cellooligosaccharides have greater conformational freedom in solution than their malto- counterparts, as a result

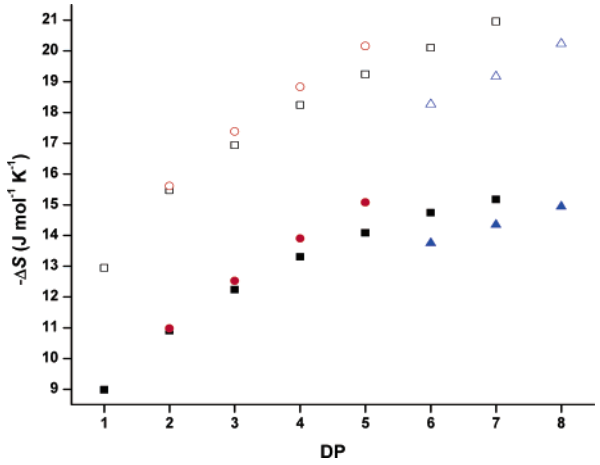

Figure 1. Solution conformational entropy ($-\Delta S$) of malto- and cellooligosaccharides and cyclodextrins as a function of degree of polymerization (DP). Circles correspond to cellooligosaccharides, squares to maltooligosaccharides, and triangles to cyclodextrins. Filled symbols denote results in DMAc, and open symbols denote results in DMAc/0.5% LiCl. All data obtained at 50 °C.

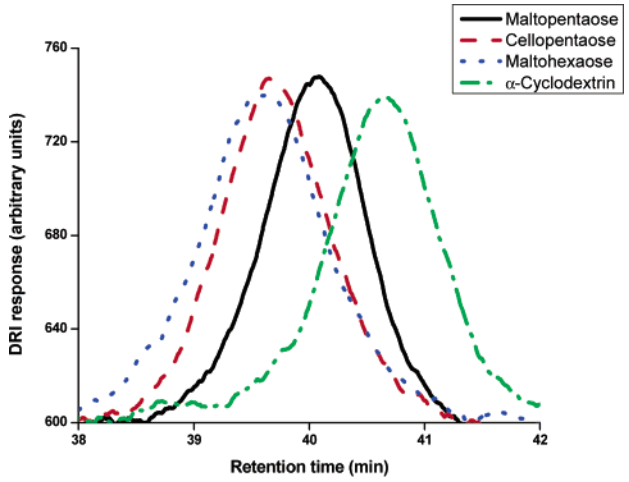

Figure 2. SEC/DRI elution profiles of maltopentaose, cellopentaose, maltohexaose, and α-cyclodextrin (DMAc/0.5% LiCl, 50 °C).

of the higher flexibility of the β anomeric configuration versus the α given identical glycosidic linkages, as in the present case. Being less hindered about the glycosidic bond than their α counterparts, the β-linked oligosaccharides are thus able to sample a larger region of the "conformational map" (the so-called "$\psi-\phi$ map")[14] than linear maltooligosaccharides of the same DP. In Figure 2, for example, the higher value of $-\Delta S$ of cellopentaose is reflected in its eluting earlier than maltopentaose (C_5 is actually seen to elute very near M_6, reflecting a $\Delta\Delta S$ of only 0.052 J mol^{-1} K^{-1} for this pair in DMAc/LiCl). This agrees with our earlier results comparing cellobiose to maltose and also isomaltose to gentiobiose.[10] It also agrees with numerous computer modeling results from various groups.[9e,f] The *qualitative* difference we observed between the α and β series of linear oligomers is independent of the presence or absence of H-bonding (quantitative differences are discussed later, under Influence of H-Bonding). The difference between the α and β series increases as a function of DP, reflecting a more rapid increase in $-\Delta S$ in cello- than in maltooligosaccharides with the addition of repeat units to the sugars. This would appear to be a direct result of the strong energy minima in the O1−C1−O4′−C4′ and in the C1−O4′−C4′−C5′ torsion angles of the α-(1→4) linkage of maltose such that continued addition of repeat units ultimately produces one strand of the starch double helix.[15]

Influence of Linearity vs Cyclicity. Third, we compare maltooligosaccharides to cyclodextrins and see that the more flexible linear oligosaccharides have higher values of $-\Delta S$, in both DMAc and DMAc/LiCl, than their cyclic counterparts. Tethering the chain ends together results in an obvious reduction of allowed conformational states, the $-\Delta S$ values of which are reported here. While our present comparison is restricted to two degrees of polymerization (DP 6 and 7), we note that over this limited range $-\Delta S$ increases more quickly as a function of DP for the CDs than for the maltooligosaccharides, an observation that is once again independent of H-bonding.

Influence of H-Bonding. We now turn to one of the most interesting aspects of this study, namely the qualitative and quantitative measure of H-bonding influence on oligosaccharide flexibility in solution. As mentioned earlier, the polar aprotic solvent DMAc is incapable of any appreciable H-bonding.[16] Addition of even a modest half-percent of LiCl, however, results in a solvent with one of the highest reported H-bond accepting abilities.[11c] Thus, we can directly attribute the $\Delta\Delta S$ between measurements in DMAc vs in DMAc/LiCl to the difference in solution conformational entropy due to H-bonding in the oligosaccharides. This derived quantity is reported in the last column of Table 1. For the malto- series we note that this $\Delta\Delta S$ increases from 4.528 J mol^{-1} K^{-1} for maltose to 5.786 J mol^{-1} K^{-1} for maltoheptaose, while for the cello- series $\Delta\Delta S$ increases from 4.632 J mol^{-1} K^{-1} for cellobiose to 5.081 J mol^{-1} K^{-1} for cellopentaose. In both series the increase in $-\Delta S$ that results from loss of intramolecular H-bonding becomes more pronounced with increasing DP. It is interesting to note, however, that over the range of DP 2−5, where we can compare the malto- and cello- series to each other, $\Delta\Delta S$ changes at virtually the same rate for both series.

For the CDs, $\Delta\Delta S$ as a result of H-bonding ranges from 4.511 J mol^{-1} K^{-1} for α-CD to 5.296 J mol^{-1} K^{-1} for γ-CD. In comparing the α- and β-CDs to maltohexaose and maltoheptaose, respectively, we note that eliminating intramolecular H-bonding results in a virtual doubling of $\Delta\Delta S$ between the linear and cyclic oligomers, i.e., $\Delta\Delta S$ between α-CD and M_6 is 0.987 J mol^{-1} K^{-1} in DMAc and 1.840 J mol^{-1} K^{-1} in DMAc/LiCl; $\Delta\Delta S$ between β-CD and M_7 is 0.826 J mol^{-1} K^{-1} in DMAc, increasing to 1.788 J mol^{-1} K^{-1} in DMAc/LiCl. Eliminating H-bonding also tends to equalize $\Delta\Delta S$, however: In neat DMAc, the difference between the $\Delta\Delta S$ of the M_6/α-CD pair and that of the M_7/β-CD pair is 0.161 J mol^{-1} K^{-1}. In DMAc/LiCl, however, this difference has now been reduced more than 3-fold, to 0.052 J mol^{-1} K^{-1}. It is difficult to ascribe this last observation to a single effect. Indeed, examination of the results in Table 1 and Figure 1 would indicate the combined influence of several of the previously described phenomena, namely of DP, linearity vs cyclicity, and H-bonding, providing another example of the complexity that is always present when studying carbohydrates in solution.

Finally, it is worth addressing two potential concerns with asserting the DMAc vs DMAc/0.5% LiCl comparisons solely represent differences due to intramolecular H-bonding in the oligosaccharides. Both questions deal with the concentration of LiCl in the solutions: (1) Does the LiCl concentration affect the H-bond-accepting ability of the solvent, such that a higher salt concentration might provide different values of $\Delta\Delta S$ between neat DMAc and DMAc with >0.5% LiCl? (2) Does LiCl concentration affect the size of the oligomers in solution; i.e., does increasing the LiCl concentration increase the ther-

modynamic goodness of the solvent, increasing the solvated size of the molecule and, consequently, increasing $\Delta\Delta S$ between measurements in neat DMAc vs in DMAc with >0.5% LiCl? To answer the first question, we turn to the solvatochromic experiments of Spange et al.,[11c] through which those authors measured the Lewis basicity or H-bond-accepting ability of a variety of solvents, including neat DMAc and DMAc with LiCl concentrations varying from 0.5% to 15.0%. While the H-bond-accepting ability of neat DMAc was only 0.78, addition of 0.5% LiCl increases that value dramatically to 1.75. Apposite to our present discussion, further increases in LiCl concentration up to 15.0% showed only an ~10% increase in H-bond-accepting ability. This indicates that while addition of LiCl provides for a complex with high H-bond-accepting ability, this ability is virtually independent of the salt concentration.

In answer to the second question, McCormick et al. studied solutions of cellulose in DMAc with varying percentages of LiCl and observed that a nearly 3-fold increase in LiCl concentration (to the point of saturation) provided for only an ~5% increase in the intrinsic viscosity of the cellulose solutions,[7a,17] meaning that molecular size in solution is virtually independent of LiCl concentration in DMAc. Therefore, while the difference in the DMAc vs DMAc/0.5% LiCl results cannot be ascribed exclusively to H-bonding, it can be ascribed predominantly to this effect.

It should also be noted that the virtual invariance in molecular size in solution (as measured by the intrinsic viscosity) with added LiCl, as observed by McCormick et al.,[7a,17] is also indicative of the fact that there are likely no residual intra- or intermolecular H-bonds remaining in the polymers, and thus in the oligomers studied here as well, after dissolution in DMAc with ≥0.5% LiCl.

Conclusions

By applying the methodology of SEC to the study of dilute oligosaccharide solutions, we have been able to measure the solution conformational entropy of homologous series of linear malto- and cellooligosaccharides and cyclodextrins. These experiments allowed us to isolate the individual contributions of a number of parameters to the ΔS of the oligosaccharides, namely how degree of polymerization, anomeric configuration, linearity vs cyclicity, and hydrogen bonding each affects the flexibility of carbohydrates in solution. Intuitive qualitative conclusions such as the increase in ΔS with increasing DP or the larger flexibility of the β anomeric configuration over the α configuration (given identical glycosidic linkages) have now been given quantitative, experimentally determined values. We have likewise quantitated the effects of linearity vs cyclicity for select α-(1→4)-linked species. Most interesting are the results showing how intramolecular H-bonding affects ΔS in all three homologous series and how this type of bonding affects the rate of change of ΔS as a function of DP, especially when comparing series that differ by either stereoisomerism or chain conformation. These studies, using a method that can be extended to other analytes and experimental conditions, not only permit a number of interesting qualitative comparisons but also provide quantitative experimental evidence as to how a number of molecular parameters define the conformational phase space in which various oligosaccharide series reside and function.

The comparisons afforded by conducting experiments in both neat DMAc and DMAc/0.5% LiCl yield quantities such as the H-bonding contribution to ΔS that would not have been as readily available from experiments conducted under aqueous conditions. Nevertheless, data under these latter conditions are highly desirable for many practical reasons, and as such, experiments in this regard are currently underway in our laboratory.

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