Estimation of Gas Phase Acidities of Glucose, Galactose, Mannose and Talose by the Kinetic Method

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Introduction

Carbohydrates are the most abundant biomolecules found in the nature. Monosaccharides, such as glucose, galactose and fructose are the basic carbohydrate units and they bind together by different types of glycosidic linkages to form different types of polysaccharides. Monosaccharides bind with nucleobases, i.e. with purines and pyrimidines, to give nucleosides and subsequently nucleotides (DNA and RNA). Carbohydrates are very important in cellular interactions and provide the major energy source required by every living organism. Among the monosaccharides, glucose, galactose and mannose are most common and which are present in mammalian physiology.

Measurement of thermochemical properties such as electron affinity, metal ion affinity, gas phase acidity (GA),\(^1\)\(^5\) and proton affinity\(^6\)\(^9\) are very important in development of ion/molecule chemistry. The information they provide is useful in the interpretation and prediction of mechanisms and reactivities. Such data provide useful information on the intrinsic properties of molecules in the absence of solvent effects. Much of the research has been focused on the determination of proton affinities but recently the attention is also focused on the estimation of gas phase acidities.

Cole et al.\(^10\) employed the negative ion ESI-tandem mass spectrometry to study the chloride adducts of monosaccharide molecules. They reported the approximate chloride affinity and gas-phase acidity order for few isomeric monosaccharides based on the decomposition of their respective [M+Cl]\(^-\) adduct ions. The dissociation of [M+Cl]\(^-\) ions produced two fragments viz. Cl\(^-\) and [M-H]\(^-\) ions. By considering the ratio of Cl\(^-\) and [M+Cl]\(^-\) ions they obtained the relative chloride affinity order and the order is: talose < glucose < sorbose < altrose ≈ tagatose ≈ mannose < fructose. Similarly, the ratio of [M-H]\(^-\) and [M+Cl]\(^-\) ions was used to rank the gas phase acidities of isomeric monosaccharides and the obtained order is: glucose ≈ mannose < altrose < sorbose < talose < fructose < tagatose ≈ psicose. Later Cai et al.\(^11\) bracketed the gas phase acidity of α-D-Glucose in between that of chloride (1373 kJ/mol) and p-chlorophenol (1407 kJ/mol) based on the dissociation their adduct ions.

Recently Salpin and Tortajada\(^12\) have determined the gas phase acidity of α-D-glucose by density functional theory and the value is1398 kJ mol\(^-1\). However, absolute gas phase acidities of glucose, mannose, galactose and talose are not reported in the literature. So we have undertaken the GA measurements of these monosaccharides by the kinetic method under electrospray ionization conditions.

Experimental

All the chemicals were obtained from Aldrich and were used as received. Stock (1mM) solutions of monosaccharides and reference acids were made up in 8:2 HPLC-grade methanol and water. Stock solutions of monosaccharides and reference compounds were mixed in appropriate volumes and diluted with methanol to achieve a final concentration of

20 μM each. Sample solutions were introduced into the source of the mass spectrometer by an inbuilt syringe pump at a flow rate of 5μL/min. Experiments were performed using a LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), equipped with an ESI source. The data acquisition was done under the control of Xcalibur software. The typical source conditions were: spray voltage, 4.5 kV; capillary voltage, -4.2; heated capillary temperature, 150 °C; tube lens offset voltage, 30V; sheath gas (N\textsubscript{2}) flow rate, 15 units; and helium was used as damping gas. For the ion trap mass analyser, the automatic gain control (AGC) settings were 2x10\textsuperscript{7} counts for full-scan mass spectrum and product ion mass spectrum with a maximum ion injection time of 200 ms. In the full-scan MS\textsuperscript{2} mode, the proton bound heterodimeric anions of interest were isolated at q\textsubscript{z} = 0.25 with a isolation width of 5. The isolated ions were then subjected to a supplementary ac signal to resonantly excite them and so cause collision induced dissociation (CID). The excitation time used was 40 ms and activation amplitude was varied from 16% to 17.5% (0.8 to 0.875 V\textsubscript{lab}) used for all the investigated heterodimers. All the isomers were done under identical experimental conditions, and spectra recorded for the average of 40 individual CID scans.

**Results and discussion**

The kinetic method developed by Cooks et al.\textsuperscript{13} has been successfully applied to a wide range of systems for the determination of gas-phase acidity (GA).\textsuperscript{1-5} This method is highly sensitive to energy differences as low as 0.1 kcal/mol and does not require sample in a pure form as it uses tandem mass spectrometry (MS/MS) to isolate the ion of interest. The determination of the GA of an unknown compound by the kinetic method starts with the formation of proton-bound heterodimeric anions between molecule of interest and a set of reference compounds whose GA is already known in the literature. [A\textsuperscript{−}H\textsuperscript{+}B\textsuperscript{−}], where A is unknown compound whose GA is to be determined and B is the reference. Selection of reference compounds is the key role in this method as the availability of the compounds with known GA values and nearer to the analyte of interest is difficult. This [A\textsuperscript{−}H\textsuperscript{+}B\textsuperscript{−}] anion is allowed to dissociate to yield the corresponding monomeric anions, A\textsuperscript{−} and B\textsuperscript{−} (eqn 1), where \( k_1 \) and \( k_2 \) are the rate constants for the competitive dissociations of the cluster ion to yield A\textsuperscript{−} and B\textsuperscript{−}, respectively.

\[
\begin{align*}
A^- \cdots H^+ \cdots B^- & \xrightleftharpoons[k_2]{k_1} A^- + BH \\
& \quad B^- + AH
\end{align*}
\]

\( \ln \frac{k_1}{k_2} = \ln \frac{[A^-]}{[B^-]} \approx \frac{\Delta (\Delta G_{\text{acid}})}{RT_{\text{eff}}} \)  \hspace{1cm} (2)

The GA can be obtained from the relative abundances of [A\textsuperscript{−}] and [B\textsuperscript{−}] ions according to the relationship given in Eqn. (2), where \( \Delta (\Delta G_{\text{acid}}) \) is the GA difference between unknown and reference compound, R is the gas constant and \( T_{\text{eff}} \) is the effective temperature of the activated dimer. The plot of \( \ln ([A^-]/[B^-]) \) vs. GA of a set of reference compounds gives a linear regression line, from which the GA of the unknown can be calculated.

The structures of the studied monosaccharides are given in Scheme 1. Proton bound heterodimeric anions [A\textsuperscript{−}H\textsuperscript{+}B\textsuperscript{−}] of each monosaccharide with a set of references are generated.
under the ESI conditions and the ions are isolated in the ion trap. We could find four suitable references for the estimation of gas phase acidities that can be used for all the studied monosaccharides. The used references along with their \( \Delta G_{\text{acid}} \) values are listed in Table 1. The CID spectra of \([A-H^+ B^-]\) anions are recorded at activation amplitudes of 16%, 16.5%, 17% and 17.5%. Below 16% activation amplitude fragmentation is not enough to provide an accurate product ion ratio; above 17.5% the spectra include peaks corresponding to water loss from \([M-H^-]\) ion of the monosaccharides. Therefore, the activation amplitudes in the range of 16% to 17.5% are considered for the kinetic method analysis, where the water loss peak is negligible in the spectra. The CID spectra showed mainly two monomeric anions, \([A^-]\) and \([B^-]\), where \(A\) = monosaccharide and \(B\) = reference whose GA is known.

![Structures of the monosaccharides studied.](image)

**Scheme 1.** Structures of the monosaccharides studied.

### Table 1. Reference acids used and their \( \Delta G_{\text{acid}} \) values.

<table>
<thead>
<tr>
<th>Reference acid</th>
<th>( \Delta G_{\text{acid}} ) (kcal/mol)</th>
</tr>
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<tbody>
<tr>
<td>o-Methyl benzoic acid</td>
<td>332.1</td>
</tr>
<tr>
<td>o-Fluoro benzoic acid</td>
<td>330.6</td>
</tr>
<tr>
<td>o-Amino benzoic acid</td>
<td>330.2</td>
</tr>
<tr>
<td>m-Fluoro benzoic acid</td>
<td>329.1</td>
</tr>
</tbody>
</table>

*All above \( \Delta G_{\text{acid}} \) values obtained from the NIST Chemistry Web book.

The CID spectrum of \([A-H^+ B^-]\) formed from Glucose (A) and o-Amino benzoic acid (B) is shown in Fig. 1 as an example. The relative abundance ratio of the product ions formed in the CID spectra is measured at different activation amplitudes and calculated their natural logarithm values, i.e., \( \ln[A^-]/[B^-] \). These values are plotted against the known \( \Delta G_{\text{acid}} \) values of the reference (substituted benzoic acids). An example plot obtained for D-Glucose is given in Fig 2 as an example. These plots (obtained for different activation amplitudes) give a straight line, from this a set of slopes and intercepts are obtained. Each slope gives the \( 1/RT_{\text{eff}} \) and each intercept gives the apparent gas-phase acidity (\( GA_{\text{app}} \)) value. From the slopes and intercepts of these plots, the \( GA_{\text{app}} \) values of all studied monosaccharides were calculated.
The calculated $G^{\text{app}}$ values of all the studied monosaccharides at different activation amplitudes along with their average value are presented in Table 2.

![Figure 1. CID spectrum of the proton-bound heterodimeric anion (m/z 316) of Glucose (A, m/z 179) and o-Amino benzoic acid (B, m/z 136).](image)

![Figure 2. Measured product ion abundance ratio $\ln(A^-/B^-)$ for Glucose vs. $\Delta G_{\text{acid}}$ of reference compounds at activation amplitudes (%) of 16, 16.5, 17, 17.5.](image)

**Table 2.** The $G^{\text{app}}$ values of monosaccharides obtained at different activation amplitudes using substituted benzoic acids as reference bases.

<table>
<thead>
<tr>
<th>Collision energy(%)</th>
<th>Glucose $G^{\text{app}}$(kcal/mol)</th>
<th>Mannose $G^{\text{app}}$(kcal/mol)</th>
<th>Galactose $G^{\text{app}}$(kcal/mol)</th>
<th>Talose $G^{\text{app}}$(kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>330.52</td>
<td>330.65</td>
<td>330.64</td>
<td>330.55</td>
</tr>
<tr>
<td>16.5</td>
<td>330.55</td>
<td>330.69</td>
<td>330.67</td>
<td>330.56</td>
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<tr>
<td>17</td>
<td>330.52</td>
<td>330.63</td>
<td>330.61</td>
<td>330.52</td>
</tr>
<tr>
<td>17.5</td>
<td>330.49</td>
<td>330.59</td>
<td>330.58</td>
<td>330.48</td>
</tr>
<tr>
<td>Average</td>
<td>330.52</td>
<td>330.64</td>
<td>330.62</td>
<td>330.53</td>
</tr>
</tbody>
</table>
Since the GA\textsuperscript{app} values measured at different activation amplitudes do not change significantly for all the studied monosaccharides, their average GA\textsuperscript{app} values can be reported as gas phase acidities as suggested by the Wesdemiotis.\textsuperscript{14} Though there is a difference in number of axial and equatorial hydroxy groups among isomeric monosaccharides i.e., glucose (all are equatorial), mannose (one axial at C\textsubscript{2}), galactose (one axial at C\textsubscript{4}) and talose (two axial, one at C\textsubscript{2} and other at C\textsubscript{4}), the differences in their gas phase acidities are marginal.

Conclusions

Proton-bound heterodimeric anions of monosaccharides with a set of suitable references are generated under negative ion electrospray ionization conditions, and dissociate appropriately in the ion trap. The relative abundances of the two monomeric product anions are used to measure the GA values of studied monosaccharides by applying the kinetic method. Since there is no change in the acidity values upon increasing the activation amplitudes, the GA values are obtained by applying the simple kinetic method. The gas phase acidity of glucose is 330.52 kcal/mol, mannose is 330.64 kcal/mol, galactose is 330.62 kcal/mol, and talose is 330.53 kcal/mol. The difference of GA value among the studied monosaccharides is less (0.12 kcal/mol), but it is reproducible.

References

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