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Estimating Collision Cross Sections of Negatively Charged *N*-Glycans using Traveling Wave Ion Mobility-Mass Spectrometry

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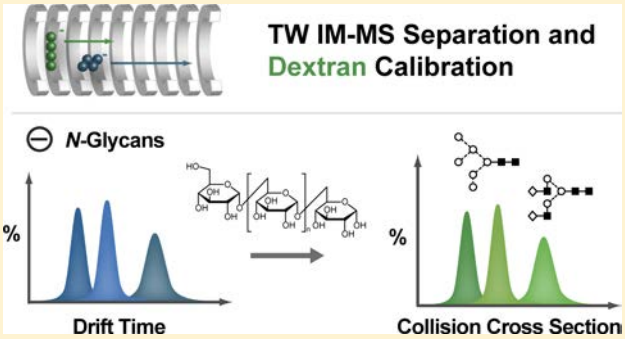
Supporting Information

ABSTRACT: Glycosylation is one of the most common post-translational modifications occurring in proteins. A detailed structural characterization of the involved carbohydrates, however, is still one of the greatest challenges in modern glycoproteomics, since multiple regio- and stereoisomers with an identical monosaccharide composition may exist. Recently, ion mobility-mass spectrometry (IM-MS), a technique in which ions are separated according to their mass, charge, and shape, has evolved as a promising technique for the separation and structural analysis of complex carbohydrates. This growing interest is based on the fact that the measured drift times can be converted into collision cross sections (CCSs), which can be compared, implemented into databases, and used as additional search criteria for structural identification. However, most of the currently used commercial IM-MS instruments utilize a nonuniform traveling wave field to propel the ions through the IM cell. As a result, CCS measurements cannot be performed directly and require calibration. Here, we present a calibration data set consisting of over 500 reference CCSs for negatively charged *N*-glycans and their fragments. Moreover, we show that dextran, already widely used as a calibrant in high performance liquid chromatography, is also a suitable calibrant for CCS estimations. Our data also indicate that a considerably increased error has to be taken into account when reference CCSs acquired in a different drift gas are used for calibration.



Carbohydrates are one of the main classes of biomacromolecules and are essential for a variety of biological processes. The attachment of oligosaccharides to proteins is the most common form of protein post-translational modification and can occur on asparagine residues (*N*-linked) or on serine/threonine amino acids (*O*-linked). These oligosaccharides, i.e. glycans, are responsible for several biological and biophysical properties that include protein folding and degradation mechanisms,[1] cell-cell-recognition,[2,3] immune response,[4] and various diseases.[5] The recent increased awareness of the importance of glycans in health, disease, and drug discovery has raised demand for better analytical methods for their characterization. The complex structure of glycans, however, makes this task one of the major challenges in current analytical chemistry.[6] Glycans consist of monosaccharide building blocks, which are often isomeric, and carry a variety of functional groups. Unlike proteins and nucleic acids, they form complex, branched structures that exist as structural isomers, but with different monosaccharide composition, connectivity, or configuration.

Mass spectrometry (MS),[7−10] high performance liquid chromatography (HPLC),[11] and liquid chromatography coupled to mass spectrometry (LC-MS) are the most commonly used techniques for glycan analysis. NMR[12] and X-ray crystallography are also used, but intrinsic limits arising from sample amount and purity requirements make these approaches problematic, especially for biological material. On the other hand, HPLC offers high throughput capabilities but often lacks the resolution required to discriminate glycans in complex mixtures. In all cases, the ability to detect and analyze structural isomers still remains a problem. A technique that has the potential to overcome these limitations is ion mobility-mass spectrometry (IM-MS), a method which has the added ability to separate ions according to their size and shape.[13−18] The recorded drift time can be converted into a collision cross section (CCS), which is a molecular property independent of

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the utilized instrument parameters and conditions. As a result, CCSs are universally comparable values with a high potential to be implemented as an additional search criterion in glycomics MS databases in the future.[19,20]

With the introduction of the commercially available traveling wave (TW) IM-MS instrument in 2006, the technique became readily available,[21] and it is today used on a routine basis for a variety of applications ranging from small molecules to large intact protein complexes.[22−24] However, due to the nonuniform electric field utilized in TW IM-MS, it is currently not possible to directly obtain absolute CCSs from the recorded drift times. Nevertheless, it has been demonstrated by several groups that the estimation of CCSs is possible using calibration approaches.[25−28] In this context, a careful choice of calibrants was shown to be essential for a successful CCS estimation. Generally, the best results are obtained when calibrants of the same molecular identity as the analytes and with comparable charge states are used. Since there were only limited amounts of CCS reference data available for complex carbohydrates,[17,29] we recently reported an extensive set of CCSs for positively charged, sodiated *N*-glycans released from commercially available glycoproteins.[30]

To complement these experiments, we have extended our investigations to include negatively charged glycans. These data are of special interest for carbohydrate analysis via IM-MS, since it has been shown that the negative ions yield highly informative fragmentation spectra.[31−34] Compared to positive ion mode, where mainly glycosidic bonds are cleaved upon collision induced dissociation (CID), a multitude of cross-ring cleavages are observed when negative ions are used. Those ions often provide more detailed information on the underlying structure of a carbohydrate and can, therefore, help to unambiguously identify and assign a particular structure. Additionally, we show that dextran, a readily available carbohydrate polymer that is used widely as a reference substance in chromatography, can also be used as a calibrant for CCSs estimations on TW IM-MS instruments. However, our data also reveal that certain care has to be taken when measurements performed in different drift gases are used within one calibration.

EXPERIMENTAL METHODS

N-Linked glycans were released by hydrazinolysis[35,36] from the well-characterized glycoproteins ribonuclease B,[37] porcine thyroglobulin,[38,39] chicken ovalbumin,[40,41] and bovine fetuin[42] obtained from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.). Subsequently, the glycans were re-*N*-acetylated using acetic anhydride in saturated $NaHCO_3$ and stored at −20 °C until required. Sialic acids were removed from the thyroglobulin and fetuin samples by heating with 1% acetic acid for 1 h at 70 °C. For the purpose of distinguishing the samples, we will further refer to the original samples as "sialylated" while the samples with additional sialic acid cleavage will be called "desialylated". The samples were dissolved in water:methanol (1:1, *v:v*) at about 1 mg/mL with the addition of 0.5 M ammonium phosphate to form stable adducts that mimic those normally found in biological samples. In this context it is important to mention that other solvents were not assessed systematically. Therefore, we cannot fully exclude that other solvent mixtures may lead to slightly altered CCSs.

Dextran from *Leuconostoc mesenteroides* was purchased from Fluka and Sigma-Aldrich (two fractions: MW = 1000 and MW = 5000) and used without further purification. Dextran samples were analyzed individually at a concentration of 0.1 mg/mL with 1 mM NaH_2PO_4 or as a mixture consisting of 0.1 mg/mL dextran1000, 0.5 mg/mL dextran5000, and 1 mM NaH_2PO_4 in water:methanol (1:1, *v:v*).

Measurements of absolute CCSs were performed with a modified Synapt G1 HDMS (Waters Co., Manchester, U.K.) instrument, described previously.[27,30] Here, the TW ion mobility cell was replaced by an rf-confining drift tube allowing the determination of absolute CCSs in helium and nitrogen. Typically, 5 μL of sample was ionized using a nanoelectrospray source (nESI) from platinum-palladium-coated borosilicate capillaries prepared in-house.[43] Each sample was measured twice at eight different drift voltages (50, 55, 60, 70, 80, 100, 125, and 150 V). Drift times t_D were extracted from raw data by fitting a Gaussian distribution to the arrival time distribution (ATD) of a given ion. At constant temperature and pressure, the drift time t_D needed to traverse a cell of length L is proportional to the inverse mobility ($1/K$) as well as the inverse electric field ($1/E$) (see eq 1). Therefore, the mobility of a given ion is determined from the slope of a t_D versus $1/V$ plot. The intercept of the fit t_0 corresponds to the time required to transport the ions from the end of the drift region to the detector.

$$t_D = \frac{L}{KE} + t_0 \tag{1}$$

The obtained mobilities K were used to calculate absolute CCSs using the Mason−Schamp equation:

$$\mathrm{CCS} = \frac{3e}{16N}\sqrt{\frac{2\pi}{\mu k_B T}}\frac{1}{K} \tag{2}$$

where N is the drift gas number density, μ the reduced mass of the ion and drift gas, k_B the Boltzmann constant, and T the temperature.

Estimated CCSs were obtained from measurements on a traveling wave quadrupole/IMS/oa-ToF MS instrument, Synapt G2-S HDMS (Waters Corporation, Manchester, U.K.) using a previously described protocol.[27,30,44] Here again, samples were ionized from in-house-prepared platinum-palladium-coated borosilicate capillaries using nESI. The drift times were extracted by fitting the ATDs of the ions with a Gaussian distribution function and corrected for their m/z dependent flight time. In addition, the corresponding absolute CCSs reference values were corrected for charge and mass. A logarithmic plot of corrected CCSs against corrected t_D could be fitted with a linear regression and was used as a calibration curve to estimate CCSs. Further details about the calibration procedure and the utilized instrument parameters can be found in the Supporting Information.

RESULTS AND DISCUSSION

***N*-Glycan Calibrants.** In this study, four different *N*-glycan mixtures that were released from the commercially available glycoproteins ribonuclease B, fetuin, thyroglobulin, and ovalbumin were analyzed by IM-MS in the negative ion mode using helium and then nitrogen as the drift gas. On ribonuclease B, typically high mannose structures are populated. Thyroglobulin carries both high mannose and complex glycans, whereas fetuin mainly carries sialylated complex-type glycans. Ovalbumin provides the most diverse carbohydrate sample with high mannose and complex and hybrid-type glycan structures.[6,45]

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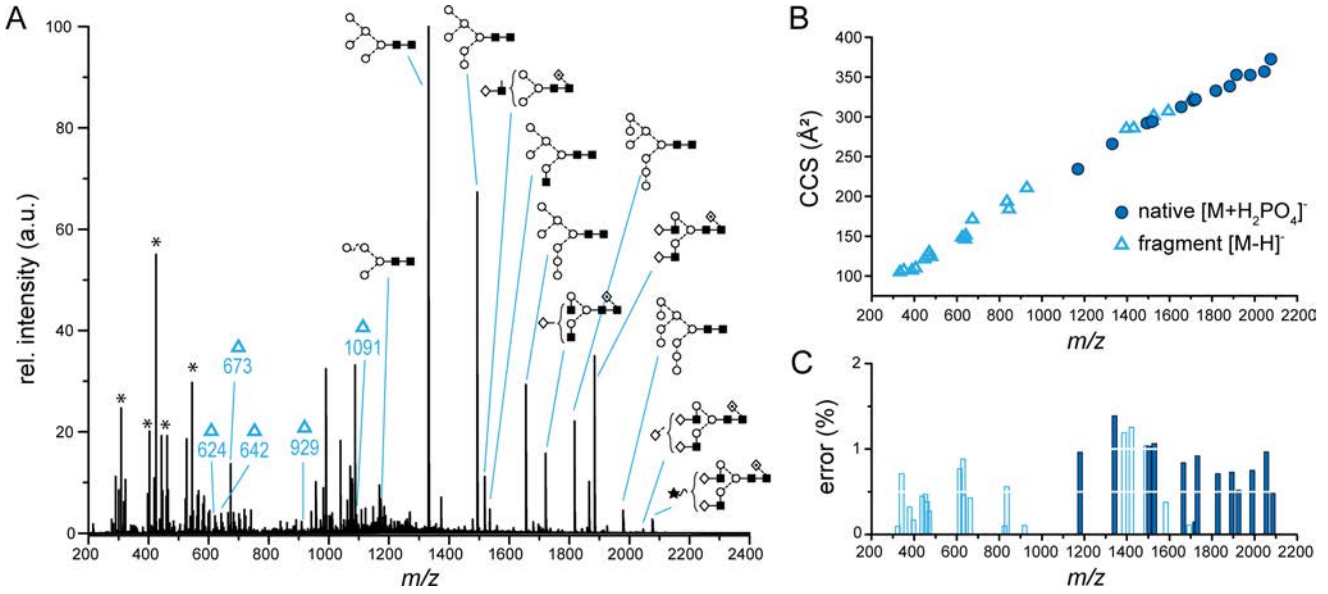

Figure 1. IM-MS data of desialylated glycans released from thyroglobulin. (A) Mass spectrum of the glycan sample at in-source activation conditions measured at a Synapt G2-S. The most abundant singly charged fragments are labeled with triangles, and the structures of the native glycans are depicted using the UOXF nomenclature.[46] The asterisks indicate background signals resulting from polydimethylsiloxane. (B) Absolute He CCSs (obtained from measurements on a modified Synapt G1) of the native glycans as phosphate adducts (circle) and the deprotonated fragments (triangle) for all singly charged ions. The corresponding relative errors (C) do not exceed 2%.

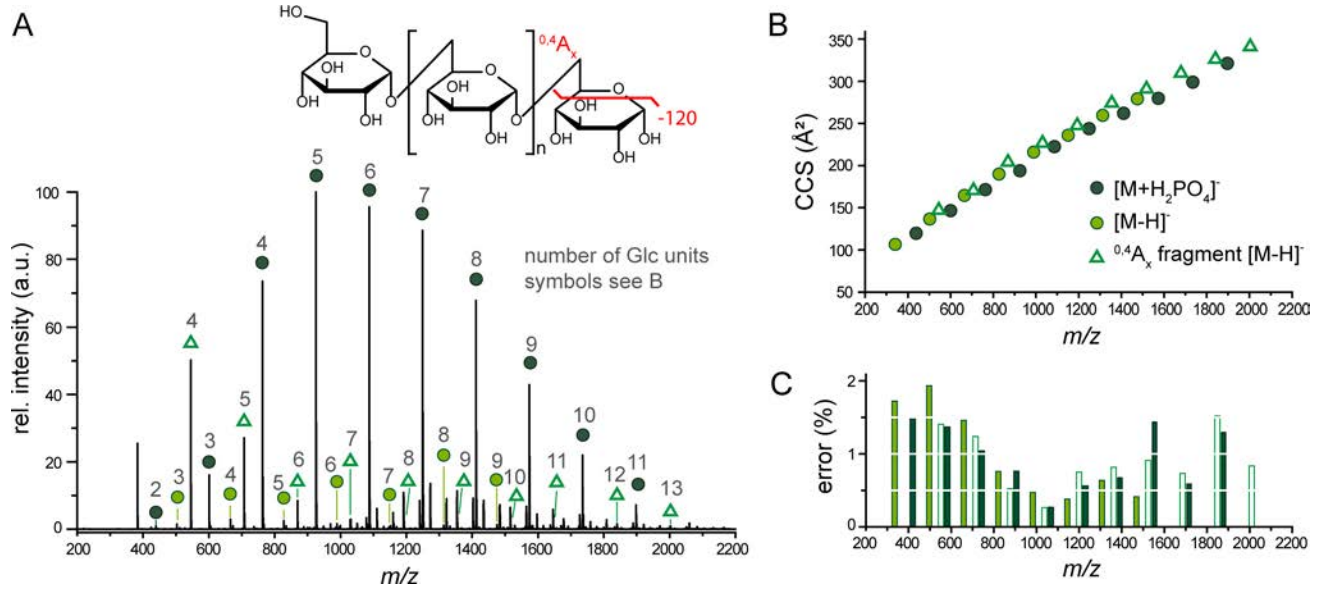

Figure 2. IM-MS data of partially hydrolyzed dextran. (A) Mass spectrum of dextran measured on a Synapt G2-S. All singly charged ions are labeled according to their type and number of glucose units with dark green circles indicating phosphate adducts, light green circles being deprotonated species, and triangles representing fragment ions. The structure of dextran is shown, and the cleavage site of the most prominent cross-ring fragment $^{0,4}A_x$ $[M_n - 120]^-$ is indicated in red. (B) The corresponding absolute He CCSs obtained from measurements on a modified Synapt G1 and (C) their associated relative errors. The covered CCS range and the errors are similar to those of *N*-glycans.

A typical mass spectrum of the glycans released from thyroglobulin is shown in Figure 1. In order to simultaneously acquire data for the intact glycans as well their resulting CID fragments, spectra were recorded at in-source fragmentation conditions with an increased cone voltage between 50 and 150 V. As a result, the signals cover a broad m/z and CCSs range within one single measurement. In order to exclude that these rather harsh conditions influence the CCSs of the investigated molecules or their fragments, we monitored the arrival time distributions (ATDs) of selected species at increasing trap collision energy after isolation in the quadruple. No significant changes in drift time were observed, which indicates that no or only minor rearrangements take place before dissociation. Besides the signals corresponding to the intact, native glycans, which (due to the added phosphate) mainly occur as phosphate adducts, a large variety of deprotonated fragment ions, which are a result of glycosidic bond or cross-ring cleavages, are observed. This observation is in good agreement with previous MS/MS measurements on these glycans in negative ion polarity, which yielded a

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Table 1. Helium and Nitrogen CCSs of Dextran (Glc_n)[a]

	He CCS in Å² (±RSTD)			N_2 CCS in Å² (±RSTD)		
n	$[M - H]^-$	$M + H_2PO_4^-$	$M + Na^+$	$[M - H]^-$	$M + H_2PO_4^-$	$M + Na^+$
2	106.6 ± 1.7%	119.6 ± 1.5%	104.1 ± 0.3%	174.6 ± 0.2%	182.6 ± 0.1%	179.5 ± 0.0%
3	136.6 ± 1.9%	146.4 ± 1.4%	137.1 ± 1.1%	202.3 ± 0.0%	212.2 ± 0.2%	215.0 ± 0.0%
4	164.5 ± 1.5%	171.5 ± 1.0%	165.1 ± 0.8%	233.9 ± 0.2%	243.8 ± 0.6%	243.4 ± 0.2%
5	189.9 ± 0.8%	193.8 ± 0.8%	194.0 ± 0.8%	265.4 ± 0.5%	271.2 ± 0.3%	273.8 ± 0.3%
6	215.9 ± 0.5%	222.4 ± 0.3%	218.9 ± 0.7%	296.7 ± 0.3%	304.8 ± 0.2%	302.1 ± 0.2%
7	235.9 ± 0.4%	243.7 ± 0.6%	243.9 ± 0.7%	319.2 ± 0.3%	327.9 ± 0.3%	330.9 ± 0.1%
8	259.6 ± 0.6%	262.0 ± 0.7%	266.2 ± 0.6%	343.6 ± 0.4%	347.8 ± 0.4%	355.8 ± 0.0%
9	279.1 ± 0.4%	279.6 ± 1.4%	286.0 ± 0.4%	365.2 ± 0.0%	365.9 ± 0.1%	377.1 ± 0.0%
10		298.8 ± 0.6%	305.9 ± 0.4%		386.5 ± 0.1%	397.8 ± 0.7%
11		321.1 ± 1.3%	318.0 ± 0.4%		408.7 ± 0.1%	412.6 ± 1.2%
12			334.8 ± 0.6%			425.5 ± 1.4%

[a] n = number of glucose units of the respective ions; RSTD = relative standard deviation of two or three independent measurements in %.

characteristic mixture of glycosidic bond and cross-ring cleavages.[31−33] For all the identified peaks, CCSs were determined for the two drift gases helium and nitrogen. Parts C and D of Figure 1 the He CCSs and the respective relative errors of the singly charged ions obtained from the thyroglobulin sample. As apparent from the data, a m/z range from 332 to 2076 and a CCS range from 105 to 372 Å² for helium and 170 to 462 Å² for nitrogen is covered for the singly charged ions. The average CCS errors observed rarely exceed 2%, which is the expected error associated with these measurements. In total, more than 450 CCSs of native glycans and their fragments were determined (see Table S1).

Calibration with Dextran. While *N*-glycans released from commercially available glycoproteins have been shown to be good calibrants in previous investigations, their spectra can be quite complex and difficult to interpret. In addition, the cleavage and sample preparation procedure for these glycans can be time-consuming and a complete removal of protein is often difficult, resulting in additional peptide signals in the mass spectrum. Therefore, the next step was to assess whether partially hydrolyzed polysaccharides can potentially be used to simplify the calibration procedure.[47] In particular, we chose dextran, a well-defined carbohydrate polymer consisting of α-1,6-linked glucose units, as a potential calibrant. Dextran is widely used as a carbohydrate calibration standard in chromatographic experiments where a so-called dextran ladder is used to convert carbohydrate retention times into comparable glucose unit (GU) values.[11] Consequently, partially hydrolyzed dextran fractions of varying length are likely to be already available in the majority of glycomics laboratories.

Generally, the MS spectra of dextran in positive and negative ion mode are easy to interpret and provide well-distributed signals in an m/z range similar to those of the *N*-glycan mixtures (Figure 2). While in positive ion mode mainly sodiated ions are observed, the most dominant signals in negative ion polarity are phosphate adducts (when phosphate is added as in this study), deprotonated molecules, and the $^{0,4}A_x$ fragment $[M - 120 - H]^-$, which was previously characterized by Čmelík et al.[48] The corresponding CCSs of these ions and their respective errors were measured using the modified Synapt G1 instrument and are shown in Figure 2 and Table 1 (see Supporting Information for full table). The covered CCS range for singly charged ions in the negative ion mode is 106−341 Å² for helium and 174−429 Å² for nitrogen and therefore is comparable to those of the above-described *N*-glycans. Moreover, for dextran, far more doubly charged ions are observed, covering a m/z range of 596−2082 and a He CCS range of 239−531 Å², which is much larger compared to *N*-glycans. For thyroglobulin, for example, only signals in the m/z range from 780 to 1183 (doubly charged) are observed with He CCS between 274 and 374 Å².

CCS Estimation. The utility of the reported CCS values for a CCS estimation was assessed by performing test calibrations on a Synapt G2-S (TW IM-MS), using a previously described procedure.[25,27,30] To do so, one carbohydrate mixture was used as a calibrant, while the other samples were treated as "unknown". This allows a direct comparison of the estimated CCSs, obtained from a TW instrument, with the absolute CCSs reported above.

As shown previously for the positive ion calibration, one glycan sample usually provides enough signals to achieve a calibration of sufficient quality.[30] This trend is also observed here, and in principle, all reported glycan samples can serve as calibrants in negative ion mode. However, similarly to the previously reported sodiated glycans, samples released from fetuin, thyroglobulin, or ovalbumin are especially recommended. The structurally diverse high-mannose glycans populated on ribonuclease B on the other hand can be an inherent source of error, since isomeric CID fragments may overlay and broaden the ATD of the native glycans.[30] Regardless of the carbohydrate mixture, different calibration curves should be used for each charge state to achieve the best CCS estimation.[30]

For the calibration, each calibrant sample (thyroglobulin and dextran) was measured under the same conditions as the analyte samples, and the obtained drift times were corrected for their m/z dependent flight time and correlated to the corrected CCSs. Each estimated CCS (CCS*) is the average value of five independent measurements at different wave velocities, with a typical standard deviation of about 1%. To evaluate the quality of the calibrants, the CCSs* for the "unknown" samples were determined and compared to their absolute CCSs, by calculating their relative deviation (d).

$$d = \frac{\mathrm{CCS}^* - \mathrm{CCS}}{\mathrm{CCS}} \times 100 \quad (3)$$

When absolute values of d from all CCSs of one sample are averaged and compared (Figure 3), it immediately becomes apparent that both investigated calibrants yield comparable errors between 1 and 2% for N_2 CCSs*. This is in good agreement with values obtained for a calibration with peptides,[44] proteins,[27] and positively charged glycans.[30] In addition, these data also indicate that dextran is a highly

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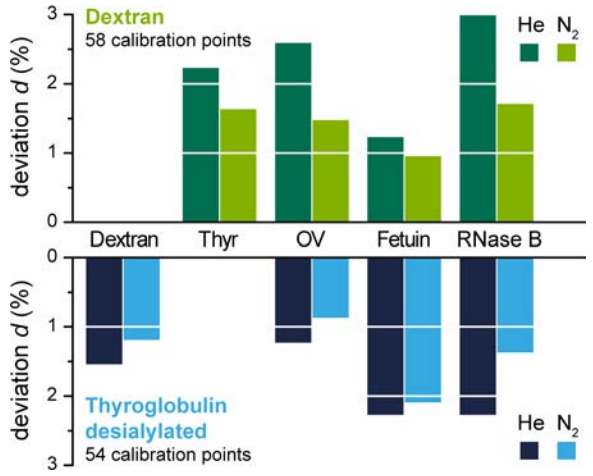

Figure 3. Average errors of estimated CCSs. Comparison between average relative errors d (eq 3) of estimated He and N_2 CCSs of dextran, thyroglobulin (Thyr), ovalbumin (OV), desialylated fetuin, and ribonuclease B (RNase B) obtained from a calibration with dextran (upper panel) and thyroglobulin glycans (lower panel). Both calibrations provide similar errors for the CCSs, but larger errors are generally observed for He CCSs than were estimated from measurements in nitrogen. Individual cross section measurements are listed in Table S1 of the Supporting Information.

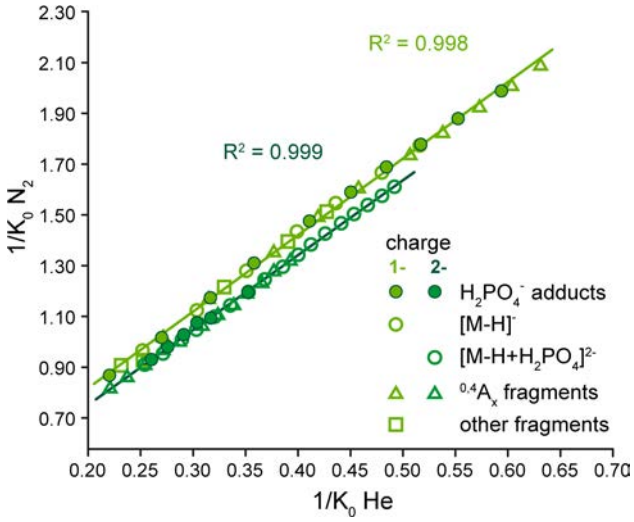

Figure 4. Correlation between inverse mobilities of dextran measured in helium and nitrogen. A linear correlation can be observed by comparing the inverse mobilities in two drift gases. Since they are proportional to the CCS, a conversion of He into N_2 values, and *vice versa*, is generally possible. The observation of two distinct trend lines highlights the necessity to use individual calibrations for each charge state.

suitable calibrant for the CCS estimation of carbohydrates on TW IM-MS instruments, because the observed errors are similar to those obtained for a native *N*-glycan calibration. For practicality reasons, however, dextran is a far superior calibrant, since it can be purchased off the shelf without the need of a time-consuming cleavage and workup procedure that is required for native glycans.

He vs N_2 CCS. Another aspect that becomes apparent from Figure 3 is the inherently larger error of the estimated He CCSs. Measurements on a Synapt instrument are typically performed using nitrogen as drift gas. Conversely, the majority of data reported in the literature and all theoretical calculated CCSs are helium values.[49,50] It has been shown, however, that it is generally possible to use data measured in nitrogen to estimate He CCSs.[27,30,44] This is possible when the inverse mobilities of ions in He and N_2 are linearly correlated, as shown exemplarily in Figure 4 for singly and doubly charged dextran ions. According to the Mason−Schamp equation, the inverse mobility is proportional to the CCS, and as a result, the CCSs in both drift gases should as well be proportional. However, recently there has been increasing evidence that estimated He CCSs obtained from measurements in nitrogen are associated with a larger experimental error.[17]

To investigate the influence of the drift gas more closely, TW IM-MS data recorded in nitrogen were used to estimate N_2 CCSs (using N_2 values for calibration) as well as pseudo-He CCSs (using He values for calibration). For all calibrants, larger errors were observed for estimated pseudo-He CCSs (Figure 3). While the N_2 CCSs can be associated with errors of about 2%, the pseudo-He CCSs differed up to 3.5% from the expected values. A possible explanation for these consistently higher errors is the changed interaction potential of the analyte with the drift gas. In general, the interaction potential depends on three different parameters: (I) the polarizability of the drift gas, (II) the atoms exposed on the exterior of a molecule, and (III) the temperature of the ions.[51] The difference in polarizability between He and N_2 should not significantly contribute to any errors, when the inverse mobilities are linearly correlated (Figure 4). However, it is still not fully understood how far the polarizability has an impact on the rather complex movement of ions in a TW field. The differences in the type of atoms exposed to the exterior, on the other hand, can be compensated for when calibrants and analyte of the same molecular identity (i.e., peptides to calibrate for peptides, carbohydrates to calibrate for carbohydrates, *etc.*) are used. The largest uncertainty is the ion temperature. It has been previously shown that the injection of ions into the TW IM-MS cell in Synapt instruments can lead to a significant increase in the effective ion temperature.[52−54] The extent of this "heating" depends on a variety of parameters, such as mass, charge, drift gas, *etc.*, and it is very likely that ions measured in N_2 have a considerably different temperature than those ions used during measurement of the "reference" He CCSs. As a result of this varying temperature, the correlation between the inverse mobilities in He and N_2 is not necessarily linear over a broad range of CCSs any longer, which, in turn, can lead to a significant increase in the error of the estimated CCS. Therefore, we recommend calibrating carbohydrate TW IM-MS data with reference CCSs measured in the same drift gas. It is nevertheless possible to estimate pseudo-He CCSs from data measured in nitrogen, for example, when the data should be compared with theoretical CCSs obtained for model structures. However, for carbohydrates a considerably larger error needs to be taken into account in this case.

Influence of Ion Polarity. In combination with previously published data on sodiated glycans,[30] the CCSs reported here form a data set that can not only be used to calibrate TW IM-MS instruments, but also provide a starting point for further investigations on the gas-phase behavior of carbohydrates. The dextran CCSs in positive and negative polarity, for example, can be used to obtain information on the influence of the ion mode and the adduct ion on the underlying gas-phase structure. Per definition, the CCS of any ion is dependent on the charge but independent of the polarity. Recent investigations revealed that large, native-like protein complexes exhibit almost identical

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CCSs in positive and negative ion polarity, which points to a very similar gas-phase structure.[55] The carbohydrates investigated here, however, are small in comparison to the above-mentioned proteins, and different adducts are likely to yield considerably different structures.

To qualitatively assess the impact of ion polarity and adduct formation on the structure, the CCSs of a dextran sample in positive and negative ion polarity are compared in Figure 5.

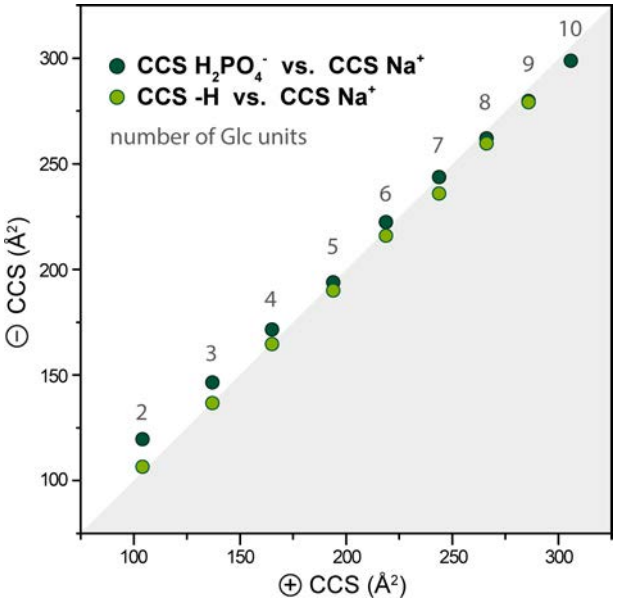

Figure 5. Comparison of dextran He CCSs in the positive and negative ion mode. The dots indicate the relation of CCSs of sodiated dextran molecules compared to CCSs of deprotonated species and phosphate adducts. The diagonal line depicts identical CCSs in positive and negative ion mode.

Positively charged, sodiated ions, and negatively charged, deprotonated ions, exhibit almost identical CCSs regardless of the number of glucose units. This observation indicates, at least on a coarse scale, that both types of ions adopt comparable gas-phase structures. Phosphate adducts, on the other hand, exhibit considerably increased CCSs for dextran molecules with up to seven glucose units. This is expected to a certain extent, because the large phosphate ions contribute to the overall size of these smaller dextran oligomers. However, these differences seem to disappear with increasing chain length, and almost identical CCSs are obtained for sodiated, deprotonated, and phosphate adducted species consisting of eight and nine glucose units. Therefore, it can be assumed that, regardless of the polarity and type of adduct, comparable structures are adopted by larger dextran molecules in the gas phase. Interestingly, similar trends have also been reported in a recent systematic study on alkali ion adducts of different oligosaccharide isomers.[56] With increasing radius of the metal ion, a noticeable increase in CCS was observed for di- and trisaccharides, while almost unchanged CCS have been obtained for larger penta- and hexa-saccharides. This influence on metal ion radius on CCSs indicates that the impact of the adduct ion, and polarity becomes negligible at a certain analyte size and further studies are needed to verify this assumption on a broader basis.

CONCLUSION

Here, we report over 500 He and N_2 CCSs of complex carbohydrates as well as their fragments measured in the negative ion mode. Our data show that CCSs of glycans and their fragments can be estimated from TW IM-MS data after careful calibration. Furthermore, we show that partially hydrolyzed dextran, an already widely used carbohydrate reference compound in liquid chromatography, is an additional suitable and convenient calibrant for CCS estimations. Especially for techniques that are based on chromatographic methods coupled to MS and IM-MS, dextran has an outstanding potential to serve as a gold standard in the future, which can be used to simultaneously obtain reference and calibration data for retention time, m/z, and CCS. In addition, these data can be easily incorporated into databases and software tools,[57] which should not only help to simplify the currently time-consuming calibration procedure, but could also pave the way for full automation of the IM-MS data analysis.

Moreover, we critically assessed the CCS estimation protocol with respect to the utilized drift gases. Our data show that a calibration using nitrogen reference CCSs for data measured in nitrogen generally yields smaller errors than those calibrated with helium reference CCSs. These findings clearly show that, besides the obvious mass and polarizability differences of the utilized drift gas, also several other factors play an important role in the separation process of ions in TW IM-MS instruments.

Furthermore, the presented data provide a good starting point for further investigations on the gas-phase structure and behavior of complex carbohydrates. As an example, we analyzed the differences in CCSs measured for dextran oligomers as different adducts and with different ion polarity. These data indicate that these parameters have a significant impact on the gas-phase structure of smaller oligosaccharides, while only minor changes are observed with increasing number of monosaccharide building blocks.

ASSOCIATED CONTENT

Supporting Information

Detailed experimental parameters and the exact calibration procedure; all CCSs from the *N*-glycan samples and dextran. This material is available free of charge via the Internet at http://pubs.acs.org/. In addition, a calibration spreadsheet with CCS reference values for carbohydrates, peptides, and oligonucleotides is available online at http://www.bcp.fu-berlin.de/chemie/pagel.

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Notes

The authors declare no competing financial interest.

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