Enhanced carbohydrate structural selectivity in ion mobility-mass spectrometry analyses by boronic acid derivatization†

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The boronic acid derivatization of carbohydrates is demonstrated as an ion mobility shift strategy to improve confidence in the identification and characterization of carbohydrate assignments using ion mobility-mass spectrometry.

Ion mobility-mass spectrometry (IM-MS) has demonstrated great potential in life sciences research, in that it provides two-dimensional separations on the basis of both analyte ion structure and mass-to-charge ratio (m/z), respectively.[1,2] Separation selectivity in the IM dimension is achieved on the basis of nearly thermal collisions of the analyte ions with a neutral drift gas (typically He) and subsequent calculation of the ion-neutral collision cross-section or apparent ion surface area. Thus, IM-MS can provide rapid (μs–ms) two-dimensional separations of structurally distinct, but isobaric species. This attribute is particularly important in the IM-MS analysis of complex biological samples, such as whole cell lysates,[3] plasma,[4] homogenized tissue[3,5] or directly from thin tissue sections.[6]

For singly-charged ions, the correlation of collision cross-section *vs.* mass is strongly dependent on the specific class of biomolecule. In general, the relative gas-phase packing efficiencies of several molecular classes have been observed to vary in the order nucleotides > carbohydrates > peptides > lipids,[2,7] which means that at a given m/z, nucleotides and lipids adopt structures corresponding to the smallest and largest surface areas, respectively. For example, Fig. 1 illustrates results for the IM-MS separation of singly-charged peptides and *N*-linked glycans for a model glycoprotein (ribonuclease B, pancreatic bovine), which was proteolytically digested with trypsin, followed by the release of *N*-linked glycans *via* PNGase F. At a given m/z, signals occurring at longer IM drift times (*i.e.* arrival time distribution (ATD)) are identified as peptides, while those at shorter ATDs are identified as carbohydrates. Many contemporary MS-based glycomic and proteomic methodologies for the characterization of protein glycoforms involve separation of the glycans from the peptides/proteins for subsequent independent analysis. This process is time-consuming and labor intensive. To simplify such studies, and to minimize sample handing and losses from liquid-phase affinity purification/separations, IM-MS could potentially provide a high throughput platform for the simultaneous identification of both peptides/proteins and carbohydrates or other glycoconjugates.

IM-MS was successfully used to study monosaccharides and could be used to differentiate between isomeric monomers.[8] To date, IM-MS studies have predominately focused on the separation of simple carbohydrates.[9] However, as illustrated in Fig. 1, in the analysis of complex biological samples, the correlation of molecular class in conformation space can be insufficient to distinguish to which class of molecule a

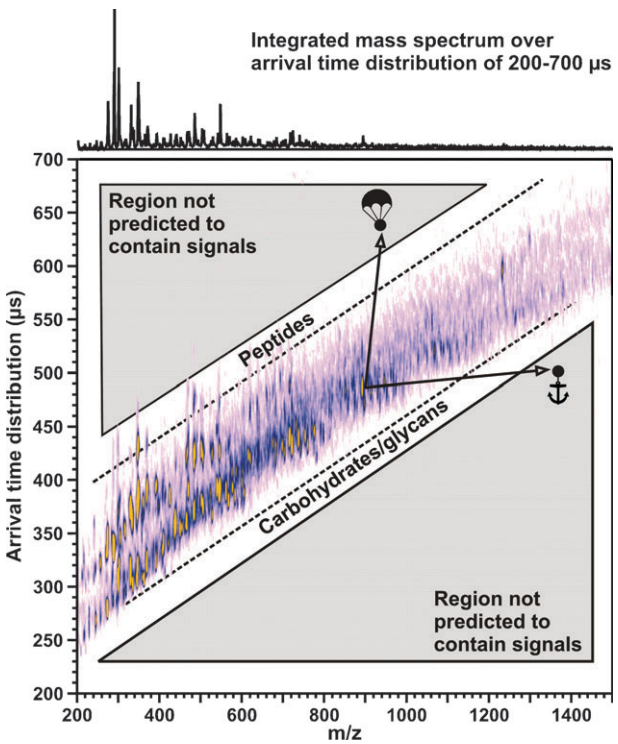

Fig. 1 A plot of conformation space in the MALDI-IM-MS analysis of the glycoprotein ribonuclease B. The protein was first proteolytically digested with trypsin, followed by *N*-glycan release using PNGase F. The carbohydrates present are from in-source decay fragmentation of the intact glycans. The dashed lines are to visualize qualitatively where the peptide and glycan signals occur. Ion mobility shift agents are used to shift specific analytes into regions of conformation space that are not expected to contain signals, as hypothetically illustrated by the parachute and anchor icons, respectively.

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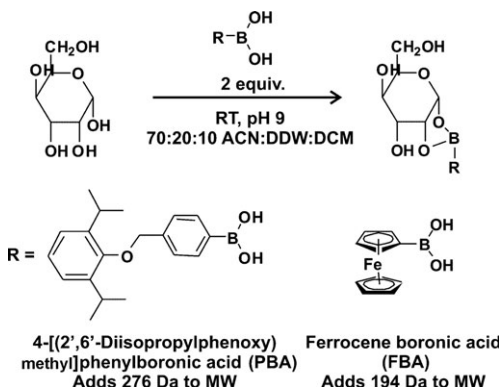
† Electronic supplementary information (ESI) available: Systematic names and structures for the carbohydrates included in this study are provided, along with tabulated values of collision cross-section for underivatized and derivatized carbohydrates, respectively. See DOI: 10.1039/b810421b

particular signal corresponds. To further increase the information derived from IM-MS carbohydrate analyses, the feasibility of a new ion mobility shift reagent strategy is demonstrated.

Ion mobility shift reagents consist of coordinating or covalently modifying a selected chemical functionality. For example, the shift reagent could be of a lower or higher density than the type of molecule to be derivatized in order to structurally shift the desired signals to regions of conformation space above or below the predicted molecular class correlation. Non-covalent ion mobility shift strategies have been demonstrated for selectively shifting isomeric amines and peptides by using crown ethers,[10] and shifting carbohydrates through coordination with different metals and metal acetates.[8] In contrast to non-covalent tagging, covalently attached ion mobility shift reagents offer several potential advantages, including: (i) quantitative derivatization of the analytes of interest, (ii) relative quantitation using isotopically labelled tags, (iii) the potential for adding affinity capture moieties onto the tag itself and (iv) the potential for inclusion of a permanent charge carrier for enhanced ionization efficiencies. Boronic acid-based functionalization was selected for carbohydrate derivatization because of its demonstrated utility in spectroscopic measurements.[11,12]

The use of boronic acids (BAs) to covalently modify saccharides was known for many years prior to the first quantitative evaluation of BA chemistry published by Lorand and Edwards in 1959.[13] In this work, they determined the equilibrium constants for the reactions of benzene boronic acid with various polyols, including a suite of monosaccharides; for the latter, these values ranged from 110–4400 mol^{-1}. Thus, although the reaction scheme depicted below is slightly reversible, the selective reaction of BAs with *cis*-diols on carbohydrates is nearly quantitative and can be tuned by optimizing the pH.

A suite of carbohydrates, including three disaccharides, two trisaccharides, two tetroses and two pentoses, were derivatized using two structurally distinct BAs under the reaction conditions depicted in Scheme 1. The collision cross-sections for the underivatized and derivatized species were calculated to evaluate



Scheme 1 A generalized scheme for the reaction of carbohydrates with the substituted BAs used in these studies. BAs prefer to attach to the reducing end of *cis*-diols, forming a stable five-membered ring. The structures and molecular weights of the nine carbohydrates investigated, ranging from disaccharides to pentoses, are provided in the ESI, Fig. S1.†

the effect of derivatization on where the resulting structural shift appeared in the IM-MS conformation space.

Specifically, the carbohydrates lacto-*N*-fucopentaose 1 (LNFP1) and lacto-*N*-fucopentaose 2 (LNFP2) from human milk were obtained from Dextra Laboratories (Reading, UK); *N*-acetyl-D-lactoseamine, maltose and lactose were obtained from Sigma (St. Louis, MO, USA). The synthetic glycans Galα1-3Galβ1-3GlcNAcβ-Sp, Galα1-4Galβ1-4GlcNAcβ-Sp, Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp and Galβ1-3-GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp (Sp = $CH_2CH_2N_3$) were obtained from the Carbohydrate Synthesis/Protein Expression Core of The Consortium for Functional Glycomics. Each carbohydrate was derivatized with both ferrocene boronic acid (FBA) and 4-[(2′,6′-diisopropylphenoxy)methyl]phenylboronic acid (PBA), obtained from Sigma. Solutions of BA were prepared in DMSO to final concentrations of 10–50 mM. Carbohydrate solutions were prepared in 70 : 20 : 10 ACN : DDW : DCM to a final concentration of 0.03 mM and were adjusted to the optimized pH for each BA (7.5 and 9.0 for PBA and FBA, respectively).[11] The BA solution was added to the carbohydrate solution, resulting in a molar ratio of *ca.* 2 : 1 BA to carbohydrate. The reaction was then allowed to proceed for 12 h, or alternatively sonicated for 5 min at room temperature.[14]

To determine collision cross-sections, MALDI analysis was performed by mixing saturated 2,5-dihydroxybenzoic acid in 50% ethanol with the analyte in a 200 : 1 molar ratio. Samples were spotted onto a stainless steel plate and dried under vacuum. The MALDI-IM-TOF mass spectrometer was equipped with a 14.7 cm ion mobility drift cell, which was maintained at a pressure of *ca.* 3 Torr helium, and an orthogonal reflectron TOF mass spectrometer with a 1 m flight path maintained at 5×10^{-8} Torr. This instrument is similar to those described previously.[2]

The ion-neutral collision cross-section (Ω) was calculated by determining the drift time (t_d) of the ion packet across the cell under low-field conditions. Collision cross-sections were calculated by the following equation:

$$\Omega = \frac{(18\pi)^{1/2}}{16}\frac{ze}{(k_b T)^{1/2}}\left[\frac{1}{m_i}+\frac{1}{m_n}\right]^{1/2}\frac{t_d E}{L}\frac{760}{p}\frac{T}{273.15}\frac{1}{N_0}$$

where L is the length of the drift cell, E is the electrostatic field strength ($\sim$90–120 V cm^{-1}), z is the charge state of the ion, e is elementary charge, m_i and m_n are the masses of the ion and the neutral drift gas, respectively, N_0 is the gas number density at STP, and p and T are the pressure ($\sim$3 Torr) and temperature ($\sim$293 K), respectively.[15]

In order to determine the effect of the BA derivatization with carbohydrates, collision cross-sections were determined for both protonated and/or sodium-coordinated species. For the FBA-derivatized species, both $[M + H]^+$ and $[M + Na]^+$ species were observed in nearly equal abundance; however, for the PBA-derivatized species, the preponderance (>98%) of the signal was for sodium-coordinated species, so protonated cross-sections are not reported for comparison purposes. The collision cross-sections of the underivatized carbohydrates are also provided (for specific values refer to the ESI, Table S2†).

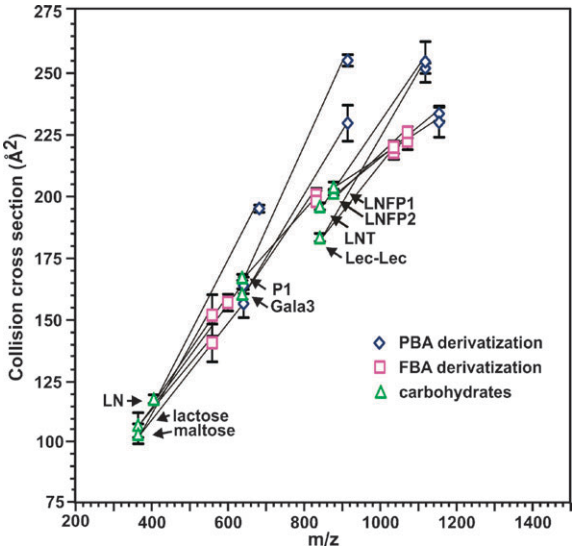


Fig. 2 A plot of collision cross-section *vs.* m/z for underivatized, and FBA- and PBA- (all present as $[M + Na]^+$) derivatized carbohydrate species. Error bars on the derivatized species represent $\pm 1\sigma$. Refer to the ESI, Table S2† for tabulated values.

The effect of derivatization on the collision cross-sections of carbohydrates is illustrated in Fig. 2. FBA was selected to serve as a high density tag (*i.e.* to serve as an anchor in Fig. 1), owing to the addition of Fe and tightly coordinated cyclopentadienyl groups. However, the increase in surface area and mass upon FBA derivatization appeared to shift signals along the carbohydrate correlation. PBA was selected to perform as a low density tag (*i.e.* to serve as a parachute in Fig. 1), owing to its sterically bulky diisopropylphenyl moiety. In this case, the desired shift in conformation space was achieved for most species, as indicated by a greater increase in surface area than that predicted for a commensurate increase in underivatized carbohydrate mass. Furthermore, the carbohydrates studied also contained four pairs of isomeric carbohydrates, which is a serious complication when using mass spectrometry alone. However, using IM-MS, these isobaric carbohydrates were separated on the basis of structure.

It should be noted that there exists a diverse collection of commercially available BAs, exhibiting a range of chemical functionality. Thus, it should be feasible to select specific BAs to promote different but particular anhydrous folding forces, which in turn should result in ion mobility shift tuneability. Furthermore, in contrast to underivatized carbohydrates, the derivatization described herein provides three distinct advantages: (i) tuneability in the separation of isobaric species, (ii) enhanced ionization efficiency (*e.g.* upon FBA derivatization, a *ca.* 2× enhancement in sensitivity is observed) and (iii) the potential to serve as fragment labels in CID[16] and as IR active species in IRMPD[14] for tandem MS studies. Owing to covalent derivatization, new classes of BAs incorporating moieties for affinity purification, isotopic enrichment for relative expression determination and the inclusion of structural standards (*e.g.* fullerenes) provide new avenues for further characterization, identification and quantification of carbohydrate species in glycomics and glycoproteomics.

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Notes and references

1 S. J. Valentine, X. Liu, M. D. Plasencia, A. E. Hilderbrand, R. T. Kurulugama, S. L. Koeniger and D. E. Clemmer, *Expert Rev. Proteomics*, 2005, **2**, 553–565; T. Wyttenbach and M. T. Bowers, *Annu. Rev. Phys. Chem.*, 2007, **58**, 511–533; A. B. Kanu, P. Dwivedi, M. Tam, L. Matz and H. H. Hill, Jr, *J. Mass Spectrom.*, 2008, **43**, 1–22.
2 J. A. McLean, B. T. Ruotolo, K. J. Gillig and D. H. Russell, *Int. J. Mass Spectrom.*, 2005, **240**, 301–315.
3 S. Myung, Y. J. Lee, M. H. Moon, J. Taraszka, R. Sowell, S. Koeniger, A. E. Hilderbrand, S. J. Valentine, L. Cherbas, P. Cherbas, T. C. Kaufmann, D. F. Miller, Y. Mechref, M. V. Novotny, M. A. Ewing, C. R. Sporleder and D. E. Clemmer, *Anal. Chem.*, 2003, **75**, 5137–5145.
4 X. Liu, M. Plasencia, S. Ragg, S. J. Valentine and D. E. Clemmer, *Briefings Funct. Genomics Proteomics*, 2004, **3**, 177–186; S. J. Valentine, M. D. Plasencia, X. Liu, M. Krishnan, S. Naylor, H. R. Udseth, R. D. Smith and D. E. Clemmer, *J. Proteome Res.*, 2006, **5**, 2977–2984; X. Liu, S. J. Valentine, M. D. Plasencia, S. Trimpin, S. Naylor and D. E. Clemmer, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 1249–1264; D. Isailovic, R. T. Kurulugama, M. D. Plasencia, S. T. Stokes, Z. Kyselova, R. Goldman, Y. Mechref, M. V. Novotny and D. E. Clemmer, *J. Proteome Res.*, 2008, **7**, 1109–1117.
5 J. A. Taraszka, R. Kurulugama, R. A. Sowell, S. J. Valentine, S. L. Koeniger, R. J. Arnold, D. F. Miller, T. C. Kaufman and D. E. Clemmer, *J. Proteome Res.*, 2005, **4**, 1223–1237; X. Liu, B. R. Miller, G. V. Rebec and D. E. Clemmer, *J. Proteome Res.*, 2007, **6**, 3134–3142.
6 S. N. Jackson, M. Ugarov, T. Egan, J. D. Post, D. Langlais, J. A. Schultz and A. S. Woods, *J. Mass Spectrom.*, 2007, **42**, 1093–1098; J. A. McLean, W. B. Ridenour and R. M. Caprioli, *J. Mass Spectrom.*, 2007, **42**, 1099–1105.
7 J. M. Koomen, B. T. Ruotolo, K. J. Gillig, J. A. McLean, D. H. Russell, M. Kang, K. R. Dunbar, K. Fuhrer, M. Gonin and J. A. Schultz, *Anal. Bioanal. Chem.*, 2002, **373**, 612–617; A. S. Woods, M. Ugarov, T. Egan, J. Koomen, K. J. Gillig, K. Fuhrer, M. Gonin and J. A. Schultz, *Anal. Chem.*, 2004, **76**, 2187–2195; L. S. Fenn and J. A. McLean, *Anal. Bioanal. Chem.*, 2008, **391**, 905–909.
8 P. Dwivedi, B. Bendiak, B. H. Clowers and H. H. Hill, Jr, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 1163–1175.
9 Y. Liu and D. E. Clemmer, *Anal. Chem.*, 1997, **69**, 2504–2509; C. S. Hoaglund, S. J. Valentine and D. E. Clemmer, *Anal. Chem.*, 1997, **69**, 4156–4161; S. Lee, T. Wyttenbach and M. T. Bowers, *Int. J. Mass Spectrom. Ion Processes*, 1997, **167/168**, 605–614; B. H. Clowers, P. Dwivedi, W. E. Steiner, H. H. Hill, Jr and B. Bendiak, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 660–669.
10 C. S. Creaser, J. R. Griffiths and B. M. Stockton, *Eur. J. Mass Spectrom.*, 2000, **6**, 213–218; A. E. Hilderbrand, S. Myung and D. E. Clemmer, *Anal. Chem.*, 2006, **78**, 6792–6800.
11 W. Wang, X. Gao and B. Wang, *Curr. Org. Chem.*, 2002, **6**, 1285–1317.
12 T. D. James, M. D. Phillips and S. Shinkai, *Boronic Acids in Saccharide Recognition*, Royal Society of Chemistry, Cambridge, UK, 2006; T. D. James, *Top. Curr. Chem.*, 2007, **277**, 107–152.
13 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769–774.
14 M. Pikulski, A. Hargrove, S. H. Shabbir, E. V. Anslyn and J. S. J. Brodbelt, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 2094–2106.
15 E. A. Mason and E. W. McDaniel, *Transport Properties of Ions in Gases*, John Wiley and Sons, Indianapolis, IN, USA, 1988.
16 D. Williams and M. K. Young, *Rapid Commun. Mass Spectrom.*, 2000, **14**, 2083–2091; M. K. Young, N. Dinh and D. Williams, *Rapid Commun. Mass Spectrom.*, 2000, **14**, 1462–1467.