cis-Diol functional group recognition by reactive desorption electrospray ionization (DESI)†

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Heterogeneous reactions at a solution/solid interface are utilized in an ambient mass spectrometry experiment to recognize the *cis*-diol functionality by its selective complexation reaction to form a cyclic boronate.

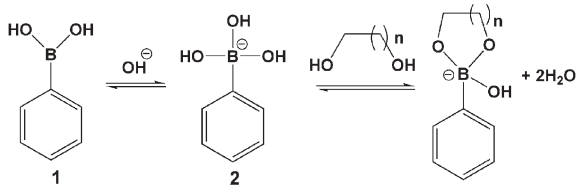
Heterogeneous reactions (*cf.* heterogeneous catalysis[1]), a class of chemical reactions in which the reactants are components of two or more phases, are of considerable practical interest. Desorption electrospray ionization (DESI)[2] is a new soft ionization method in which a pneumatically-assisted electrospray is directed onto a surface under ambient conditions; the secondary ions generated by the interaction of charged microdroplets with analyte molecules on the surface are collected and analyzed. When the spray solvent is doped with particular reagents, the unique sampling and ionization protocol of DESI also allows one to perform ion/molecule reactions at the interface between the charged microdroplets and the solid surface bearing the condensed phase analyte. Such ion/molecule reactions differ in several important characteristics from conventional ion/molecule reactions: i) they take place under heterogeneous not homogenous conditions with respect to physical phase, at the solution/solid interface; ii) they occur under ambient conditions, specifically at atmospheric pressure instead of vacuum; iii) the neutral analyte molecule and the molecular precursor to the reactant ion can be heavy, polar and non-volatile; iv) *in-situ* derivatization of the analyte on the surface can occur through the reaction and offer a fast and selective method of detection of the analyte; v) standard solution-phase reagents can be used to produce finely controlled reactions based on well-known solution chemistry. These characteristics have the potential to extend the range and increase the richness of the ion chemistry accessible to mass spectrometry and also to provide new analytical advantages. It is the goal of this study to test and verify these predictions using a functional group specific reaction which is well-established in solution.

The general type of experiment discussed here can be categorized as a reactive DESI experiment, one which uses a neutral reagent selected to generate solution phase reagent ion which can undergo characteristic bond-forming reactions with the adsorbed analyte being examined. Previous reports on reactive DESI are restricted to simple adduct formation, including the generation of a trimeric cluster of Cu^{2+} and L-tryptophan with phenylalanine[2a] and the complexation of RDX with the anion CF_3COO^-,[2a,c] or of TNT with the methoxide anion.[2c] In parallel with the present study, Eberlin reactions under DESI conditions[2i] involving the transacetalization of acylium cations and cyclic acetals have been carried out.

In this study, we begin a systematic study of the scope and value of heterogeneous reactions in mass spectrometry by considering the *cis*-diol functionality. We have selected several biologically important cases and attempted their recognition *via* cyclization with benzeneboronate anions $PhB(OH)_3^-$ using DESI. The biomolecules chosen include both aliphatic diols (carbohydrates and steroids) and aromatic diols (flavanols and catecholamines). The rapid detection of these substances by mass spectrometry under ambient conditions is normally challenging because most of them are not ionized efficiently by electrospray (ESI) and matrix assisted laser desorption/ionization (MALDI).[3] The rapid and reversible covalent complexation of phenylboronic acid **1** with 1,2- or 1,3-diols to form cyclic boronates in basic aqueous medium is well known (Scheme 1, n = 1 or 2).[4] It is believed[4b] that the reaction involves a tetrahedral arylboronate intermediate $PhB(OH)_3^-$ **2** rather than deprotonated phenylboronic acid $PhB(OH)O^-$. In solution this characteristic reaction[3b,5,6] is selective for rigid *cis*-diols as opposed to *trans*-diols.

Reactive DESI experiments were performed using, as the spray solvent, 1.0–3.0 mM aqueous solutions of phenylboronic acid $PhB(OH)_2$ adjusted to be slightly basic (pH 9∼10) with ammonium hydroxide or NaOH. Analyte solutions in methanol or in water were deposited onto the chosen surface for DESI experiments drying. All compounds used are commercially available. The surface onto which the analyte of interest was placed was either paper or metal. The mass spectrometer was a standard ion trap instrument (Thermo Finnigan LTQ, San Jose, CA) and was operated in the negative ion mode. Details of the DESI parameters are given elsewhere.[2c]

One similarity between the reactive DESI experiments and those encountered in conventional heterogeneous reactions at liquid/solid surfaces is that the reactant ions in DESI are present in solution phase, in the fine charged droplets generated from the electrospray. (Studies of the ionization process using laser



Scheme 1

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tomography indicate that completely desolvated primary ions can be generated by choice of different ionization conditions.) The experiment is unusual, however, in that the products of the heterogeneous reactions are directly detected by mass spectrometry after desolvation in the atmospheric interface some microseconds after the reactive interaction event.

Fig. 1a shows the negatively charged reactive DESI mass spectrum of a blank paper surface (*i.e.* no sample present). The ion of *m*/*z* 121 corresponds to deprotonated phenylboronic acid $PhB(OH)O^-$ and that at *m*/*z* 139 is the tetrahedral anion $PhB(OH)_3^-$ **2**. These two anions are generated in solution when the pH of the spray solvent containing phenylboronic acid $PhB(OH)_2$ is adjusted to be basic using ammonium hydroxide. These solvated anions are desolvated in the atmospheric interface of the mass spectrometer and appear in the mass spectrum in this form. Collision-induced dissociation (CID) experiments on the mass-selected ion **2** yield the deprotonated phenylboronic acid anion $PhB(OH)O^-$ (*m*/*z* 121) and $H_2BO_3^-$ (*m*/*z* 61) by loss of water and benzene, respectively, confirming its structure. The ion of *m*/*z* 243 in Fig. 1a is assigned as the condensation product of the anion $PhB(OH)_3^-$ (*m*/*z* 139) and phenylboronic acid $PhB(OH)_2$, since it yields fragment ions of *m*/*z* 121, 139 and 225 upon CID. The ion of *m*/*z* 154 is likely generated by electrochemical oxidation of $PhB(OH)_2$ during electrospray and this structural assignment is supported by the observation that no *m*/*z* 154 was generated when the spray voltage was lower than 2 keV and also by the fact that the ion dissociates to yield $PhB(OH)O^-$ (*m*/*z* 121) by loss of $HO_2^{\bullet}$. Also, note that adduct ions of phenylboronic acid $PhB(OH)_2$ with *m*/*z* 139 and 154 are observed at *m*/*z* 261 and 276, respectively.

Figs. 1b and 1c display the mass spectra recorded from the reactions of microdroplets containing anion **2** with solid fructose present on a surface and with an aqueous glucose solution adsorbed on a cotton tip, respectively. In both cases, sampling took place in the ambient environment not in the mass spectrometer. Compared with the blank spectrum (Fig. 1a), Figs. 1b and 1c clearly show product ions (*m*/*z* 283) due to complexation reactions of anion **2** with D-fructose and D-glucose, respectively. In agreement with the 1 : 5 natural isotopic abundance ratio of ^{10}B to ^{11}B, the ratio of *m*/*z* 282 to *m*/*z* 283 is close to 1 : 5. Scheme 2 illustrates these observed reactions of anion **2** with D-fructose and D-glucose. Note the furanose conformation of D-glucose drawn in Scheme 2(b): it is based on the fact that boronic acids bind glucose preferentially in the α-furanose form and not in the more abundant α-pyranose form.[7] Scheme 2 also proposes as the most likely structures for the product ions **3** and **4**, the cyclic boronates in which the boron binds at the *cis* 1,2-diol functionality of the β-D-fructofuranose and α-D-glucofuranose, although binding at the hydroxyl groups on C3, C5 or C6 is also possible in the case of glucose. Tandem mass spectrometry data were recorded to provide information on the nature of these covalent complexes (refer to their CID data in Fig. 1S, ESI†). The ion **3** (*m*/*z* 283) upon CID fragments by sequential losses of water, giving ions of *m*/*z* 265 and 247. Two other characteristic fragment ions, with *m*/*z* 179 and 205, were generated by the loss of Ph–B=O and benzene, respectively. The CID spectrum of **4** shows some differences from that of the fructose product ion **3**, and these can serve to differentiate fructose from its isomer, glucose. There are sequential losses of three water molecules to form the ions of *m*/*z* 265, 247 and 229 from **4**. The major fragment ion (*m*/*z* 265) of **3** and **4** is also present in the single stage mass spectra (Figs. 1b and 1c), respectively, as is the ion of *m*/*z* 301, the adduct of $PhB(OH)O^-$ (*m*/*z* 121) with monosaccharide. Simultaneous generation of the characteristic product ion at *m*/*z* 283 and the ions at *m*/*z* 265 and 301 by reactive DESI provides a new *in-situ* and selective method for detection of fructose and glucose without any sample pre-treatment. Note that in the case of D-glucose, the glucose on the cotton tip probably was still in solution during the reaction (the experimental design was chosen in order to test the possibility of the direct detection of aqueous glucose given the importance of glucose-containing physiological samples). The analogous heterogeneous reaction with solid glucose on the surface was also successfully observed (Fig. 2S, ESI†). Another experiment was carried out in which electrospray ionization was performed on a basic aqueous solution containing glucose and phenylboronic acid. The MS^2 spectrum of

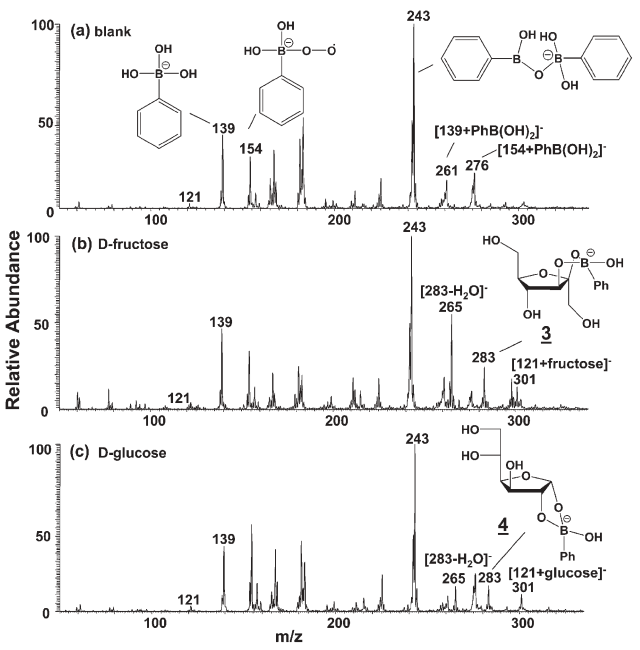
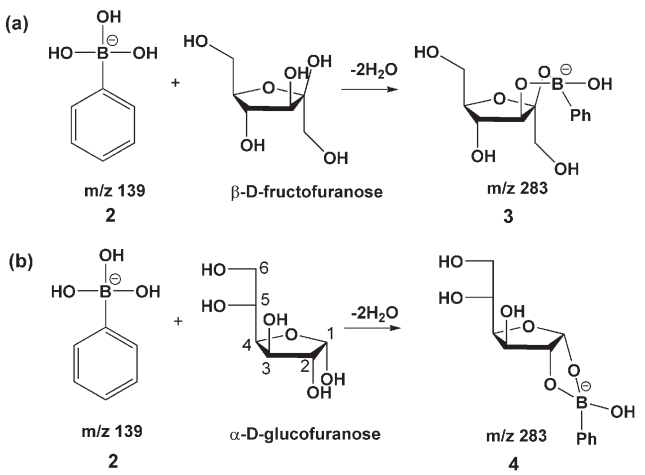


Fig. 1 Reactive DESI mass spectra showing the ionic species generated from $PhB(OH)_3^-$ anions upon interaction with (a) a blank surface, (b) D-fructose on the surface and (c) D-glucose absorbed in a cotton tip.



Scheme 2

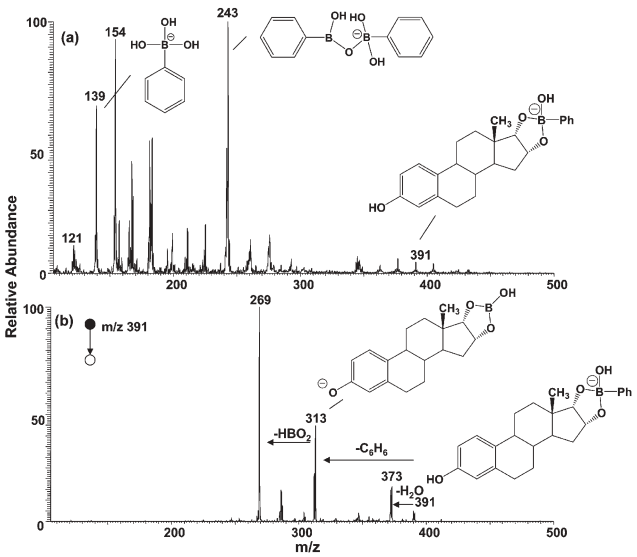


Fig. 2 (a) Reactive DESI spectrum showing the products of heterogeneous reactions of $PhB(OH)_3^-$ anions with solid *cis*-estriol on a stainless metal surface; (b) MS^2 product ion spectrum of the reaction product ion of *cis*-estriol (*m*/*z* 391).

the observed product (*m*/*z* 283, Fig. 3S, ESI) is in good agreement with that of ion **4**, further confirming the structure of the reactive DESI product.

Other biologically-active aliphatic diols examined were 1,3,5(10)-estratrien-3,16α,17α-triol (*cis*-estriol) and 1,3,5(10)-estratrien-3,16α,17β-triol (*trans*-estriol). Differentiation of *cis*- and *trans*-diols by conventional ion/molecule reactions[8] with phosphenium ions and trimethyl-Group 14 cations in the mass spectrometer has been reported. The major difference in the present reactive DESI study from the previous reports[8] is that the estriol sample was present as the solid on a surface and the reaction was performed under ambient conditions, making it easier to manipulate the sample and perform the practical application. Fig. 2a displays the reactive DESI mass spectrum recorded for *cis*-estriol. The corresponding product ion of the complexation is seen at *m*/*z* 391. Product characterization by CID analysis shows that *m*/*z* 391 forms fragment ions of *m*/*z* 373 and 313 by loss of water and benzene, respectively. The major fragment ion at *m*/*z* 269 is probably due to further loss of HBO_2 from *m*/*z* 313. When *cis*-estriol was replaced by *trans*-estriol, no cyclization product ion of *m*/*z* 391 was observed. This expected result is in agreement with the fact[9] that complexation of phenylboronic acid in solution is stereoselective and occurs with *cis*-1,2-cyclopentanediols but not with *trans*-cyclopentanediols.

In addition to the aliphatic diols discussed above, model aromatic diols such as catechol and 3-fluorocatechol as well as aromatic diol biomolecules such as quercetin and (−)-epinephrine were also investigated using reactive DESI. Quercetin is an important natural pigment and antioxidant widely distributed in vegetables, berries and fruit. Epinephrine is a hormone and a neurotransmitter released by neurons to regulate activity of target tissues including brain cells and muscle cells. The detection of antioxidants and neurotransmitters is receiving increasing attention. The occurrence of heterogeneous reactions between $PhB(OH)_3^-$ and each of these compounds on surfaces has been successfully observed (Figs. 4S and 5S, ESI†). Characteristic dissociation channels, the losses of benzene and Ph–B=O, were observed for the product ions upon CID, confirming their structures. Also, in the case of catechol and 3-fluorocatechol, the product ions in the reactive DESI mass spectra were more abundant than those of the aliphatic diols discussed before, probably because the increased acidity of OH of the diols increases the forward rate constant and decreases the reverse rate constant kinetically in the complex formation reaction of boronic acid.[10]

In conclusion, this study has described novel reactive DESI experiments for the rapid and selective detection of some biologically important compounds using heterogeneous ambient reactions of the $PhB(OH)_3^-$ anion with *cis*-diols. It also provides an example of the fact that the ion chemistry traditionally performed in the gas phase at low pressure can be studied at the interface of microdroplets and surfaces at atmospheric pressure. The application of boronic acid reactions for the detection of glycopeptides from tryptic protein digests is under investigation.

The successful prediction and demonstration of the heterogeneous reactions reported here encourages the view that additional examples will be encountered and that the scope of ion/molecule chemistry will indeed be widened through the reactive desorption electrospray ionization method.

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