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Desorption/Ionization on Porous Silicon Mass Spectrometry Studies on Pentose–Borate Complexes

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Desorption/ionization on porous silicon mass spectrometry (DIOS-MS) was used to investigate the binding affinities between aldopentose isomers and boron. Boron has been recognized for its importance in pentose synthesis and stabilization in prebiotic conditions. Boron may also account for the fact that ribose, among other aldopentoses, is the favored building block in RNA synthesis. This research started with the detection of aldopentoses in the positive mode through cationization and the aldopentose–borate complexes in the negative mode. Then two competition schemes, one using a pentose structure analogue and the other using ^{13}C-labeled ribose, were designed to compare the relative binding affinities of four aldopentoses (xylose, lyxose, arabinose, and ribose) to boron. Both approaches determined the binding preference to be ribose > lyxose > arabinose > xylose. This work illustrates the potential of DIOS-MS in the analyses of nonvolatile, small molecules in delicate chemical equilibria. Without externally introduced matrices, background signals are not a limiting factor. Furthermore, the possible dramatic change of pH associated with the matrix introduction, which may disturb the equilibria of interest, is avoided.

*D*esorption/*i*onization *o*n porous *s*ilicon *m*ass *s*pectrometry (DIOS-MS) is a surface-enhanced laser desorption/ionization technique featured with simple sample preparation where no additional matrix compounds are needed.[1] DIOS can be readily employed in instruments with a MALDI ion source, and the system is capable of high throughput analysis. The technique has found applications in many areas, and a wide range of analytes has been investigated by DIOS-MS, including peptides, drug molecules, protein digests, and carbohydrates.[2–6] Researchers have also explored the technique under atmospheric pressures,[7,8] using an infrared laser,[9,10] as well as in areas of forensics,[11,12] polymer,[12,13] quantitative analysis,[14] and automated enzymatic activity assays.[15] Most recently, surface modifications on porous silicon introduced new research opportunities,[16,17] underlined by the highly sensitive detection of ~480 molecules.[18]

In this report, DIOS was used as an ionization technique for simple carbohydrate molecules in both the positive and negative modes. The low molecular weight, negatively charged pentose–borate complexes were successfully observed in a parallel, high-throughput fashion. The results were used to determine, for the first time by mass spectrometry, the relative binding affinities of pentose–borate complexes.

An important question in the evolution of ribonucleic acids (RNAs) is why ribose is favored over other pentose isomers. It has been shown that the borate ion, present in tourmalines and evaporites in deserts, plays an important role in the synthesis of pentoses (5-carbon monosaccharides) from simple organic precursors: formaldehyde and glycolaldehyde in the laboratory.[19] The borate ion is also proven to stabilize pentoses through the formation of boron–diol complexes, which prevents them from undergoing "browning" degradation.[20] Verchere and Hlaibi conducted the first comprehensive analysis that considered the effect

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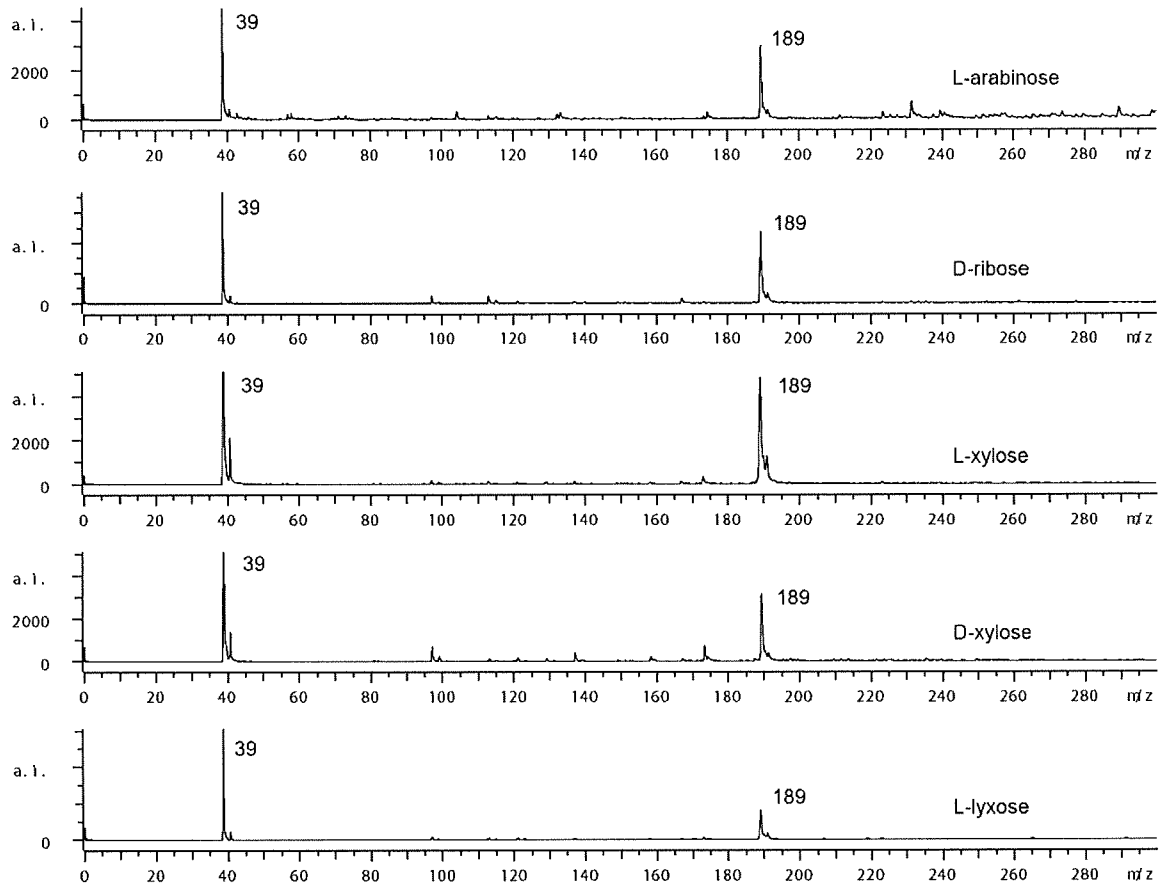

Figure 1. Five pentoses (m.w. 150), D-ribose, L-arabinose, L-xylose, and D-xylose, L-lyxose, formed adduct ions [pentose + K]$^+$ of *m/z* 189. Clean DIOS mass spectra were obtained at the pentose to K^+ ratio of 10:1, where K^+ and [pentose + K]$^+$ peaks were the only prominent signals.

of boron in the conformational equilibrium of carbohydrates. Later these authors included this effect in the calculation of the association constants by using the combination of potentiometric titration and $^{11}B/^{13}C$ NMR spectroscopy.[21,22]

While boron has been recognized for its importance in pentose synthesis and stabilization, explicit structural interactions need to be investigated. Could boron also account for the fact that ribose, among the four isomers (ribose, xylose, arabinose, and lyxose), is the favored building block for RNA? Does boron have a higher binding affinity to ribose than to the other isomeric aldopentoses, so that ribose is stabilized and enriched to be available for RNA synthesis? These questions were approached with DIOS-MS.

This application illustrates the "matrix-free" advantage of DIOS-MS; i.e., no matrix compounds are added. The absence of the matrix not only eased the background problems present in MALDI-MS but also allowed the analysis free of the chemical environments introduced by the matrix, such as low pH. The unique features suggest DIOS-MS as a promising technique in applications where MALDI is not compatible with the experimental conditions, or the analyte ions are in the low mass range.

EXPERIMENTAL METHODS

Chemicals and Reagents. All reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO) if not otherwise stated. $^{13}C_5$-ribose was purchased from Omicron Biochemicals Inc. (South Bend, IN). All solutions were aqueous and deionized water was used. Crystalline silicon wafers were purchased from Silicon Sense Inc. (Nashua, NH).

Mass Spectrometry Analysis. DIOS-MS analysis was performed on a Bruker Daltonics (Billerica, MA) Reflex II MALDI-TOF mass spectrometer retrofitted with delayed extraction in either the positive or the negative ion detection mode. Accelerating voltage was set at 20 kV, and the laser power was estimated to be a few microjoules. Most analyses were carried out in the linear mode, while the reflectron mode was used in $^{13}C_5$-labeled ribose competition experiments. An aliquot of 0.5 μL test solution was spotted on individual porous silicon (PSi) spots.

Manufacture of Porous Silicon Chip. The porous silicon surface was manufactured with the HOME (H_2O_2-*Me*tal) etching method.[23] The optimized etching procedure was as follows: a silicon chip was cut from a low-resistance (0.005–0.02 Ω/cm), n-doped, (111)-oriented crystalline silicon wafer (Silicon Sense, Nashua, NH). Covered by a patterned aluminum mask, the chip was sputter-coated with a thin layer of Au in a Hummer TM 6.2 sputter coater (Ladd Research, Williston, VT). The Au-coated silicon chip was immersed in an etching solution composed of 49% HF:30% H_2O_2:ethanol (1:1:1 by volume) for 20 s to give an arrayed chip of 16 PSi spots approximately 800 μm in diameter.

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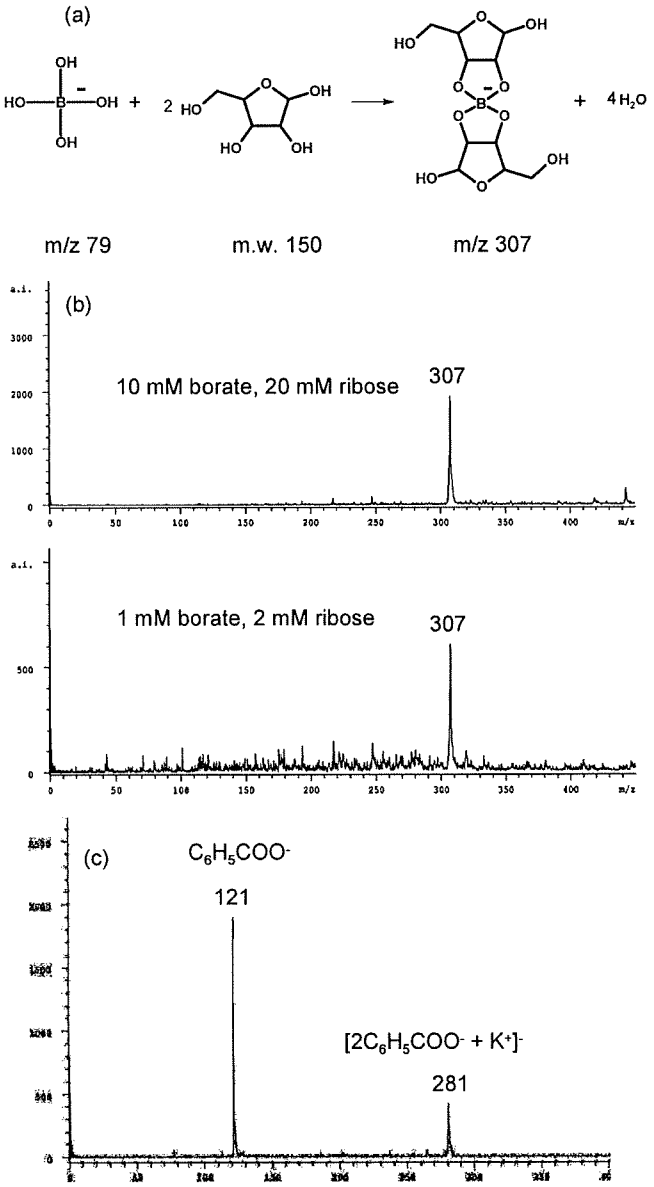

Figure 2. (a) In the presence of borate ion, pentoses adopt the cyclic five-member ring structure and form a spirane complex through the interaction between the boron atom and two diol moieties. (b) Mass spectra of ribose/$Na_2B_4O_7$ mixtures at different concentrations in the linear negative ion mode. In both spectra, the *m/z* 307 ion was the dominant signal while S/N was improved at a higher pentose/$Na_2B_4O_7$ concentration. (c) A benzoic acid/KOH mixture was used for mass calibration in the negative ion mode.

Detection of [Pentose+ K]$^+$ Adduct Ions. A potassium acetate (KAc) master solution (100 mM) was diluted into a series of solutions of 0.5, 1.0, 2.0, 10, and 50 mM. Pentose solutions (10 mM) were mixed with the KAc solutions at equal volume respectively to achieve molar ratios of pentose: K^+ = 20:1, 10:1, 5:1, 1:1, 1:5, 1:10. The five pentose isomers tested were D-ribose, L-arabinose, L-xylose, D-xylose, and L-lyxose.

Detection of Pentose–Borate Complexes. Each pentose isomer (10 mM, 20 μL) was mixed separately with a sodium borate solution ($Na_2B_4O_7$, 10 mM, 2.5 μL). The signal at *m/z* 307 was monitored in the negative mode. Further dilutions were made to determine the optimum analyte concentration.

Competition Experiments between 1,4-Anhydroerythritol and Each Pentose Isomers. 1,4-Anhydroerythritol (AHE, 0.1 M, 100 μL) and the corresponding pentose isomer (0.1 M, 100 μL) were mixed in a Eppendorf tube containing deionized water (700 μL) and vortexed thoroughly. A sodium borate solution ($Na_2B_4O_7$, 0.025 M, 100 μL) was added, and the resulting mixture was vortexed and equilibrated for 2 h at room temperature. An aliquot of 0.5 μL was spotted on the DIOS plate and allowed to dry before DIOS-MS analysis. Ions of *m/z* 251, 261, 307 were monitored in the linear negative ion mode.

Competition Experiments between $^{13}C_5$-Ribose and Each Pentose Isomers. $^{13}C_5$-ribose (10 μL, 0.1 M) and each unlabeled pentose isomer (0.1 M, 10 μL) were mixed separately in Eppendorf tubes containing deionized water (70 μL) and vortexed thoroughly. A sodium borate solution ($Na_2B_4O_7$, 0.025 M, 70 μL) was added, and the resulting mixture was vortexed and equilibrated for 2 h at room temperature. An aliquot of 0.5 μL was spotted on the DIOS plate and allowed to dry before DIOS-MS analysis. Ions of *m/z* 307, 312, 317 were monitored in the reflectron negative ion mode.

RESULTS AND DISCUSSION

Porous Silicon Chip. Two approaches are commonly used in preparing porous silicon surfaces for mass spectrometry analysis: anodic etching[1] and metal-assisted HOME (*H_2O_2-Metal*) etching.[23] Both methods were attempted and the latter approach was used routinely in this work due to the improved reproducibility, higher throughput, and less contact with hydrofluoric acid.

The characteristic physical features of a porous silicon surface, such as pore size and overall porosity, are believed to affect its performance in DIOS-MS analysis.[23] Therefore, manufacture parameters were optimized to make PSi chips reproducibly to ensure the quality of MS analysis.

In metal-assisted HOME etching, different PSi surface morphologies are achieved by varying the thickness of the deposited Au layer and/or etching duration. The Au coating was obtained by a "sputtering" technique, using argon as the processing gas (40 Torr) at 15 mA current for 40 s. The thickness of Au layer was determined to be ~10 nm.[24] Because the morphology with smaller pores was believed to give better DIOS signals,[23] the sputter current was decreased to 10 mA to achieve reduced coating thickness and a more compact pore structure. Moreover, a 20 s etching duration was chosen over longer durations for the same reason.

The final etching conditions for HOME etching were 40 s Au sputtering at 10 mA, followed by 20 s HF etching. Scanning electron microscopy (SEM) images showed a homogeneous surface structure, composed of closely packed small columns of ~60 nm in diameter and ~150 nm in height was obtained using these conditions. The nanoscaled silicon columns were perpendicular to the surface, and the pores formed between were ~30–60 nm in diameter.

Detection of [Pentose + K]$^+$ Adduct Ions. Although DIOS-MS is capable of detecting a variety of compounds, direct analyses of monosaccharides are difficult, and sometimes cationization is required to form detectable adducts.[6] No significant ion signal associated with any pentose isomers was detected when tested

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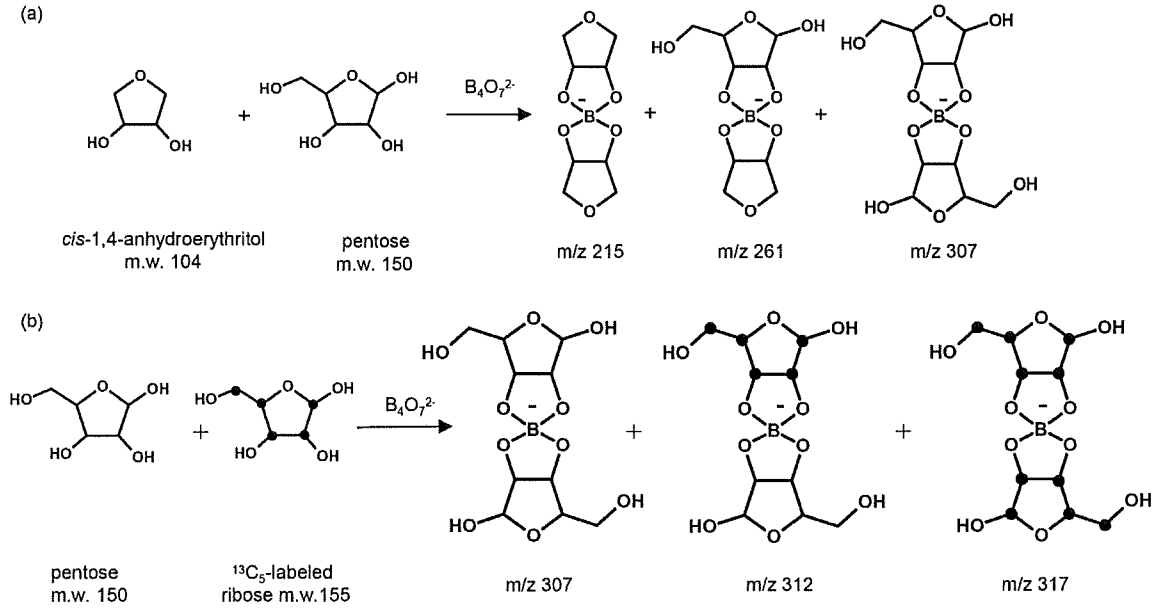

Figure 3. (a) A pentose structural analogue, 1,4-anhydroerythritol (AHE), was used in the competition test against a pentose isomer in the presence of the borate ion. Three negatively charged complexes, suggested structures shown here, have *m/z* of 215, 261, and 307. (b) In the case of $^{13}C_5$-labeled ribose being the competition compound, with ^{13}C depicted as black dots, the complexes have *m/z* of 307, 312, and 317 instead.

by themselves. However, in the presence of potassium ion, pentose isomers (m.w. 150) form adduct ions [pentose + K]$^+$ of *m/z* 189. For all five pentose isomers tested (D-ribose, L-arabinose, L-xylose, D-xylose, and L-lyxose), clean DIOS-MS spectra were obtained, with K^+ and [pentose + K]$^+$ peaks as the only prominent signals (Figure 1).

Another observation is that the pentose to K^+ molar ratio affected the ion peak profile and the overall spectrum quality. A pentose to K^+ ratio of 10:1, not 1:1 as one might expect, resulted in clean mass spectra with good S/N. The result is consistent with the fact that, in electrospray ionization mass spectrometry, addition of a small amount of salt is sometimes necessary to achieve desired ionization.[25] In fact, no *m/z* 189 of the adduct ion was observed at pentose to K^+ molar ratios less than 1. It appears that although the potassium ion assists the detection of neutral pentose molecules by forming charged adducts, higher K^+ concentrations may suppress adduct ion signals.

Detection of Pentose–Borate Complexes. After pentose isomers were successfully detected in the positive ion mode through the formation of cation adducts, the analytes of interest, dehydration products of borate ion and pentose, were tested. The mixture of pentose/$Na_2B_4O_7$ produces an *m/z* 307 pentose–borate complex ion (Figure 2a) and was detected in the negative DIOS-MS (Figure 2b). The formation of the spirane structure, confirmed by NMR techniques, is a result of the coordination between the *cis*-diol groups and boron.[20,22]

Shown in Figure 2b are mass spectra of ribose/$Na_2B_4O_7$ mixtures at different initial concentrations. The concentrations of two initial solutions are calculated to give a boron:pentose molar ratio of 1:2. In both spectra, the ion of *m/z* 307 is the only dominant signal. The S/N was improved with higher initial pentose/$Na_2B_4O_7$ concentration (10 mM borate ion, 20 mM pentose), which was used in future experiments. Compared to peptide analysis reported[5] and practiced in our lab, the sensitivity in the detection of the pentose–borate complexes is considerably reduced. For negative mode mass calibration (Figure 2c), a benzoic acid (C_6H_5COOH) /potassium hydroxide (KOH) mixture was used to give ions of *m/z* 121 and 281, corresponding to $C_6H_5COO^-$ and $[2C_6H_5COO^- + K^+]^-$.

Demonstrated in these experiments, the advantage of using DIOS-MS over other MS techniques is the minimum sample preparation which retains the native pH (~ 8.5) essential for the stability of the complex. In the following competition experiments, it was also important not to have any external chemicals which would potentially affect the equilibrium of the system.

Competition Experiments 1,4-Anhydroerythritol and Each Pentose Isomer. 1,4-Anhydroerythritol (AHE), which possesses a *cis*-diol moiety as a pentose, was used as the reference compound to compete with individual pentose isomers. Shown in Figure 3a is the reaction scheme.

When mixed at a 1:1 ratio, both AHE and the pentose are capable of forming the spirane complexes composed of two identical molecules and one boron atom. At the same time, a third type of the complex acquires a structure of two different competing molecules and one boron atom. The yields of the three complexes reflect the binding affinities of AHE and pentose with boron. Accordingly, four pentose isomers of interest (ribose, xylose, lyxose, and arabinose) were made to compete against AHE. The results of the four sets of experiments were used to study the relative binding affinity to boron among different pentose isomers.

Here, ion intensities of the pentose–borate complex (*m/z* 307) and AHE–borate complex (*m/z* 215) are used for comparison. Although the relative responses of the various complexes in this experiment are not known, an assumption is made that four pentose–borate complexes have similar ionization efficiencies. It is worth noting that the AHE–borate complex (*m/z* 215) is not exactly an internal standard since its concentrations in four

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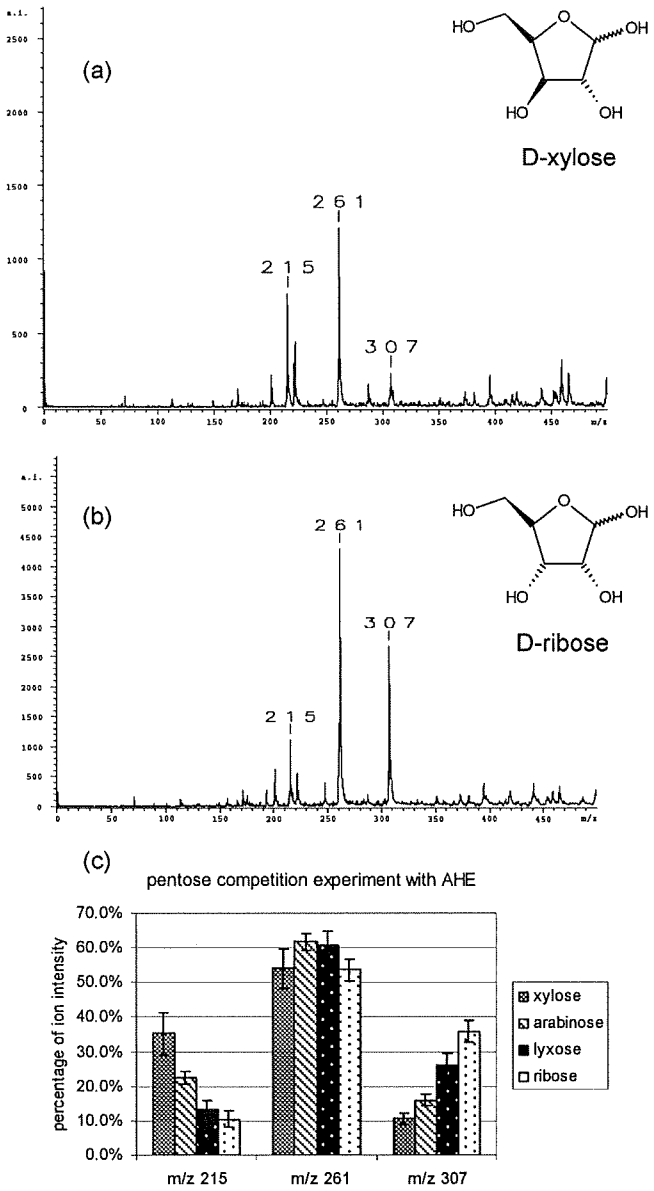

Figure 4. Representative DIOS mass spectra of mixtures of (a) xylose or (b) ribose with AHE and the borate ion. The structure of xylose and ribose are also shown. Results of four sets of competition experiments for four aldopentose isomers of interest (ribose, xylose, lyxose, and arabinose) are summarized in (c). Ion intensity percentages of total ion intensity of *m/z* 215, 261, and 307 are the averages of six spectra acquired on the same DIOS spot; each spectrum consisted of 50 laser shots. Error bars represent the relative standard deviations (RSDs). Ribose/AHE/borate mixture shows highest ion intensity percentage at *m/z* 307 than the other three aldopentoses.

equilibra can be different. However, the ionization efficiency of the AHE–borate complex is not a concern. Since the final evaluation is among four pentose–borate complexes after they are first compared to the AHE–borate complex, the possible ionization efficiency differences of ion *m/z* 307 and *m/z* 215 is canceled out.

Shown in Figure 4a,b are DIOS-MS mass spectra of mixtures of xylose or ribose with AHE in the presence of borate ion. As demonstrated in the mass spectra, the ion intensity fraction of *m/z* 307 in the ribose mixture is much higher than that of *m/z* 215, while for the xylose mixture, the *m/z* 307 ion is less intense. This indicates that ribose bound better to boron than xylose.

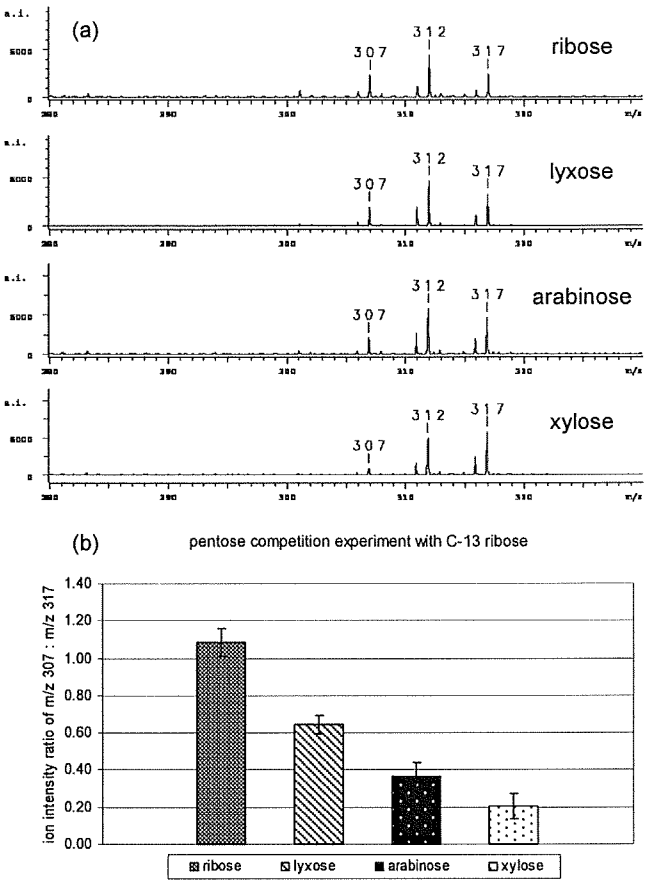

Figure 5. Results of the competition experiments between $^{13}C_5$-ribose and pentose isomers. (a) Four DIOS-MS mass spectra of the mixtures of the borate ion: ^{13}C5-ribose with ribose, xylose, lyxose, and arabinose, respectively. (b) Ion intensity ratios of *m/z* 307 to *m/z* 317 in four mixtures. The preferential order of binding to boron is determined to be ribose > lyxose > arabinose > xylose.

The results of four sets of competition experiments are summarized in Figure 4c. Each intensity ratio is plotted as the average from six spectra acquired on the same DIOS spot, each spectrum consisting of 50 laser shots. The ion intensity fraction of each complex is calculated as a percentage of the total ion intensity of all three complexes formed in the equilibrium. The error bars represent the relative standard deviations (RSDs). The ribose/AHE/borate mixture shows the highest ion intensity fraction at *m/z* 307 than the other mixtures. It is concluded that ribose has a higher affinity to boron than the other pentose isomers. Moreover, the order of affinites is determined as ribose (1.0, ± 9%) > lyxose (0.73, ±13%) > arabinose (0.44, ±10%) > xylose (0.30, ±15%); the first numbers in the parentheses are ion intensity fractions of *m/z* 307 normalized against that of ribose, and the second numbers are the RSDs.

Competition Experiments $^{13}C_5$-Ribose and Each Pentose Isomer. In addition to using a structural analogue as the competition reagent to investigate the relative binding affinities of pentose isomers toward boron, a ^{13}C-labeled ribose was used in a direct competition experiment against other pentose isomers. Shown in Figure 3b is the suggested reaction scheme.

An isotopically labeled pentose would allow for more direct comparison between the pentose isomers. Naturally, $^{13}C_5$-ribose was chosen to be the reagent. Similar to the previous experiment,

the competition reagent was mixed with each of the four pentose isomers (ribose, xylose, lyxose, and arabinose) at a 1:1 molar ratio in the presence of the borate ion. Ions of *m/z* 307, 312, 317 were monitored in the reflectron negative ion mode (Figure 5a). The ion intensity ratios of *m/z* 307 to *m/z* 317 are plotted in Figure 5b. The average values and standard deviations of ion intensities are calculated from six DIOS-MS mass spectra obtained on the same spot, each being a sum of 50 laser shots.

As shown in Figure 5a, ions at *m/z* 307, 312, and 317 were observed in all four mixtures. Ion signals at *m/z* 311 and 316 (one mass unit lower than the expected *m/z* values) are believed to associate with nonfully $^{13}C_5$-labeled species. In Figure 5a, the ion intensity ratio of *m/z* 307 to *m/z* 317 is about 1 in the $^{13}C_5$-ribose/ribose/borate mixture. It is expected that the isotopically labeled species should behave very similarly to its counterpart in terms of chemical reactivity and ionization efficiency. Due to the inherent impurity problem of the isotopic labeling, there is more unlabeled ribose than fully labeled ^{13}C-ribose initially, even though the two species were mixed at a 1:1 molar ratio. As a result, more of the ribose–borate complex formed at equilibrium, so the ion intensity ratio of *m/z* 307 to *m/z* 317 is slightly greater than 1 (Figure 5b).

More significantly, the results show that, in the other three mixtures, the $^{13}C_5$-ribose–borate complex (*m/z* 317) displays higher ion intensity than the borate complexes of competing pentose isomers (xylose–borate, arabinose–borate, and lyxose–borate, *m/z* 307). With the assumption that the involved complexes have similar ionization efficiencies, it is reasonable to say that the $^{13}C_5$-ribose–borate complex has a higher yield in the corresponding equilibrium, meaning that $^{13}C_5$-ribose has a better binding affinity to boron. Figure 5b shows the preferential order of binding to boron: ribose (1.08, ±7%) > lyxose (0.65, ±8%) > arabinose (0.37, ±20%) > xylose (0.20, ±33%); the first numbers in the parentheses are ion intensity ratios of *m/z* 307 to *m/z* 317, and the second numbers are the corresponding RSDs. The results confirmed the observation in the AHE competition experiments.

As previously mentioned, stability constants of borate complexes have been determined by using a combination of potentiometric titration and ^{11}B NMR spectroscopy, and the reported stability constant trend for aldopentoses was ribose > xylose > lyxose > arabinose.[21,22] Although the published order did not agree with our result, the ribose nonetheless was determined to have the highest stability constant.

The degradation rates of pentose isomers under alkaline/colemanite conditions were also determined using the ^{1}H NMR technique by estimating the loss of selected signals. The results also demonstrated that all the aldopentoses are stabilized by boron to a different extent, following the trend: ribose > lyxose > arabinose > xylose (data not shown).

CONCLUSIONS

We applied DIOS-MS to monosaccharide analyses. First, the pentose isomers were detected as cation adducts in the positive mode and charged complexes in the negative mode. Then, an aldopentose structural analogue was used to compete with individual aldopentoses in forming borate complexes to determine the binding preference among the four aldopentoses. Finally, $^{13}C_5$-labeled ribose was included in another set of competition experiments which further confirmed the first competition results. Ribose exhibited higher affinity to boron than other aldopentoses, and the binding preference was determined to be ribose > lyxose > arabinose > xylose. The result indicates that the favored binding between ribose and boron can be an important factor in RNA evolution.

This work also illustrates the potential of DIOS-MS in the analysis of nonvolatile, heat-labile, small molecules in delicate chemical equilibria. Without external matrix compounds, background signals are not a limiting factor. Moreover, the possible dramatic change in pH, which may disturb the equilibria of interest, is avoided.

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