Carbohydrate and steroid analysis by desorption electrospray ionization mass spectrometry†

Tiina J. Kauppila,[a] Nari Talaty,[b] Ayanna U. Jackson,[b] Tapio Kotiaho,[ac] Risto Kostiainen*[a] and R. Graham Cooks*[b]

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Desorption electrospray ionization mass spectrometry (DESI-MS) is applied to the analysis of carbohydrates and steroids; the detection limits are significantly improved by the addition of low concentrations of salts to the spray solvent.

Desorption electrospray ionization (DESI) is an ambient desorption ionization technique which has been applied to rapid *in situ* analysis with minimal or without any sample pretreatment.[1,2] Due to its speed, ease of use and high salt tolerance, DESI is becoming a useful tool in forensic,[3–5] pharmaceutical[6,7] and biological[8] applications. Analytes present on the target substrate are dissolved in the spray solvent and released from the wet sample surface into the secondary microdroplets formed by high velocity charged droplet impact.[9] Optimum performance in DESI is usually obtained for polar analytes that can readily be protonated or deprotonated. However, many biologically important molecules, including carbohydrates and steroids, do not have these characteristics. They may be successfully characterized by other mass spectrometry methods[10,11] but direct ambient analysis is highly desirable. Reactive DESI,[12,13] an experiment in which the solvent spray is doped with a reagent that selectively reacts with the analyte to improve the analytical specificity and detection limits, has proven successful for some carbohydrates and steroids. Simple adduct formation is known to increase sensitivity and ionization efficiency of some analytes such as lipids in ESI.[14]

We here discuss the application of DESI-MS to the analysis of neutral carbohydrates and steroids by adding controlled amounts of salts (*e.g.* lithium, sodium, potassium, ammonium or silver salts) to the solvent spray. Improvements in detection limits and selectivity are achieved by this simple procedure. This method is also applied to the direct analysis of carbohydrates and steroids from raw urine samples and the results are compared to the corresponding reactive DESI experiments. All experiments employed a Thermo Fisher LTQ 2D linear ion trap mass spectrometer (San Jose, CA, USA) in both the positive and negative ion modes. The mass spectrometer was equipped with a desorption electrospray ion source, a prototype of the OmniSpray® source of Prosolia Inc. (Indianapolis, IN, USA) described elsewhere.[1] The experimental conditions include: a spray flow rate of 3 μL min^{-1}, a capillary temperature of 150 °C, a gas pressure of 150 psi and an electrospray voltage of 5 kV.

The analytes included six carbohydrates of different molecular masses (xylose, glucose, glucuronic acid, maltose, maltotriose and maltoheptaose) and four steroids (testosterone, dehydroepiandrosterone [DHEA], 5β-pregnan-3,20-dione and cortisone), all having different proton affinities (Fig. S1 supplementary material†). Spray solvents with and without salt additives were compared (H_2O–MeOH with 0.1% HCOOH, 100 μM LiCl, NaCl, KCl, $AgNO_3$ or NH_4OAc in the positive ion mode, and H_2O–MeOH with 0.1% NH_4OH, 10 μM NH_4Cl, 10 μM NH_4Br, 0.01% HCOOH, 0.01% CH_3COOH, 10 μM H_2SO_4 or 100 μM NH_4NO_3 in the negative ion mode). A detailed list of the solvents used and ions monitored is given in Table S1 (supplementary material†).

The salt concentrations were optimized for the analysis of maltose and DHEA; above the optimum concentrations the analyte signal was somewhat suppressed by added salt, whereas below the optimum concentrations the analyte was not observed easily. These results corroborate the recent systematic investigations of salt effects on DESI at physiological salt concentrations.[15] However in the earlier study, the salt was present in the analyte (condensed phase sample) and not in the spray solvent. This meant that little adduct formation was observed (compared to traditional ESI). Here, adduct formation is sought and achieved. In either case, DESI is found to be tolerant toward the presence of salt. Typically the salts in this study were present in 10–100 μM concentrations in the spray solution. For each analyte, a 0.5 μL sample was spotted on a Teflon surface, allowed to dry in the ambient laboratory environment, and analyzed using DESI-MS. In the positive ion mode, the highest signals and S/N ratios for the carbohydrates were obtained when solvents doped with NaCl, KCl and NH_4OAc were used (Fig. 1a shows the spectrum for xylose). These results are in good agreement with previously reported ESI and MALDI results.[16,17] For the carbohydrates, the presence of salt in the spray solvent seemed to be necessary for successful ionization; without salt and using H_2O–MeOH–HCOOH (0.1%) only maltoheptaose, the largest carbohydrate, was ionized forming $[M + H]^+$.

The steroids testosterone, DHEA, pregnandione and cortisone yielded protonated molecules when the standard spray solvent H_2O–MeOH–HCOOH (0.1%) was used but, except for testosterone, the ionization efficiency was enhanced when salts were added (Fig. 2a and S2†). The differences in the behavior of the

[a] *Faculty of Pharmacy, Division of Pharmaceutical Chemistry, University of Helsinki, 00014 Helsinki, Finland. E-mail: risto.kostiainen@helsinki.fi*
[b] *Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA. E-mail: cooks@purdue.edu*
[c] *Department of Chemistry, Laboratory of Analytical Chemistry, 00014 Helsinki, Finland*

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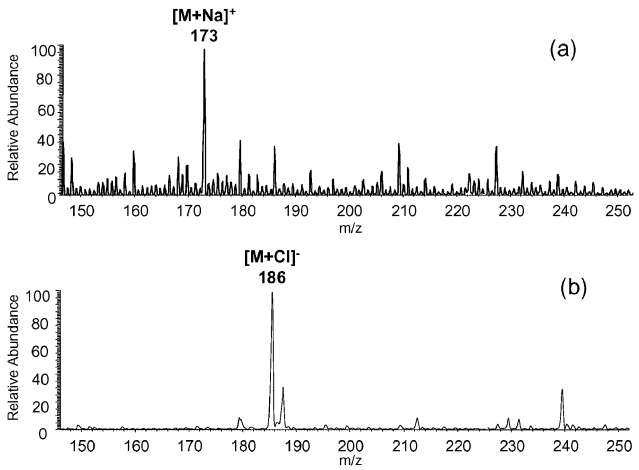

Fig. 1 DESI mass spectra of 1 mM xylose (50 nmol) (a) positive ions recorded using H_2O–MeOH–NaCl (100 μM) (b) negative ions recorded using H_2O–MeOH–NH_4Cl (10 μM) as the spray solvent.

steroids can be explained by the differences in their proton affinities (PAs), which are approximately 825 kJ mol^{-1} for DHEA (reference compound cyclopentanone), 840 kJ mol^{-1} for pregnandione (reference compound cyclohexanone) and 870 kJ mol^{-1} for cortisone and testosterone (reference compound 5,5-dimethyl-2-cyclohexenone) by considering group equivalent effects on the known proton affinities[18] of simple analogs. Because of its higher PA, testosterone is protonated more easily than the other compounds which more readily form salt adducts. DHEA, with the lowest PA, gave the smallest protonated signal and the greatest signal increase in the presence of salt. The greater salt cation affinity of cortisone than testosterone, in spite of their similar PAs, is ascribed to the adjacent hydroxyl groups of cortisone compared to the single hydroxyl group of testosterone. The S/N ratios for each analyte ion, when analyzed using the different spray solvents in the positive ion mode, are shown in Fig. S2.†

In the negative ion mode, the carbohydrates gave signals due to $[M - H]^-$ when spraying H_2O–MeOH–NH_4OH (0.1%) in the cases of glucuronic acid, maltose, maltotriose and maltoheptaose spectra. The smaller carbohydrates, xylose and glucose, could not be ionized through simple deprotonation. However, for all the carbohydrates, even for glucuronic acid, the signal of the analyte adduct (*e.g.* $[M + Cl]^-$) was significantly increased by the use of salt additives (Fig. S3†). The greatest signal intensities for the carbohydrates xylose, glucose, glucuronic acid, maltose and maltotriose were obtained when using H_2O–MeOH–NH_4Cl (10 μM) as the spray solvent, and observing chloride adduct formation (Fig. 1b). This is in good agreement with previous studies, where chloride ions have been utilized in the selective ionization of carbohydrates in ESI.[19] For maltoheptaose, the greatest signal was obtained when H_2O–MeOH–H_2SO_4 (10 μM) was used as the spray solvent and the doubly-charged ion $[M + SO_4]^{2-}$ was observed. Enhanced signals for carbohydrates using H_2SO_4 have also been obtained in experiments with MALDI, but it has been reported that the effect requires that the analyte should have at least four sugar units.[20] This seems to be true also in DESI, since intense signals with H_2SO_4 were only obtained for maltoheptaose, the largest carbohydrate examined.

Of the steroids, only cortisone was ionized in the negative ion mode using H_2O–MeOH–NH_4OH (0.1%) as the spray solvent; in this case $[M - H]^-$ was the base peak. Other solvents gave salt adducts, although with lower intensities (Fig. 2b and S3†). The S/N ratios for each analyte when ionized using different spray solvents in the negative ion mode are shown in Fig. S3.†

An alternative to the formation of salt adducts is the use of reactive DESI[12] to generate covalently-bonded adducts. Adduct formation by cortisone (500 pmol) was compared directly with a recently reported reactive DESI experiment in which heterogeneous reactions of hydroxylamine (NH_2OH) occur with the carbonyl group of the steroid during ionization.[13] Both methods involve the addition of reagents to the DESI spray and give similar performance when the intensities of the $[cortisone + Na]^+$ peak and the oxime reaction product are compared (Fig. S4†). Simple adduct formation occurred with an improvement in S/N ratio of a factor of three compared to that for the protonated species. A comparison was made for glucose (500 pmol) where simple adduct formation was compared to the occurrence of a heterogeneous reaction at a solution/solid interface (*cis*-diol reaction upon selective complexation to form a cyclic boronate).[12] The improvement in S/N ratio was ~15 for the simple adduct $[glucose + Cl]^-$. No signal was observed in the absence of salt for this amount of analyte. In this case, while the reactive DESI experiments are more selective than simple salt adduct formation, the spectra resulting from the adduct formation have a higher signal intensity resulting in improved detection limits.

Limits of detection (LOD) for the compounds studied were determined in both the positive and negative ion modes. The data were collected in full MS scan mode and peaks corresponding to the analytes with a S/N ratio of 3 were chosen to represent the LOD. In the positive ion mode the solvents H_2O–MeOH–HCOOH (0.1%) and H_2O–MeOH–NaCl (100 μM) gave typical LODs in the range of 250 fmol–500 pmol (72 pg–580 ng, Table 1), being lowest for the steroids (250 fmol–2.5 pmol). For the carbohydrates, the LODs were in the range of 500 fmol–500 pmol, being lowest for maltoheptaose (500 fmol) and highest for the acidic glucuronic acid (500 pmol) when H_2O–MeOH–NaCl was the spray solvent. Clearly, the presence of NaCl improved the LODs for all the

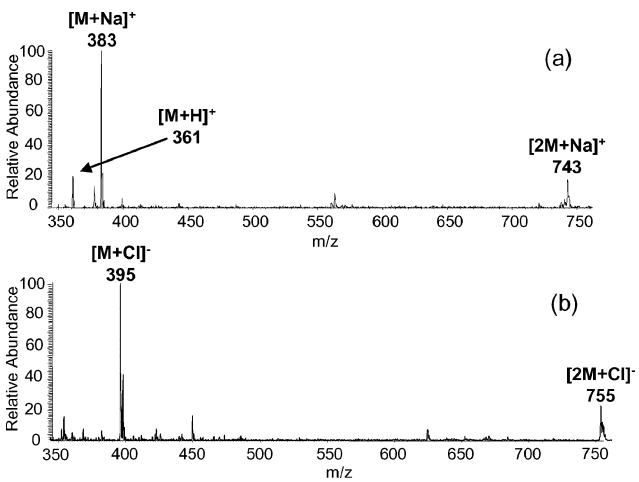

Fig. 2 DESI mass spectra of 100 μM cortisone (50 pmol) (a) positive ions recorded using H_2O–MeOH–NaCl (100 μM) (b) negative ions recorded using H_2O–MeOH–NH_4Cl (10 μM) as the spray solvent.

Table 1 Limits of detection (S/N > 3) using H_2O–MeOH–HCOOH (0.1%) and H_2O–MeOH–NaCl (100 μM) as spray solvents in the positive ion mode (+ve) and H_2O–MeOH–NH_4Cl (10 μM) in negative ion mode (−ve)

Analyte	+ve (HCOOH) (pmol)	+ve (NaCl) (pmol)	−ve (NH_4Cl) (pmol)
Xylose	[a]	250	25
Glucose	[a]	50	25
Maltose	[a]	2.5	5
Maltotriose	[a]	25	5
Maltoheptaose	50	0.5	500
Glucuronic acid	[a]	500	2.5
DHEA	0.5	0.25	[a]
Cortisone	0.5	0.25	0.25
5β-Pregnan-3,20-dione	2.5	0.25	[a]
Testosterone	0.25	0.25	[a]

[a] The limit of detection was not determined since the analyte could not be detected in the particular solvent.

compounds except testosterone which yielded the same LOD with and without added salt in the spray solution.

In the negative ion mode, the limits of detection were determined only using H_2O–MeOH–NH_4Cl (10 μM) as the spray solvent as it yielded the highest ionization efficiency for most analytes. The LODs obtained in the negative ion mode were in the same range as in the positive ion mode (250 fmol–500 pmol) but for xylose, glucose, maltotriose and glucuronic acid, the LODs were even lower than in the positive ion mode (250 fmol–25 pmol, Table 1). This is probably due to the generally lower background in the negative ion mode, which results in increased S/N ratios. For glucuronic acid, the reason for the improvement in LOD is obviously its acidity which causes efficient ionization by deprotonation in the negative ion mode.

Finally, the use of H_2O–MeOH–NaCl (100 μM) as the spray solvent was tested in the analysis of steroids and carbohydrates from complex matrices. Fig. 3a shows the analysis of a urine sample spiked with 1 mM of DHEA, 5β-pregnan-3,20-dione and cortisone and diluted 100 times, with examination of ~5 pmol of each analyte. Abundant sodiated molecules of each steroid were observed at *m*/*z* 311, *m*/*z* 339 and *m*/*z* 383. In addition, sodiated dimers were observed at higher masses. Fig. 3b shows the spectrum of a urine sample spiked with 10 mM glucose and diluted 100 times (~50 pmol analyte). The sodiated glucose molecule was observed at *m*/*z* 203. Suppression of the analyte signal by the matrix was not observed in either of these applications, although the only sample pretreatment method used was dilution of the sample. This method clearly can be used in the analysis of carbohydrates and steroids in complex matrices.

The signal of carbohydrates and steroids in DESI-MS is significantly increased by adding suitable concentrations of salts to the solvent spray. The adduct effects of the salts described here is especially useful in the analysis of carbohydrates which are not easily protonated. The method is less selective than reactive DESI but is shown to work well in the analysis of carbohydrates and steroids from urine, where signals for the analytes were obtained without significant suppression by the matrix. The improved spectral quality associated with the salt sprays may be due to decreased solubility of the organic analytes in the wet surface film due to a "salting out" phenomenon. This would increase the surface activity of the organic and so increase the efficiency of analyte sampling into the secondary microdroplets generated upon primary droplet impact. This study was financially supported by ONR Grant #N00014-02-1-0834, NSF CHE-0412782 and by the Academy of Finland (Project #1209189).

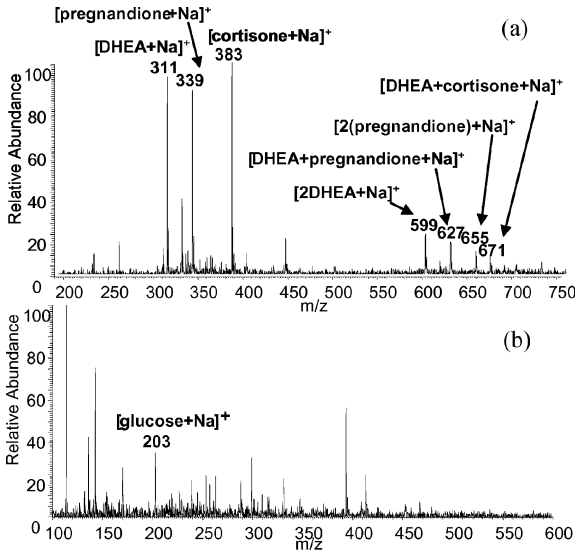

Fig. 3 Positive ion DESI spectra of ×100 diluted raw urine: (a) spiked with DHEA, pregnandione and cortisone (~5 pmol of each analyte) in H_2O–MeOH (b) spiked with ~50 pmol of glucose analyte in H_2O–MeOH. Spray solvent H_2O–MeOH–NaCl (100 μM).

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