HHS Public Access

Author manuscript

Anal Chem. Author manuscript; available in PMC 2017 June 21.

Published in final edited form as:

Anal Chem. 2015 September 15; 87(18): 9396–9402. doi:10.1021/acs.analchem.5b02242.

High-Throughput Bioconjugation for Enhanced 193 nm Photodissociation via Droplet Phase Initiated Ion/Ion Chemistry using a Front-end Dual Spray Reactor

Victoria C. Cotham, **Jared B. Shaw**, and **Jennifer S. Brodbelt***

Department of Chemistry, The University of Texas at Austin, 105 E. 24th St, Austin, TX, USA 78712

Abstract

Fast on-line chemical derivatization of peptides with an aromatic label for enhanced 193 nm ultraviolet photodissociation (UVPD) is demonstrated using a dual electrospray reactor implemented on the front-end of a linear ion trap (LIT) mass spectrometer. The reactor facilitates the intersection of protonated peptides with a second population of chromogenic 4-formyl-1,3-benzenedisulfonic acid (FBDSA) anions to promote real-time formation of ion/ion complexes at atmospheric pressure. Subsequent collisional activation of the ion/ion intermediate results in Schiff base formation generated via reaction between a primary amine in the peptide cation and the aldehyde moiety of the FBDSA anion. Utilizing 193 nm UVPD as the subsequent activation step in the MS^3 workflow results in acquisition of greater primary sequence information relative to conventional collision induced dissociation (CID). Furthermore, Schiff base modified peptides exhibit on average a 20% increase in UVPD efficiency compared to their unmodified counterparts. Due to the efficiency of covalent labeling achieved with the dual spray reactor, we demonstrate that this strategy can be integrated into a high-throughput LC-MS^n workflow for rapid derivatization of peptide mixtures.

TOC image

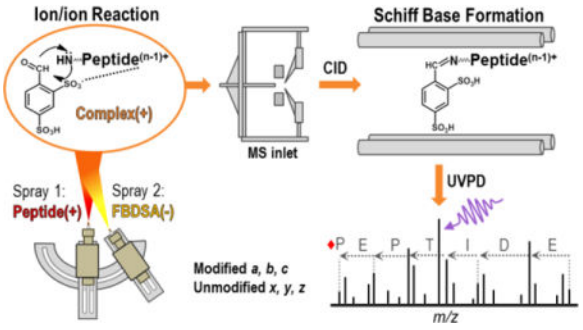
*Corresponding Author: jbrodbelt@cm.utexas.edu.

Supporting Information.

The contents of the Supporting Information include diagrams of the dual spray reactor design, control and dual spray reaction mass spectra for direct infusion and chromatographically separated peptides, comparative CID and UVPD mass spectra for Schiff base modified peptides, and energy variable CID plots for unmodified and Schiff base reacted peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

Notes

The authors declare no competing financial interest.



Bioconjugation techniques have long been used to extend the versatility of mass spectrometry (MS) for proteomic analysis.[1,2] The number of reactive moieties incorporated into polymeric amino acid chains makes them amenable to a diverse array of site-selective chemistries, which have been exploited in both MS and MS^n modes for purposes such as enhancing ionization efficiencies,[3,4] incorporating isotopic labels for quantification,[5–7] modulating fragmentation,[8–10] and promoting selective dissociation.[11–13] Despite their utility, derivatization reactions are often the rate-limiting step in MS-based workflows since most require off-line solution phase chemistry prior to mass spectrometric analysis. Recently, this shortcoming has been addressed by several compelling strategies that utilize the mass spectrometer as a tool to facilitate rapid functional group derivatization analogous to that performed in bulk solution. These strategies apply the principles of two fundamentally distinct chemical platforms: 1) microdroplet chemistry[14] and 2) gas phase ion/ion chemistry.[15] Other approaches, including the use of theta capillaries and a sheath gas reaction mode, have been used to modulate electrospray ionization in an on-line manner via fast mixing of droplets or exposure of droplets to gaseous acids or bases.[16–20]

MS-based microdroplet chemistry exploits the interfacial region between the ion source and the vacuum inlet of the mass spectrometer to promote rapid chemical modification during the ionization process.[14,21–26] Cooks and co-workers demonstrated that the confined volume of a charged evaporating droplet acted as a microreactor for heterogeneous spray mixtures and promotes accelerated bond formation relative to bulk solution.[23–25] The enhanced reaction kinetics were attributed to extremes in pH and concentration within the shrinking droplet environment, as well as increased collision frequencies at atmospheric pressure.[23] This phenomenon was most prominent at sub-nanospray volumes, such as that of secondary droplets formed during reactive desorption electrospray ionization (rDESI).[24] Strategies incorporating on-line microdroplet derivatization have primarily been limited to small molecule applications, with only a select few studies adapting these methods for peptide or protein analysis.[14] These exceptions include the in-situ crosslinking of primary amines in single peptides with bis(sulfosuccinimidyl) suberate (BS3) using reactive DESI[25] and online dithiothreitol (DTT) reduction of disulfides in intact proteins via reactive electrospray-assisted laser desorption/ionization (rELDI).[27] Although these techniques established the feasibility of on-line microdroplet-assisted modification of several functionalities in polymeric amino acid chains, the potential for applying these methods to high-throughput proteomic mixtures remains largely unexplored.

Gas phase ion/ion reactions represent the second major MS-based platform for on-line bioconjugation of peptides and proteins. These strategies move the chemical reaction step from the atmospheric pressure interface into the vacuum chamber of the mass spectrometer.[15] Implementation of such methods requires the use of sophisticated instrumentation with bipolar trapping capabilities for mutual storage of populations of oppositely charged reagent and analyte ions.[28] To date, a number of covalent chemistries have been used to selectively functionalize moieties of polypeptide chains in the gas phase, including Schiff base and NHS-ester modification of primary amines,[29–31] carbodiimide derivatization of carboxylic acids,[32] as well as directed peptide bond formation via sulfo-NHS ester N-terminal coupling.[33] Unlike analogous condensed phase reactions, which offer limited control over reacting species and are thus subject to undesirable side products, *in vacuo* ion/ion reactions afford fine-tuned selection of reactant ions and a high degree of conversion into the products of interest.[15] Despite these merits, complete gas-phase transformation often comes at the cost of long activation times (>100 ms) that are not amenable to on-line chromatographic separations, thereby limiting analysis to low complexity samples.

In this work we demonstrated a method that combines the principles of droplet phase and gas phase ion/ion chemistry to promote rapid bioconjugation using a front-end dual spray reactor. The reactor is designed to be readily coupled to LC platforms for high-throughput proteomic applications that were either not possible or unexplored using previously described droplet phase or gas phase strategies alone. To demonstrate the utility of the reactor for droplet-phase initiated ion/ion reactions, we borrow from covalent Schiff base chemistry that has been extensively explored in the gas phase.[29,30,34,35] We expand on this further by taking advantage of the chromophore addition at peptide N-termini following Schiff base formation with 4-formyl-1,3-benzenedisulfonic acid (FBDSA) to enhance photodissociation at 193 nm.[36] This workflow is summarized in Scheme 1.

EXPERIMENTAL SECTION

Chemical and Materials

Model peptides KMVELVHFL, KLVANNTRL, RPPGFSPFR, ASHLGLAR, DRVYIHPFHLVIHN and DAEFRHDSGYQVHHQK were purchased from AnaSpec Inc. (Fremont, CA). SYSMEHFRWG was purchased from American Peptide Company (Sunnyvale, CA). DRVYIHPFHL and 4-formyl-1,3-benzenedisulfonic acid (FBDSA) were obtained from Sigma-Aldrich (St. Louis, MO). Peptides were used without purification. All other solvents were purchased from Fisher Scientific (Fairlawn, NJ).

Implementation of a Front-end Dual Spray Reactor

Design and fabrication of the dual spray reactor mounting system was performed in house according to the illustration shown in Supplemental Figure S1. The reactor was equipped with two electrosonic spray ionization (ESSI) sources (Prosolia Inc., Indianapolis, IN) that were integrated on the front-end of the mass spectrometer by way of a free-standing mount attached to a U-shaped rail that surrounded the MS inlet. The curved rail allowed for independent adjustment of the angle between the two sources in addition to the angle of each

source relative to the axis of the mass spectrometer. Both sources were fixed to sliding supports that enabled adjustment of the y-dimensional distance between the emitter tip and the MS inlet. These supports were further mounted to a precision 1D translational stage for positional control in the z-direction. The first source was completely integrated, allowing direct control of polarity and spray voltage through the instrument control software. The spray voltage of the second source was supplied by an external 5 kV high voltage power supply (Stanford Research Systems Inc., Sunnyvale, CA) operated in negative polarity. Nebulizing sheath gas was introduced using an external nitrogen line equipped with a tee fitting and adjustable metering valve to evenly split the gas flow to both sources and allow manipulation of gas flow rates, respectively.

Droplet Phase Initiated Ion/Ion Chemistry, Mass Spectrometry and Photodissociation

All experiments were conducted on a Thermo Velos Pro dual linear ion trap mass spectrometer (San Jose, CA) outfitted with a GAM EX5 or Coherent Excistar XS 500 Hz ArF excimer laser operated at 193 nm. The back flange of the mass spectrometer was modified to allow introduction of the laser beam coaxial to the dual cell linear ion trap through a CaF2 window and 2 mm stainless steel aperture, as previously described.[37] In all experiments, peptide cations were generated by positive mode electrospray ionization of 5 μM peptide working solutions prepared in equal parts water and acetonitrile containing 0.1% formic acid and infused at rate of 1.5 μL/min. For ion/ion reactions, a second ion population consisting of a large excess of reagent anions was produced by negative mode electrospray of 1 mM FBDSA in 50:50 water/methanol at an infusion rate of 3 μL/min. The peptide source was positioned on axis to the inlet of the mass spectrometer, whereas the reagent ion source was position approximately 45 degrees off axis. Complex formation was accomplished at atmospheric pressure by intersecting analyte sprays of opposite polarity in the reaction region prior to the capillary inlet. The spray voltage for the cation source was held constant at 1.75 kV, while the anion spray voltage was varied between −2.5 and −3 kV depending on optimal complex formation. Electrostatic adducts formed in the overlapping sprays were isolated and subjected to low energy collisional activation using a normalized collision energy (NCE) between 10–20% to promote loss of water and formation of the stable Schiff base imine product. Isolation and MS^3 activation of the Schiff base was carried out using either collisional induced dissociation (CID) or 193 nm ultraviolet photodissociation (UVPD). MS^3 experiments based on UVPD were performed using a single 2 mJ, 5 ns laser pulse. A q-value of 0.125 was used to extend the low m/z cutoff. UVPD efficiencies were calculated as previously described.[36]

High-Throughput Bioconjugation on an LC Timescale

The dual spray reactor was coupled to a Dionex Ultimate 3000 (Sunnyvale, CA) capillary flow system for all high-throughput bioconjugation experiments. Peptides were separated on an Agilent ZORBAX 300Extend-C18 column (150 × 0.3 mm, 3.5 μm particle size) held at a constant temperature of 30°C. Eluent A consisted of 0.1% formic acid in water and eluent B was 0.1% formic acid in acetonitrile. A linear gradient from 3% to 35% B over 30 min at 4 μL/min was used. An auxiliary syringe method was programed to infuse FBDSA at a rate of 3 μL/min throughout the course of separation. The spray voltage for the cation and anion source was held constant at 1.75 kV and −2.5 kV, respectively. Rapid derivatization was

accomplished using an automated data-dependent neutral loss (DDNL) MS^3 program (Scheme 2) similar to that described by Gygi *et al.*[38] The top five most abundant ions in the full MS scan were subjected to low energy collisional activation (18% NCE, isolation width of 5 Th, *q*-value 0.15) for 50 ms. MS^3 UVPD (1 pulse, 2 mJ, *q*-value of 0.125) was triggered if a neutral loss product was detected at −18 Da (−18 Th, −9 th and −6 Th to account for the 1+, 2+ and 3+ charge states, respectively) and was within the top 3 most abundant ions in the MS^2 spectrum. All resulting data was filtered such that only grouped MS^2 and MS^3 scans were manually interrogated.

RESULTS AND DISCUSSION

Covalent modification in the gas phase has been shown to occur via stable, long-lived electrostatic intermediates formed between polypeptide cations and bi-functional reagent anions.[15,29,31] Within this context, bi-functionality refers to the ability of reagent anions to engage in stabilizing non-covalent interactions that favor complex formation over competing proton transfer, as well as facilitate functional group derivatization.[15] Han and McLuckey reported the first example of gas phase bioconjugation within the confines of an electrodynamic ion trap mass spectrometer using the aldehyde-containing reagent 4-formyl-1,3-benzenedisulfonic acid (FBDSA) to covalently modify primary amines of peptide cations via Schiff base ion/ion chemistry.[29] The resulting derivatized peptides were found to yield more informative fragmentation upon collisional activation compared to their unmodified counterparts. Similarly, gas phase FBDSA-based chemistry has been used to enhance collisional dissociation in the negative ion mode for charge inverted Schiff base modified peptides.[34,35,39] These seminal studies demonstrated the feasibility of online ion/ion mediated bioconjugation, as well as its utility for improving the structural characterization of modified species. Despite the improved throughput of this online approach relative to orthogonal in-solution chemistry, the complex scan functions and average reaction times (50–1000 msec) required for formation of electrostatic intermediates *in vacuo*, followed by covalent conversion and subsequent activation to obtain structurally relevant information, have rendered these methods not well adapted for chromatographic timescales. These limitations have prompted our efforts to develop a streamlined approach that utilizes a front-end dual spray reactor to initiate ion/ion reactions in the droplet phase prior to introduction into the mass spectrometer. To benchmark the performance of the dual spray reactor, we employed aforementioned Schiff base reactions between peptide cations and FBDSA anions for comparison with previously reported gas phase ion/ion covalent chemistry.

The process for dual spray assisted bioconjugation of peptides with FBDSA is summarized in Scheme 1. The reactor utilizes two electrospray sources biased at opposite polarity to simultaneously generate overlapping populations of peptide cations and FBDSA anions. This configuration was designed to maximize collisions between reactive species in the droplet, pseudo-droplet, and gas phase at the high pressure interface of the mass spectrometer, similar in concept to the Y-shaped reactor inlet used to merge ions from two independently biased sources in early ion/ion proton transfer reactions,[40] or that used in extractive electrospray (EESI) configurations.[41,42] The role of the sulfonate groups of FBDSA is two-fold: first, they provide acidic sites that are readily deprotonated under

negative electrospray conditions to form 1- and 2- anions (Figure S2a, Supporting Information), and secondly they engage in stabilizing acid-base interactions with multiply charged peptide cations to form charge-reduced non-covalent complexes that persist into the gas phase. This process is demonstrated in Supplemental Figure S2, which compares the MS^1 spectrum of the peptide DRVYIHPFHLVIHN before (Figure S2b) and after dual spray infusion with anionic FBDSA (Figure S2c). The major products in the post ion/ion reaction spectrum are charge-reduced relative to the unmodified peptide and arise from partial neutralization of multiply charged peptide cations (up to five sites of protonation) with singly and doubly deprotonated FBDSA anions. This is clearly indicated by the absence of the 5+ charge state and attenuation of the relative abundances of the 3+ and 4+ charge states in the resulting dual spray spectrum. Moreover, new products are observed at *m/z* values consistent with the formation of 2+ and 3+ peptide/FBDSA complexes as indicated by a mass shift of +266 Da (denoted by the "Δ" symbol) relative to the unmodified peptide. As shown in Supplemental Figure S3, a direct relationship was observed between the magnitude of the anion source voltage and the relative contribution of complexes to the total ion current of the dual spray spectrum. The absence of peptide/FBDSA complexes when the voltage is set to zero (akin to ion/molecule reaction conditions), in addition to the observed shift towards more abundant complex formation as the anion source voltage is stepped to increasingly negative potentials confirms that the reaction occurs exclusively through an ion/ion mediated pathway.

An example of an optimized dual spray spectrum is shown in Figure 1a for FBDSA-reacted DRVYIHPFHLVIHN. Here, signal from the non-covalent complex accounts for approximately 37% of the total ion current, thus demonstrating the efficiency of the front-end ion/ion reaction. It should be noted that competing proton transfer reactions may also contribute to the observed attenuation of peptide charge state; however, an approach for isolating and quantifying this contribution relative to complex formation is beyond the scope of this work.

Following optimization of the front-end ion/ion reaction, the second step of the process requires isolation and gentle collisional activation of the electrostatic complex to promote covalent bioconjugation. This input of energy into the system is likely required to overcome the activation barrier necessary for nucleophilic attack of the FBDSA aldehyde by the free N-terminus of the peptide. Since water represents the major byproduct of imine formation, the dominant water loss product observed in the MS^2 spectrum (Figure 1b) is highly indicative of covalent Schiff base derivatization. An additional ion activation event results in an MS^3 spectrum that confirms Schiff base formation (Figure 1c). Unlike previously reported *in vacuo* methods, which accomplish MS^3 by using a second collisional activation step, we integrate 193 nm ultraviolet photodissociation (UVPD) as an alternative activation method to both verify Schiff base formation and achieve comprehensive structural characterization of resulting modified peptides. The use of 193 nm UVPD is strategic for several reasons: 1) UVPD can be accomplished in a much shorter activation period than required for CID, thus improving the throughput of the analysis, 2) in many cases, UVPD provides more extensive coverage of the peptide backbone compared to CID,[43] and finally 3) N-terminal modification of peptides with chromogenic labels, such as FBDSA, has been shown to enhance UVPD efficiencies,[36] thereby offering an additional metric by which to

evaluate covalent attachment. The exceptional sequence coverage of the peptide backbone afforded by 193 nm UVPD is demonstrated in Figure 1c. The combined information obtained by the extensive array of complementary N- and C-terminal ions (in this case *a/b* and *x/y/z*, respectively) allows for unambiguous localization of Schiff base modification at the peptide N-terminus. This lack of ambiguity arises from the fact that all N-terminally derived ions exhibit a conserved mass shift of +248 Da (denoted in the spectrum by the addition of "◆" to the ion labels), whereas the entire set of complementary C-terminal product ions remain unmodified. The differentiation of several isobaric leucine and isoleucine residues across the peptide backbone is also possible by unique *v*- and *w*-type side-chain ions produced upon UV activation. Moreover, the non-resonant nature of UVPD allows access to a lower *m/z* trapping limit, whereas CID suffers from a low mass cutoff (LMCO) restriction imposed by the RF amplitude applied to the trap. By overcoming this limitation, UVPD provides greater depth of coverage for high *m/z* (low charge) precursor ions commonly observed following the formation of charge-reduced peptide/FBDSA complexes during front-end ion/ion reactions (Figure S4).

Previous work in our lab demonstrated enhanced 193 nm photodissociation of peptides containing native aromatic residues,[43] as well as for those whose intrinsic aromaticity was increased through N-terminal derivatization with chromogenic labels.[36] Based on these findings, we anticipated that front-end ion/ion mediated N-terminal modification with chromogenic FBDSA would enhance the UVPD dissociation behavior of peptides. To test this hypothesis, UVPD was evaluated for a series of model peptides in both the unmodified and FBDSA-labeled states. Notably, spectral changes were most prominent for peptides lacking intrinsic aromatic residues such as in the case of KLVANNTRL (Figure 2). Despite complete backbone coverage of the unmodified peptide, the fragment ion abundance only accounts for approximately 17% of the total ion intensity, therefore indicating rather poor dissociation of the singly charged precursor (Figure 2a). Alternatively, photoactivation of the Schiff base modified form of this peptide showed nearly 27% increase in the resulting photodissociation efficiency (Figure 2b), as indicated by a significant increase in fragment ion intensity relative to the surviving precursor. Similar results were obtained for all peptides investigated in this study, with Schiff base modification accounting for an average enhancement in photodissociation efficiency of approximately 20% (Figure 3). This value is highly consistent with previous findings for peptides modified with SPITC and PPITC chromogenic N-terminal labels.[36] To verify that this change in dissociation behavior is the direct result of enhanced gas-phase photoabsorption as opposed to changes in the critical energies of the modified peptides, an energy variable collisional activation analysis was performed on unmodified and Schiff base labeled KLVANNTRL (1+). Supplemental Figure S5 shows the normalized precursor ion intensity for both forms of KLVANNTRL (1+) as a function of increasing collision energy. The high degree of overlap in the variable CID profiles suggests that FBDSA predominantly enhances the photoexcitation energy, while having no appreciable impact on the critical energy of the peptide. Despite improved dissociation efficiency, decreased UVPD sensitivity is possible for FBDSA-labeled peptides due to incomplete reaction conversion to the Schiff base product.

Finally, we wanted to assess the feasibility of coupling the dual spray reactor to a liquid chromatograph in order to show the first example of high-throughput ion/ion mediated

bioconjugation on a chromatographic timescale. For these proof-of-principle studies, a five peptide mixture was utilized to simulate the complexity of a small, single protein digest. Unlike direct infusion experiments, which allow source parameters to be independently adjusted in order to maximize complex abundance, integration of the reactor into a high-throughput workflow requires using a fixed set of source parameters to generate sufficiently high levels of ion/ion complex for selection during automated data-dependent acquisition. Figure S6 provides a comparison of spectra for each peptide following LC-MS analysis of the mixture under control (Figure S6a–e) and dual source reaction conditions (Figure S6f–j). In all cases, spectral differences between the samples arise from the presence of electrostatic complexes. Interestingly, the degree of complex formation appears greatest for early eluting peptides where the mobile phase composition is predominantly aqueous. This corresponds to larger droplet sizes on the basis of the higher surface tension of water relative to organic solvents, and thus more dilute droplet conditions. Contrary to microdroplet reaction theory, which states that accelerated rates of reaction are observed as droplet size decreases,[24] our results point to a greater emphasis on stabilizing the electrostatic interactions for ion/ion mediated reactions as opposed to increasing the relative concentration of reactants per unit volume. Once formed and transferred into gas phase, the extent to which complexes are converted to covalent Schiff base products is highly dependent on the amount of time allotted for the reaction to proceed in the MS^2 step via low energy collisional activation (Figure S7). Alternatively, rapid activation at high collision energies (NCE>25%) resulted in dominant loss of FBDSA (data not shown). This observation indicates that covalent conversion occurs optimally under slow heating conditions as opposed to fast energy transfer. Efficient conversion within narrow elution windows is critical for compatibility with front-end separations, and the extent to which this conversion occurs has an immediate impact on the quality of subsequent UVPD spectra necessary for characterization of Schiff base labeled peptides. For all non-covalent complexes observed in these experiments, a reaction time of 50 ms combined with 18% normalized collision energy (NCE) resulted in predominant conversion to Schiff base products. These MS^2 spectra were easily distinguished based on the abundance of the water loss product; whereas activation at 18% NCE was sufficiently high to promote some extent of backbone cleavage for unreacted peptides (data not shown).

The ability to establish a set of dual source parameters capable of promoting ion/ion mediated covalent derivatization for all peptides in a mixture provided confidence that online bioconjugation followed by fast UVPD characterization of modified peptides was possible using an automated LC-MS^n approach. To test this strategy, a data-dependent neutral loss MS^3 (DDNLMS^3) method was developed (Scheme 2) in which the top five most abundant ions in the MS^1 spectrum were subjected to MS^2 using the aforementioned activation parameters. If the product ion corresponding to the neutral loss of water was within the top three most abundant ions in the low energy CID spectrum, a subsequent 193 nm UVPD activation event was initiated. Automation of the complete ion/ion mediated bioconjugation process is shown via consecutive scan events in Figure 4 for the peptide ASHLGLAR corresponding to the chromatographic peak at approximately 17 minutes of the elution profile (Figure 4a). The 1+ charge state of the electrostatic complex appears as the second most abundant ion in the MS^1 spectrum (Figure 4b), allowing efficient selection

during data-dependent acquisition. Collisional activation of the complex results in a dominant neutral loss of water (Figure 4c), thus triggering 193 nm UVPD (Figure 4d). The array of fragment ions generated provides extensive structural characterization of the modified peptide. Moreover, greater than 20% improvement in UVPD efficiency was observed relative to the unmodified analog (Figure S8), thus showing excellent agreement of the high-throughput LC-MS^n results with those obtained via dual source infusion set-up.

CONCLUSION

The development and implementation of a front-end dual electrospray reactor used for online ion/ion mediated bioconjugation was demonstrated for rapid Schiff base derivatization of peptide cations with FBDSA anions. Dual spray-assisted covalent chemistry was found to be highly consistent with analogous reactions performed *in vacuo*; however, by shifting the ion/ion reaction step to the interface of the mass spectrometer, both the throughput and adaptability of this approach was streamlined for integration into chromatographic workflows. Furthermore, we showed the advantage of utilizing fast online reactions to enhance the photodissociation efficiencies of peptides using 193 nm photoactivation as well as to improve the structural characterization of modified peptides.

Methods to maximize non-covalent complex formation are currently underway to improve compatibility of the dual spray reactor with high-throughput proteomic applications. Modifications include utilizing static nano ESI for the delivery of reagent anions as a means to reduce droplet size and improve reactant mixing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding from the NIH (Grant R21 GM103553), the Welch Foundation (Grant F1155), and the NSF Graduate Research Fellowship Program (Grant DGE-110007) is gratefully acknowledged. The assistance of Dustin Holden for the illustrative drawings of the dual source set-up is also acknowledged.

References

1. Hermanson, GT. Bioconjugate Techniques. Academic Press; 2013.
2. Stephanopoulos N, Francis MB. Nat Chem Biol. 2011; 7(12):876–884. [PubMed: 22086289]
3. Mirzaei H, Regnier F. Anal Chem. 2006; 78(12):4175–4183. [PubMed: 16771548]
4. Pashkova A, Moskovets E, Karger BL. Anal Chem. 2004; 76(15):4550–4557. [PubMed: 15283601]
5. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Nat Biotechnol. 1999; 17(10):994–999. [PubMed: 10504701]
6. Wiese S, Reidegeld KA, Meyer HE, Warscheid B. PROTEOMICS. 2007; 7(3):340–350. [PubMed: 17177251]
7. Ulbrich A, Merrill AE, Hebert AS, Westphall MS, Keller MP, Attie AD, Coon JJ. J Am Soc Mass Spectrom. 2014; 25(1):6–9. [PubMed: 24178922]
8. Keough T, Youngquist RS, Lacey MP. Proc Natl Acad Sci. 1999; 96(13):7131–7136. [PubMed: 10377380]
9. Madsen JA, Brodbelt JS. Anal Chem. 2009; 81(9):3645–3653. [PubMed: 19326898]

10. Robinson MR, Madsen JA, Brodbelt JS. Anal Chem. 2012; 84(5):2433–2439. [PubMed: 22283738]
11. Cotham VC, Wine Y, Brodbelt JS. Anal Chem. 2013; 85(11):5577–5585. [PubMed: 23641966]
12. Diedrich JK, Julian RR. Anal Chem. 2010; 82(10):4006–4014. [PubMed: 20405909]
13. Vasicek L, O'Brien JP, Browning KS, Tao Z, Liu H-W, Brodbelt JS. Mol Cell Proteomics. 2012; 11(7):1–10.
14. Espy RD, Wleklinski M, Yan X, Cooks RG. TrAC Trends Anal Chem. 2014; 57:135–146.
15. Prentice BM, McLuckey SA. Chem Commun. 2013; 49(10):947–965.
16. Mortensen DN, Williams ER. Anal Chem. 2014; 86(18):9315–9321. [PubMed: 25160559]
17. Mortensen DN, Williams ER. Anal Chem. 2014
18. Fisher CM, Kharlamova A, McLuckey SA. Anal Chem. 2014; 86(9):4581–4588. [PubMed: 24702054]
19. Miladinović SM, Fornelli L, Lu Y, Piech KM, Girault HH, Tsybin YO. Anal Chem. 2012; 84(11): 4647–4651. [PubMed: 22571167]
20. Kharlamova A, McLuckey SA. Anal Chem. 2011; 83(1):431–439. [PubMed: 21141935]
21. Grimm RL, Hodyss R, Beauchamp JL. Anal Chem. 2006; 78(11):3800–3806. [PubMed: 16737240]
22. Kim HI, Kim H, Shin YS, Beegle LW, Jang SS, Neidholdt EL, Goddard WA, Heath JR, Kanik I, Beauchamp JL. J Am Chem Soc. 2010; 132(7):2254–2263. [PubMed: 20121208]
23. Müller T, Badu-Tawiah A, Cooks RG. Angew Chem Int Ed. 2012; 51(47):11832–11835.
24. Girod M, Moyano E, Campbell DI, Cooks RG. Chem Sci. 2011; 2(3):501–510.
25. Badu-Tawiah AK, Li A, Jjunju FPM, Cooks RG. Angew Chem Int Ed. 2012; 51(37):9417–9421.
26. Chen H, Wortmann A, Zhang W, Zenobi R. Angew Chem Int Ed. 2007; 46(4):580–583.
27. Peng IX, Ogorzalek Loo RR, Shiea J, Loo JA. Anal Chem. 2008; 80(18):6995–7003. [PubMed: 18683952]
28. Xia Y, McLuckey SA. J Am Soc Mass Spectrom. 2011; 19(2):173–189.
29. Han H, McLuckey SA. J Am Chem Soc. 2009; 131(36):12884–12885. [PubMed: 19702304]
30. Stutzman JR, Luongo CA, McLuckey SA. J Mass Spectrom. 2012; 47(6):669–675. [PubMed: 22707160]
31. Mentinova M, McLuckey SA. J Am Chem Soc. 2010; 132(51):18248–18257. [PubMed: 21128662]
32. Prentice BM, Gilbert JD, Stutzman JR, Forrest WP, McLuckey SA. J Am Soc Mass Spectrom. 2013; 24(1):30–37. [PubMed: 23208744]
33. McGee WM, McLuckey SA. Proc Natl Acad Sci. 2014; 111(4):1288–1292. [PubMed: 24474750]
34. Hassell KM, Stutzman JR, McLuckey SA. Anal Chem. 2010; 82(5):1594–1597. [PubMed: 20121142]
35. Stutzman JR, McLuckey SA. Anal Chem. 2012; 84(24):10679–10685. [PubMed: 23078018]
36. Vasicek L, Brodbelt JS. Anal Chem. 2010; 82(22):9441–9446. [PubMed: 20961088]
37. Gardner MW, Smith SI, Ledvina AR, Madsen JA, Coon JJ, Schwartz JC, Stafford GC, Brodbelt JS. Anal Chem. 2009; 81(19):8109–8118. [PubMed: 19739654]
38. Villén J, Beausoleil SA, Gygi SP. PROTEOMICS. 2008; 8(21):4444–4452. [PubMed: 18972524]
39. Stutzman JR, Hassell KM, McLuckey SA. Int J Mass Spectrom. 2012; 312:195–200. [PubMed: 22408389]
40. Ogorzalek Loo RR, Udseth HR, Smith RD. J Am Soc Mass Spectrom. 1992; 3(7):695–705. [PubMed: 24234636]
41. Chen H, Venter A, Cooks RG. Chem Commun. 2006; (19):2042–2044.
42. Law WS, Wang R, Hu B, Berchtold C, Meier L, Chen H, Zenobi R. Anal Chem. 2010; 82(11): 4494–4500. [PubMed: 20443546]
43. Madsen JA, Boutz DR, Brodbelt JS. J Proteome Res. 2010; 9(8):4205–4214. [PubMed: 20578723]

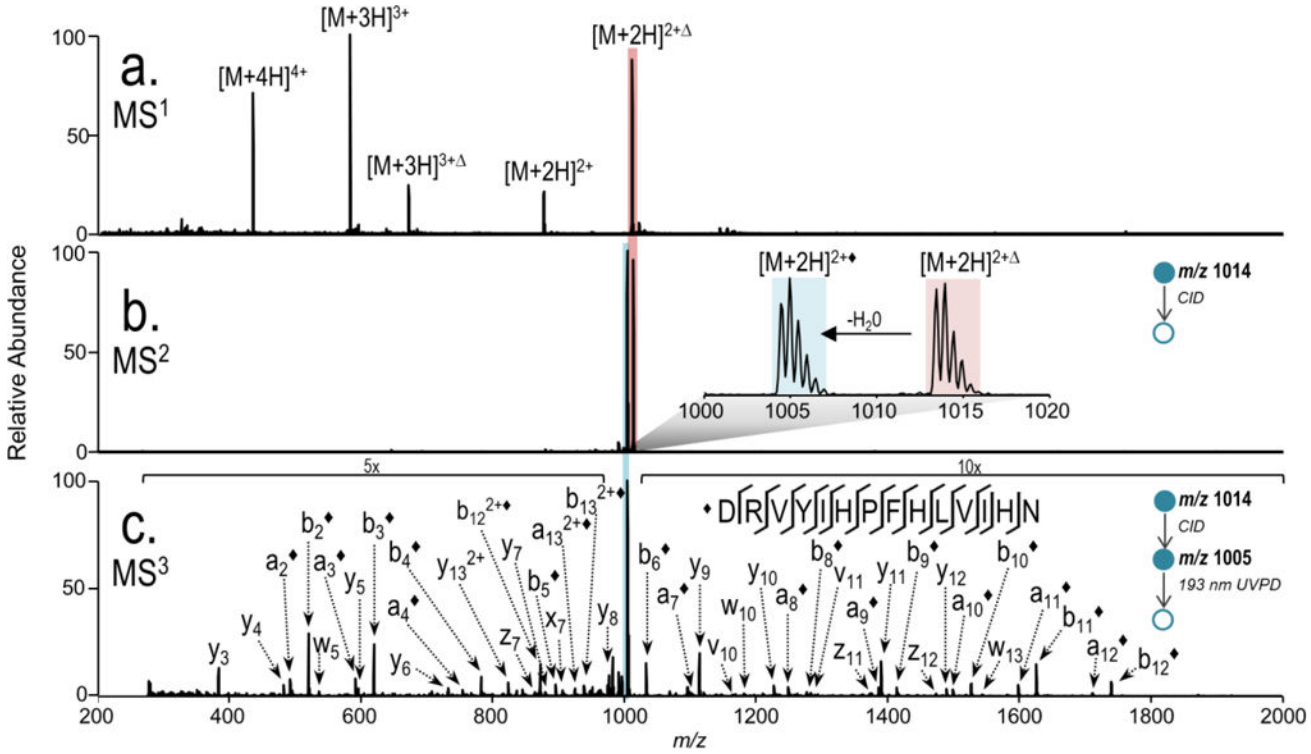


Figure 1.

Process for on-line modification of peptides with FBDSA using a dual spray reactor. (a) Electrostatic complexes are formed at atmospheric pressure between multiply charged peptide cations and FBDSA anions. These ion/ion intermediates are denoted with a "Δ" superscript. (b) Low energy collisional activation of the intermediate ion/ion species promotes Schiff base formation via the concerted formation of an imine bond and loss of a water molecule, resulting in a chromophore-labeled Schiff base product (◆) with a mass shift of-18 Da relative to the electrostatic complex. (c) 193 nm UVPD of the labeled peptide exhibits extensive backbone fragmentation.

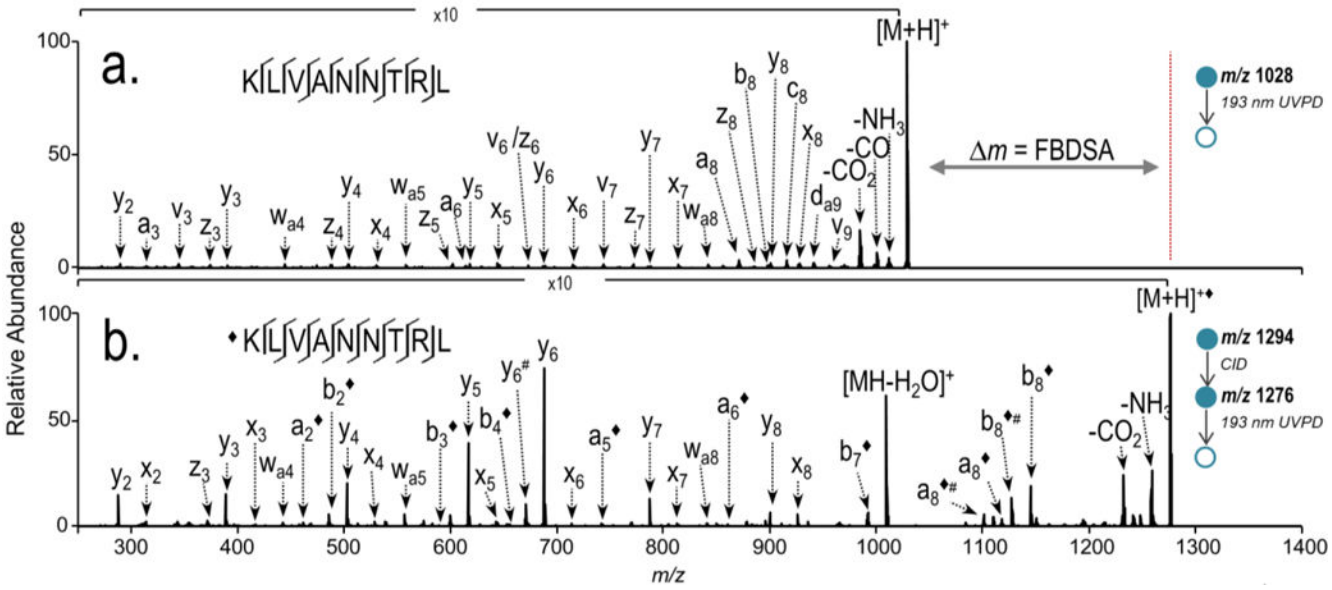


Figure 2.

Comparison of 193 nm UVPD efficiencies before and after Schiff base modification of KLVANNTRL (1+). (a) MS^2 UVPD mass spectrum for unlabeled peptide and (b) MS^3 UVPD mass spectrum following on-line derivatization using the dual spray reactor.

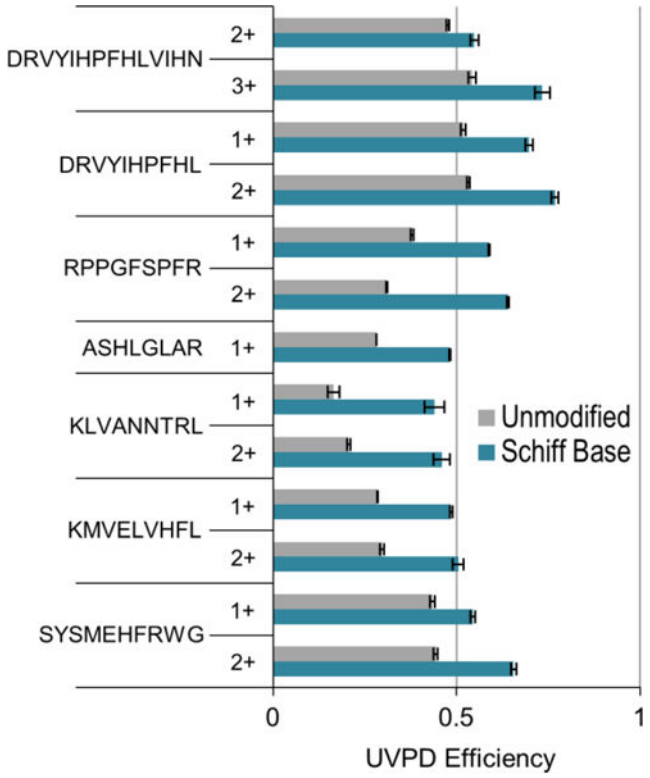


Figure 3.

Comparison of 193 nm UVPD efficiencies before and after Schiff base modification of KLVANNTRL (1+). (a) MS^2 UVPD mass spectrum for unlabeled control and (b) MS^3 UVPD mass spectrum following online derivatization using the dual spray reactor.

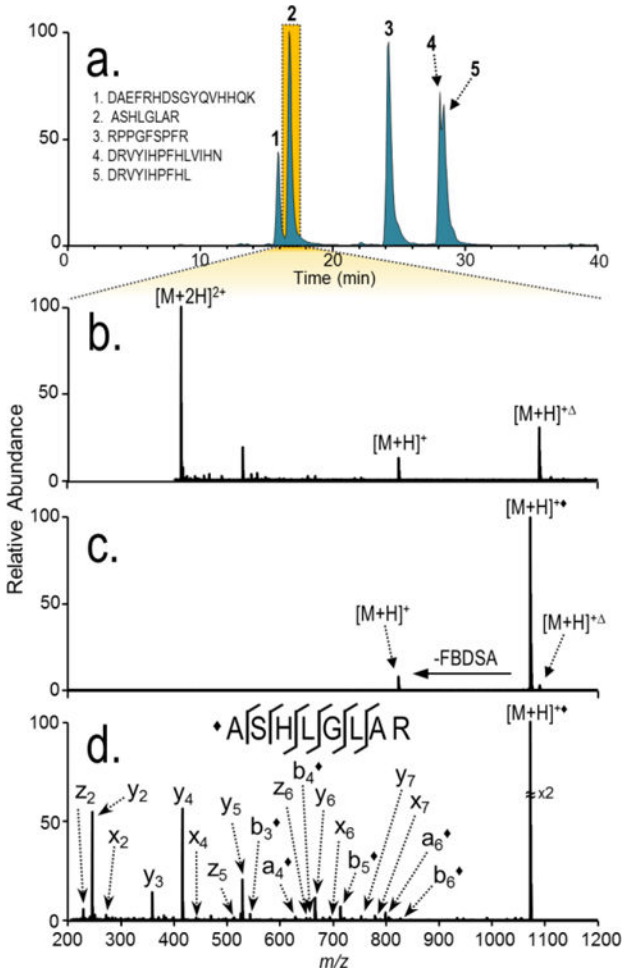
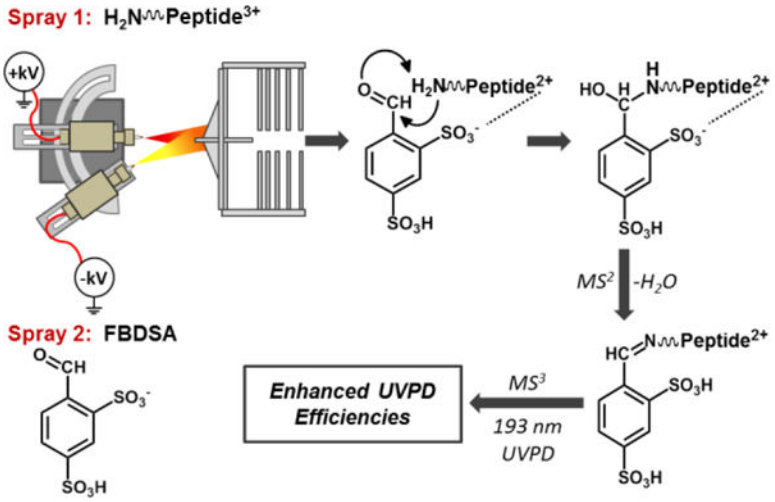


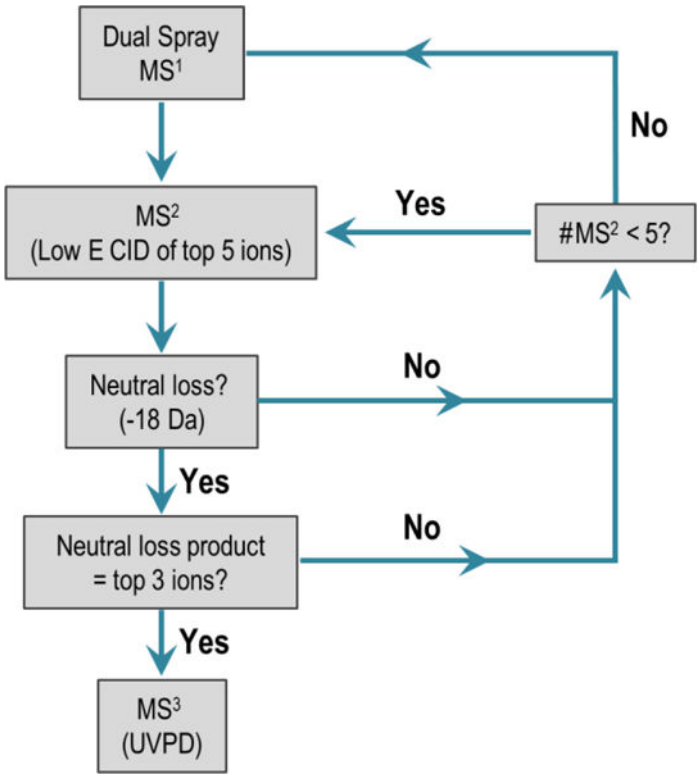
Figure 4.

Online derivatization of a peptide mixture using the dual spray reactor combined with an LC-MS based data-dependent neutral loss MS^3 scan program. (a) Base peak chromatogram for the peptide mixture. (b) MS^1 scan during the elution of ASHLGLAR showing a mixture of unreacted peptide and peptide/FBDSA complexes (Δ). The electrostatic complex is selected by the DDNLMS^3 program to undergo (c) low energy CID (NCE = 18%). A neutral loss product is detected at −18 Da (◆), which triggers (d) 193 nm UVPD of the Schiff base product.



Scheme 1.

Summary of Dual Spray initiated Bioconjugation of a Peptide Cation with an FBDSA Anion for Enhanced Ultraviolet Photodissociation.



Scheme 2.

Data-dependent neutral loss MS^3 decision tree for online derivatization using an LC-MS^3 platform.