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Femtosecond Laser-Induced Ionization/Dissociation of Protonated Peptides

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The application of electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) techniques coupled with tandem mass spectrometry (MS/MS) for the identification and characterization of proteolytically derived peptide ions has underpinned the emergent field of proteomics.[1] However, the ability of MS/MS approaches to generate sufficient product ions from which the sequence of an unknown peptide can be determined, or to unambiguously characterize the specific site(s) of post-translational modifications within these peptides, is highly dependent on the specific method employed for ion activation,[2] as well as the sequence and charge state of the precursor ion selected for analysis.[3] In practice, collision induced dissociation (CID),[4] whereby energy deposition occurs through ion−molecule collisions followed by internal vibrational energy redistribution prior to dissociation, often results in incomplete backbone fragmentation, or the dominant loss of labile groups from side chains containing post-translational modifications such as phosphorylation, particularly for peptides observed at low charge states.[3,5,6] Thus, there has been great interest in the development of alternate activation methods, including surface induced dissociation (SID),[7] infrared multiphoton dissociation (IRMPD),[8,9] ultraviolet photodissociation (UVPD),[10,11] electron capture and electron transfer dissociation (ECD, ETD),[12,13] and metastable atom dissociation,[14,15] that yield greater sequence information and that provide selective control over the fragmentation chemistry, independent of the identity of the precursor ion. However, each of these methods suffers from certain limitations. For example, IRMPD and UVPD efficiencies require the presence of a suitable chromophore for photon absorption, while ECD and ETD are applicable only to the analysis of multiply charged precursor ions. Herein, to overcome these limitations, we describe the implementation and initial results obtained from a novel strategy for peptide sequence and modification analysis, termed femtosecond laser-induced ionization/dissociation (fs-LID), involving the use of ultrashort (<35 fsec) laser pulses[16] for energy deposition and nonergodic dissociation[17] in a quadrupole ion trap mass spectrometer.

Specific details of the instrumentation and experimental conditions employed in this study can be found in the Supporting Information. Briefly, an amplified Ti:Al_2O_3 laser was interfaced with a modified Thermo Scientific LCQ DECA XP Plus ion trap mass spectrometer to provide transform-limited pulses 33 fs in duration (300 μJ/pulse, 3×10^{13} W/cm^2 calculated peak power at the center of the trap) at a repetition rate of 1 kHz with a 28 nm bandwidth centered around 800 nm (see Figures S1 and S2). Individual peptides were introduced to the mass spectrometer by ESI, then selected precursor ions were isolated and subjected to MS/MS and MS^3 by fs-LID or CID.

fs-LID of the $[M + H]^+$ precursor ion of angiotensin II (200 msec irradiation, 31.5% precursor ion dissociation efficiency) (Figure 1A) yielded more than 40 assignable products, including 23 of 42 possible a-, b-, c-, x-, y-, and z-type "sequence" ions, from which 100% sequence coverage was obtained. In comparison, the CID spectrum from the same precursor ion was dominated by selective cleavage at the C-terminal side of the aspartic acid residue to yield the y_7 ion (Figure S3A), with only 17 assignable product ions (12 of 42 possible sequence ions). fs-LID for a 30 msec irradiation period of (Figure S3B) resulted in an essentially identical spectrum to that shown in Figure 1A, albeit with a lower precursor ion dissociation efficiency of 9.7%. Notably, an odd electron doubly charged ($[M + H]^{2+}\bullet$) product ion, formed via photoionization of the even electron singly protonated precursor, was observed in Figures 1A and S3B. Comparison of the high resolution zoomscan spectrum acquired for this ion with that of the even electron $[M + 2H]^{2+}$ precursor ion of angiotensin II (Figures S4A and S4B, respectively) confirmed the presence of the odd-electron doubly charged photoionization product. $[M + H]^{2+}\bullet$ ions have previously been produced by electron ionization[18] as Penning ionization products following metastable ion activation[15] or by electron transfer upon CID of triply charged $[Cu^{II}(terpy)(M + H)]^{3+}\bullet$ complexes.[19] However, these ions have not previously been observed via conventional photoionization techniques,[20] and their involvement in the dissociation pathways responsible for the formation of sequence type product ions have not previously been reported. CID MS^3 (Figure 1B) and fs-LID MS^3 (Figure S5) revealed that the majority of the product ions observed in Figure 1A were formed from this radical species. Other products formed via the losses of *p*-quinomethide (−106) and CO_2 (−44), indicative of specific side chain functional groups in the peptide, were also observed in Figure 1A, consistent with prior studies on the fragmentation reactions of peptide radicals.[21,22] Importantly, the fs-LID technique was also demonstrated to be applicable to the analysis of multiply protonated precursor ions, as shown for the $[M + 2H]^{2+}$ and $[M + 3H]^{3+}$ precursor ions of angiotensin II (shown in Figures S6A and S6B, respectively, for a 200 msec irradiation period, and in Figures S7A and S7B, respectively, for a 30 msec irradiation period), and for the $[M + 2H]^{2+}$ precursor ion from an additional peptide, Glu-fibrinopeptide B (120 msec irradiation period, Figure S8A). In each case, fs-LID resulted in the formation of extensive sequence ions from which equivalent or greater sequence coverage could be obtained compared to CID (see Figures S9A and S9B and Figure S8B). Comparison of the 200 msec and 30 msec fs-LID MS/MS spectra for the singly and doubly protonated precursor ions of angiotensin II revealed that the abundance of the odd-electron photoionization product ions decreased with increasing irradiation times, relative to the abundance of the "sequence ions", indicating that some of the radical ion population that is initially formed can undergo secondary dissocia-

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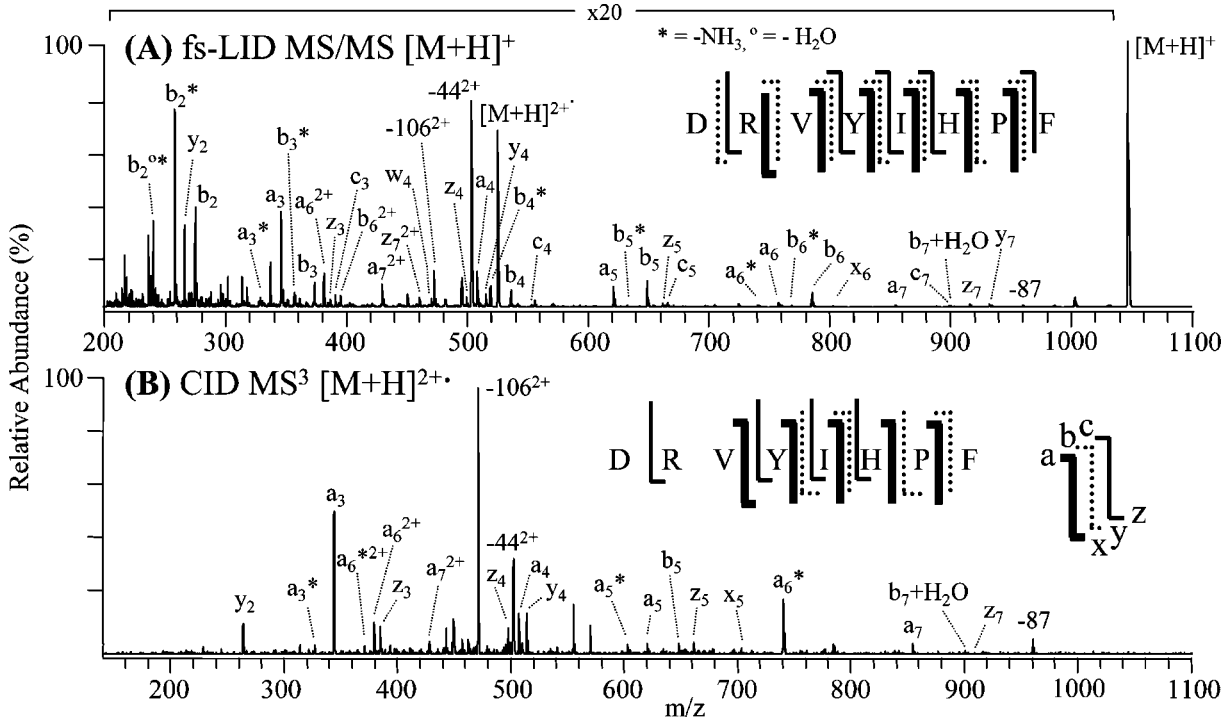

Figure 1. (A) fs-LID MS/MS (200 msec irradiation) of the $[M + H]^+$ precursor ion of angiotensin II and (B) CID MS^3 of the $[M + H]^{2+}\cdot$ photoionization product from panel A.

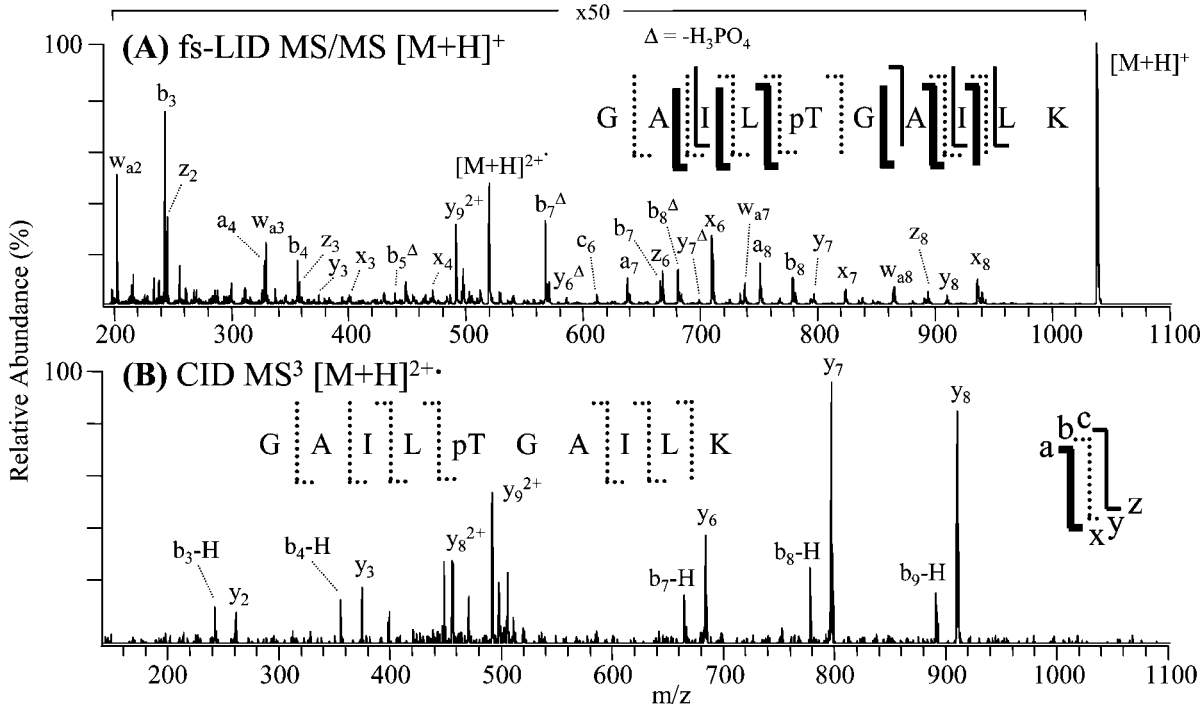

Figure 2. (A) fs-LID MS/MS (200 msec irradiation) of the $[M + H]^+$ precursor ion of GAILpTGAILK. (B) CID MS^3 of the $[M + H]^{2+}\cdot$ photoionization product from panel A.

tion as the number of laser pulses increases. This result is also consistent with the CID MS^3 and fs-LID MS^3 data described above.

To assess the utility of the fs-LID technique for the characterization of peptides containing post-translational modifications, the fragmentation reactions of the singly and doubly protonated precursor ions from the model synthetic phosphopeptide GAILpTGAILK (pTK),[5] and the singly, doubly, and triply protonated precursor ions of the model synthetic phosphopeptide LFpTGHPESLER (pTSR)[6] were examined. It can be seen from the spectrum in Figure 2A, for the singly protonated precursor ion of the pTK peptide, that 100% sequence coverage was obtained upon fs-LID (21 of the 54 possible a-, b-, c-, x-, y-, and z-type "sequence" product ions), with negligible loss of the phosphate group ($-H_3PO_4$ (98 Da) or $-HPO_3$ (80 Da)) observed from the precursor ion and only minimal loss from the product ions. In contrast, CID MS/MS (Figure S10A) resulted in only limited sequence ion formation, with

dominant loss of H_3PO_4 from both the precursor and product ions, that precluded the ability to localize the site of phosphorylation to a single amino acid residue. Similar to that observed for the angiotensin II peptide, fs-LID MS^3 ($q = 0.25$, 200 msec irradiation) of the $[M + H]^{2+}\cdot$ photoionization product ion in Figure 2A (Figure S11) resulted in relatively limited sequence ion formation. However, when combined with the sequence information observed by CID MS^3 (Figure 2B), it was clear that the majority of the "sequence" ions in the fs-LID MS/MS spectrum in Figure 2A originated via secondary dissociation of the odd-electron photoionization product. For the doubly protonated precursor ion of the pTK peptide, equivalent sequence coverage was obtained from both CID MS/MS (Figure S10B) and fs-LID MS/MS (Figure S10C). Figure S12A and S12B contain the CID MS/MS and fs-LID MS/MS (120 msec irradiation period) spectra obtained from the singly protonated precursor ion of the pTSR peptide. Similar to the CID data obtained from the pTK peptide in Figure S9A, the loss of 98 Da was observed as the dominant fragmentation pathway. In addition, several product ions formed via gas-phase transfer of the phosphate group from the phosphorylated threonine residue to the unmodified serine residue following dissociation (an example labeled $y_6^{\blacksquare}$ is shown in Figure S12A) were observed. The formation of these "rearrangement" ions has recently been shown to be relatively common under typical CID conditions in ion trap mass spectrometers and can limit the ability to unambiguously assign the correct site of phosphorylation or lead to erroneous assignment of the phosphorylation site.[6] In contrast, although fs-LID MS/MS (41.4% precursor ion dissociation efficiency) of the singly protonated precursor ion of the pTSR peptide (Figure S12B) did not yield as extensive sequence coverage as that observed by CID, the loss of 98 Da was significantly lower in abundance, and no evidence was observed for formation of rearrangement ions. CID MS/MS and fs-LID MS/MS (120 msec irradiation) of the doubly and triply protonated precursor ions of the pTSR peptide are shown in Figures S13A and S13B (44.2% precursor ion dissociation efficiency) and Figures S14A and S14B (42.3% precursor ion dissociation efficiency), respectively. Similar to the results described above for the other multiply protonated precursor ions, fs-LID again resulted in the formation of extensive sequence ions from which equivalent sequence coverage could be obtained compared to CID, but with only minimal loss of 98 Da.

Note that some phosphate loss was observed from either the product ions or the precursor ions of the pTK and pTSR peptides following fs-LID, perhaps suggesting that the fs-LID dissociation process may not be nonergodic. Zewail and co-workers have concluded that nonergodic dissociation processes dominate the initial fragmentation reactions observed following femtosecond photon absorption.[17] However, in systems containing a large number of vibrational modes, and with extensive intramolecular solvation, limited vibrational energy redistribution could potentially occur in a time frame similar to that for nonergodic fragmentation, resulting in the observation of some ergodic fragmentation derived product ions (e.g., loss of 98 Da from the precursor ions). Alternatively, excess internal energy within the product ions initially formed via nonergodic pathways, following the 3 or 4 photon absorption that would be required for photoactivation under the current experimental conditions, may allow for some sequential ergodic fragmentation, with subsequent loss of the phosphate group.

Although further optimization of the fs-LID technique, and statistical evaluation of the fs-LID fragmentation behavior compared to that observed by CID or ECD/ETD, will require the acquisition of data from a significantly larger number of peptides, the initial results outlined above suggest that fs-LID is a viable alternate ion activation strategy for peptide sequence and modification analysis, with great promise for improving the capabilities of tandem mass spectrometry methods for comprehensive proteome analysis, particularly for the sequence analysis and characterization of singly protonated peptides (i.e., those formed by MALDI), where alternate dissociation methodologies to CID are currently lacking.

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Supporting Information Available: Details of the instrumental and experimental conditions, and additional fs-LID and CID MS/MS and MS^3 spectra of angiotensin II, Glu-fibrinopeptide B, and the model synthetic phosphothreonine containing peptides GAILpTGAILK (pTK) and LFpTGHPESLER (pTSR). This material is available free of charge via the Internet at http://pubs.acs.org.

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