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Gas-Phase Ion Unimolecular Dissociation for Rapid Phosphopeptide Mapping by IRMPD in a Penning Ion Trap: An Energetically Favored Process

Jason W. Flora and David C. Muddiman*

Department of Chemistry, Virginia Commonwealth University, Box 842006, Richmond, Virginia 23284

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One of mass spectrometry's (MS) foremost contributions to the biochemical community is the recognition of diverse forms of the same proteins transformed by posttranslational modifications associated with their functional character.[1] The most important and common posttranslational modification is the phosphorylation of serine, threonine, and tyrosine,[2] which regulates cellular processes such as metabolism, growth, and reproduction.[3]

Mass spectrometry has become a powerful analytical tool for the structural characterization of biomolecules and several methods of phosphopeptide mapping have been proposed that take advantage of the relative speed, sensitivity, and adaptability of MS. These methods include the use of phosphatases prior to MS analysis,[4] chemical tagging of the phosphorylation site,[5] metastable decomposition,[6] collision induced dissociation (CID),[7] precursor ion scanning,[7b,8] neutral loss scanning,[9] nozzle-skimmer dissociation,[10] electron capture,[2c] and ^{31}P monitoring by inductively coupled plasma (ICP)-MS.[11] A method for screening complex proteolytic digests with electrospray ionization[12] Fourier transform ion cyclotron resonance[13] (ESI-FTICR) MS and infrared multiphoton dissociation[14] (IRMPD) has recently been developed by this research group[15] where all phosphopeptides within a complex protein digest can be simultaneously identified by a single IR laser irradiation event.[15] The scope of this research is to evaluate the energetics of phosphopeptide dissociation and confirm that it is energetically more favorable than unmodified peptide dissociation by IRMPD.

MS for the unimolecular dissociation of trapped gas-phase ions can provide valuable information with regards to the kinetics and energetics of the ion's structure. Dunbar first determined bond dissociation energies using IR irradiation produced by a continuous wave (CW) CO_2 laser for small molecules.[16] Dunbar and McMahon then demonstrated that a vacuum chamber can act as a blackbody source of IR photons when heated to a particular temperature[17] resulting in a Boltzmann distribution of trapped ion internal energies at the temperature of the ion trap.[18] This blackbody induced radiative dissociation (BIRD) has been demonstrated for the determination of bond dissociation energies of clusters[19] and biomolecules.[20] Marshall and co-workers recently demonstrated that IRMPD is a practical method for the determination of the relative energies of activation for unimolecular dissociation for large gas-phase biomolecules (>50 atoms) which are in close agreement with values previously obtained by BIRD.[18] This technique has also been used to determine the relative E_a for dissociation of oligonucleotides and modified (7-deaza purine) analogues.[21]

We are able to evaluate the energetics associated with IRMPD of gas-phase peptide ions for phosphopeptide mapping[22] using the kinase domain of the insulin receptor (KDIR) (sequence: TRDIYETDYYRK) unmodified (M), monophosphorylated (M_p), and triphosphorylated (M_{ppp}) as model systems, which are analogous to a typical proteolytic fragment with varying degrees of tyrosine phosphorylation. Figure 1 is the ESI-FTICR mass spectra of $[M_{ppp} - 2H^+]^{2-}$ after ion isolation (Figure 1A) and with 600 ms of IR irradiation at 2 W (Figure 1B). At this short irradiation time almost all of the M_{ppp} has dissociated into the signature ions indicative of tyrosine phosphorylation.

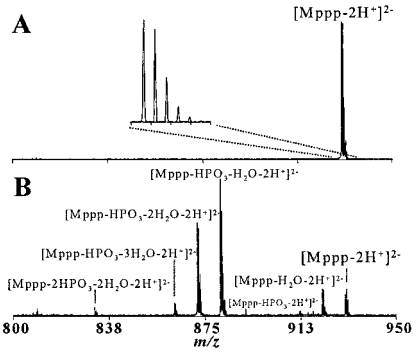

Figure 1. ESI-FTICR mass spectra of the doubly charged M_{ppp} (A) without IRMPD and (B) with IR irradiation at 2 W for 600 ms.

Relative energies of activation (E_a) for gas-phase dissociation are determined for M, M_p, and M_{ppp} by an equation derived by Dunbar[16] based upon the approximate relation between laser intensity and temperature where υ is the frequency of radiation (10.6 μm = 2.83×10^{13} Hz), h is Plank's constants, and I_{laser} is the laser intensity (W/cm^2):

$$E_a = -\frac{\mathrm{d}\ln k_{diss}}{\mathrm{d}1/(kT)} = qh\upsilon\frac{\mathrm{d}\ln k_{diss}}{\mathrm{d}\ln I_{laser}}$$

The partition function for the vibrational mode that absorbs the IR radiation, q, is fixed at 1.05 as determined by Marshall and co-workers.[18]

First-order rate constants (k_{diss}) were determined for M, M_p, and M_{ppp} at five laser powers[23] (data not shown) from the slope of the natural log of the relative ion abundance of the precursor ions versus the duration of IR irradiation. All precursor ions were isolated followed by thermalization (collisional cooling with N_2 gas at $\sim 1 \times 10^6$ Torr) prior to IRMPD to remove any internal energy accumulated during the isolation event due to the off-resonant absorption of rf radiation. It should be noted that initial E_a studies, without post isolation collisional cooling, showed no induction periods and negative y-intercepts for all kinetic plots due to internal energy acquired during the isolation event.

Figure 2 is a plot of the natural log of k_{diss} versus the natural log of laser intensity for the doubly charged KDIR M, M_p, and M_{ppp}. As predicted, the unmodified peptide has a higher relative activation energy for dissociation (E_a for $[M - 2H^+]^{2-} = 0.51$ eV) than the monophosphorylated species (E_a for $[M_p - 2H^+]^{2-} = 0.43$ eV) as well as the multiply phosphorylated peptide (E_a for $[M_{ppp} - 2H^+]^{2-} = 0.18$ eV).

* Corresponding author. E-mail: dcmuddim@mail1.vcu.edu.

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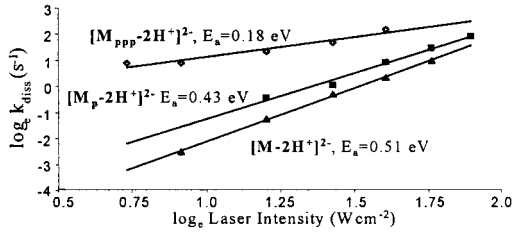

Figure 2. Plot of the $\log_e k_{diss}$ versus the $\log_e$ of laser intensity for the doubly charged M (△), M_p (□), and M_{ppp} (◇). E_a are listed.

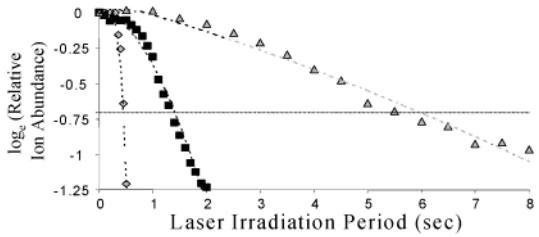

Figure 3. The $\log_e$ of the precursor ion relative ion abundance versus the laser irradiation period for the IRMPD of the KDIR M (△), M_p (□), and M_{ppp} (◇) during IR laser irradiation at 2 W.

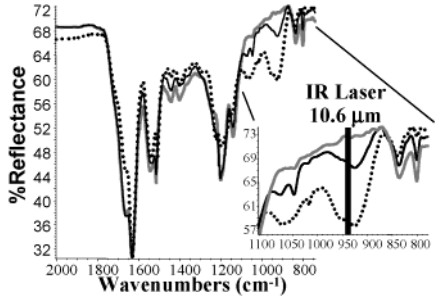

Figure 4. FTIR spectra (normalized to 1635 cm^{-1}) of the KDIR M (gray line), M_p (black line), and M_{ppp} (dashed line). The phosphonate stretching region is expanded and the IR laser frequency is shown.

Figure 3 shows a plot of the natural log of the precursor ion relative ion abundance versus the laser irradiation period at 2 W. Fifty percent of the M_{ppp} and M_p ions have dissociated after 0.45 and 1.3 s, respectively, while 50% of the unmodified KDIR precursor ion has not dissociated after more than 5 s. Irradiation times required to dissociate the three KDIR peptides undoubtedly differ because of the different relative E_a discussed above. However, this is not the only contributing factor to the preferred dissociation indicative of the phosphopeptides by IRMPD. As previously reported, the P−O stretch (9.6−11 μm or 1042−909 cm^{-1}) is in direct resonance with the CO_2 IR laser (10.6 μm or 943 cm^{-1}) used for IRMPD.[14]

Figure 4 shows the attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectra of M, M_p, and M_{ppp}.[24] The extinction coefficients (k) of M_p and M_{ppp} relative to M ($k = 1$) at the frequency of the IR irradiation are 1.2 and 1.6, respectively. While these k values represent only moderate increases in absorptivity, they can contribute to the rate of first-order decay of the precursor ion population of the phosphopeptides. The E_a for dissociation of phosphopeptides is the physical reason for their selective dissociation; however, the vibrational frequency of the phosphate moiety may be a contributing factor affording a higher internal energy at the rapid exchange limit.[18a,20] Thus, IRMPD in a Penning ion trap is an ideal platform for rapid phosphopeptide mapping amenable to online separation techniques.

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(22) All experiments were conducted using nanospray ESI with a modified Ionspec (Irvine, CA) FTICR-MS (7 T). IRMPD on trapped ions with a continuous wave 25-Watt Synrad 48-2(W) (Mukilteo, WA) CO_2 laser. The KDIRs were purchased from ANASPEC (San Jose, CA).

(23) All experiments were conducted at the zero-pressure limit. For all IRMPD events, the entire trapped ion population is uniformly irradiated as the diffused laser beam has a diameter of 8.75 mm while the thermal radius of the ion cloud is ~0.07 mm.

(24) All FTIR experiments were conducted using a Nicolet Nexus 670 with a multibounce ATR module with 20 μL of 1 mM KDIR (M, M_p, M_{ppp}) methanol solutions dried on the ATR plate (150 spectra). k is determined from the absorbance mode assuming reflectance is proportional to the transmittance and 100% reflectance corresponds to 100% transmittance.

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