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Biomolecular condensation via ultraviolet excitation in vacuo

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ABSTRACT

Recently, we reported that 157 nm vacuum ultraviolet irradiation (VUV) of proton-bound peptide dimers trapped in a vacuum, results in the elimination of water and formation of a peptide bond [J. Am. Chem. Soc. 133 (2011) 15834–15837]. Here, we further explore the ability to form a covalent bond between biomolecular ions with photoexcitation. Photoexcitation of long-lived charge-bound complexes appears to be a general phenomenon, resulting in the loss of water and the formation of covalent bonds between many types of molecules. Several examples are described, including: the linear coupling of amino acid chains that produce octapeptides from tetrapeptide complexes; inter-molecular cross-linking of amino acid side chains, and glycosidic bond formation between disaccharide complexes. Simple mechanisms for each case are proposed.

1. Introduction

Recently, we reported that photoactivation of non-covalent, proton-bound dimer complexes of peptide ions stored in an ion trap results in the elimination of water and the formation of peptide bonds [1]. Although this reaction is inefficient (<1% yield in some cases), the idea that the proton-bound complex exists as a long-lived intermediate along a pathway that results in the formation of longer polypeptide chains is interesting. Such reactions may be relevant to the origin of biological molecules [2,3]. Of particular note is that charge-bound complexes are formed by many ion sources and are often observed upon electrospraying solutions containing peptides. In this paper, we explore these reactions in more detail. We present new data for several types of charge-bound complexes, including peptide bound complexes, demonstrate the ability to cross link two peptides (that have been modified to prevent reactions at the N- and C-terminus), and show an illustration involving activation of a sodium-bound dimer made up of disaccharides.

The ability to produce covalent bonds in the controlled environment of a mass spectrometer opens up many new possibilities for synthesizing both well-known and exotic systems for study. A general approach would attract considerable attention; however, to date there have been relatively few examples of gas phase syntheses in mass spectrometers. For example, Beauchamp and co-workers generated highly reactive carbenes by collisional activation of a diazo precursor in order to covalently couple a host–guest complex [4]. In another paper, Julian and Beauchamp showed that sequential collision-induced dissociation (CID) of an adenosine 5′-monophosphate trimer precursor can be used to form an adenosine 5′-triphosphate by the successive elimination of two adenosines [5]. McLuckey and co-workers have carried out reactions between cations and anions to produce covalent bonds. They have shown that the amino terminus of protonated peptides stored in an ion trap can be covalently modified upon association with a 4-formyl-1,3-benzenedisulfonic acid anion [6]. More recently they showed that it was possible to cross-link peptide ions (both intra- and inter-molecular cross-links have been produced) [7]. Importantly, all of these previous syntheses have relied on activation processes which are slow with respect to intramolecular vibrational energy redistribution, which restricts the synthetic approach to the lowest energy pathways.

In the present work we note that peptides and oligosaccharides themselves contain several functional groups that have the potential to condense, and have utilized a novel synthetic route initiated by fast dissociation chemistry to achieve condensation. More specifically, Norrish Type I homolytic cleavage [8], imposed by Reilly and co-workers [9] to explain products of 157 nm photodissociation, is a fast process, providing access to highly reactive species via direct dissociation in the excited state. In our adaptation of this mechanism, photoexcitation of a long-lived peptide complex initiates a Norrish-like cleavage, and subsequent radical rearrangements give rise to water elimination and formation of a covalent bond. The reaction takes place on a singly charged reagent complex and the result is that a covalent bond is formed between either N- and C-termini of peptides or amino acid side chains of modified

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peptides. Additionally, radical cleavage between ring carbon and hydroxyl oxygen of oligosaccharides can also result from photoexcitation, which leads to glycosic bond formation via a similar radical recombination.

2. Experimental

2.1. Materials and preparation

Tetrapeptides, Gly-Pro-Gly-Gly (GPGG), acetyl-Val-Lys-Met-Asp-7-amido-4-trifluoromethylcoumarin (ac-VKMD-cu), cellobiose, and cellotetraose have been purchased from Sigma–Aldrich (St. Louis, MO) and used without further purification. Electrospray solutions of GPGG and ac-VKMD-cu peptides at a concentration of 2×10^{-4} M were prepared in 49:49:2 (vol.%) water:methanol:acetic acid. Cellobiose and cellotetraose ions were produced from a solution containing 2×10^{-4} M of the oligosaccharide in a 50:50 (vol.%) water:acetonitrile and 2 mM NaCl solution. The solutions are infused through a pulled capillary (75 μm i.d., 360 μm o.d.) tip at a flow rate of 0.30 μL min^{-1} using a syringe pump (KD Scientific, Holliston, MA). The capillary tip is maintained at a DC bias of ~2.0 kV above the voltage of the entrance plate to the desolvation region.

2.2. Instrumentation

A detailed description of the IMS/ion trap instrument used for this study can be found elsewhere [10]. Briefly, a home-built drift tube is coupled to an ion trap mass spectrometer. The electrosprayed ions travel through a drift region (at 300 K under a uniform electric field of $\sim 2.3 \times 10^3$ V m^{-1}) and enter the LTQ Velos instrument (Thermo Electron, San Jose, CA). The ions are accumulated and mass-selected to isolate singly charged dimer ions in a linear ion trap. The dimer complex can be collisionally activated by applying a resonant rf excitation waveform for 10 ms with activation q of 0.25 and normalized collision energy of 30%. The stored complex can also be activated by 157 nm irradiation. Details regarding the modification of the LTQ instrument to provide the ability to perform photoexcitation experiments have been described previously [10,11]. Briefly, a F_2 laser (EX100HF-60, GAM Laser, Orlando, FL) has been aligned to the ion trap with a vacuum line. A single pulse of laser light (157 nm) is introduced into the ion trap at the beginning of a 10 ms activation step with 0% normalized collision energy and an activation q of 0.1. Product ions of interest obtained from photoexcitation are subjected to MS^3 analysis by collisional activation under the same ion isolation and CID conditions as the MS^2 experiments.

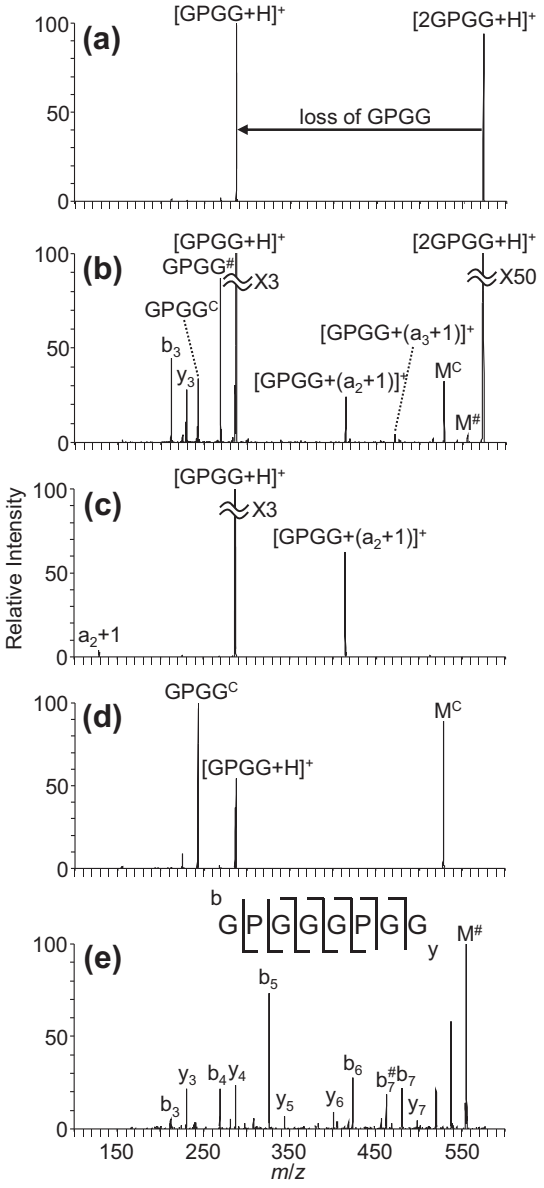
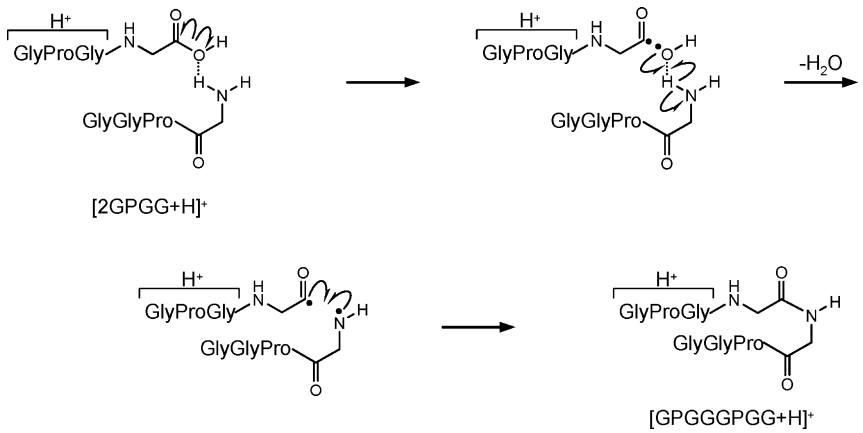


Fig. 1. Fragmentation spectra of $[2GPGG+H]^+$ obtained by (a) CID and (b) 157 nm irradiation. The superscripted C and # indicate the losses of CHO_2 and H_2O, respectively. CID spectra of (c) $[GPGG+(a_2+1)]^+$, (d) the CHO_2-loss product (M^C), and (e) the water-loss product ($M^\#$) in spectrum b. The peaks in spectrum e are assigned according to the peptide sequence, GPGGGPGG.

3. Results and discussion

3.1. Linear coupling of amino acid chains

It is well known that electrospraying relatively concentrated peptide solutions ($\geq 10^{-5}$ M) leads to formation of proton-bound peptide dimer ions [12,13]. A precursor mass spectrum (not shown) indicates that proton-bound $[2GPGG+H]^+$ dimer ions (observed at $m/z = 573$) are formed by electrospraying a 2×10^{-4} M solution of the GPGG tetrapeptide. Fig. 1 shows the mass spectrum obtained upon collisional activation, which causes the complex to heat up and dissociate. Thus, CID on the $[2GPGG+H]^+$ ions results in disassembly of the complex into $[GPGG+H]^+$ monomer ions appearing at $m/z = 287$ (Fig. 1a). In contrast, photoexcitation of the singly protonated $[2GPGG+H]^+$ complex ions results in a significantly more complicated spectrum. As shown in Fig. 1b, 157 nm irradiation of the complex generates several product ions involving inter-molecular and intra-molecular fragments as well as a peak showing neutral loss of 18 Da ($M^\#$ in Fig. 1b, discussed below). The latter peak has been attributed to water loss by the complex ion. In addition to the peak obtained from dissociation of the noncovalent complex, small intra-molecular fragments at $m/z = 212$, 230, and 243 that can be assigned to b_3, y_3, and $[GPGG - CHO_2]^+$ ($GPGG^C$ in Fig. 1b) ions are observed. It is noteworthy that peaks at $m/z = 414$, 471, and 528 corresponding to $[GPGG+(a_2+1)]^+$, $[GPGG+(a_3+1)]^+$, and $[2GPGG - CHO_2 + H]^+$ (M^c in Fig. 1b), respectively, involve cleavages of the covalent backbone of one of the $[GPGG+H]^+$ ions without inter-molecular dissociation. The CID analyses of the product ions at $m/z = 414$ and 528 are consistent with our assignments of $[GPGG+(a_2+1)]^+$ and M^C as noncovalent complexes (Fig. 1c and d). This aspect (preservation of non-covalent complex species) of the fragmentation pattern is similar to well-known characteristics of electron capture dissociation [14,15]; the fragmentation pattern is also dissimilar to ECD in that the peptide complexes include



Scheme 1. Proposed mechanism for formation of a GPGGGPGG octapeptide from the $[2GPGG+H]^+$ complex upon 157 nm irradiation.

high-energy fragments (a- and x-type ions) and c- and z-type ions are noticeably absent. The observation of these peptide fragment complexes without disruption of the weak noncovalent bonding upon 157 nm photoexcitaton confirms that UV photon absorption can induce covalent bond breaking (close to the absorption site) prior to intramolecular vibrational energy redistribution [9,16].

As mentioned above, the VUV PD spectrum of the $[2GPGG+H]^+$ complex ions also presents a neutral loss of water at $m/z = 555$ ($M^{\#}$ in Fig. 1b). It is well known that the CID spectrum of a single peptide often contains fragment ions due to losses of small neutrals like water and ammonia [17]. There are no reports of water-loss products formed from noncovalent peptide complexes. The elimination process of water from the complex is shown in Scheme 1. The mechanism to form a peptide bond between the peptide monomers involves homolytic radical cleavage via a Norrish Type I photoreaction as the initial step [8,9]. The Norrish Type I cleavage occurs between the carbonyl carbon and the hydroxyl oxygen on the C-terminus of one peptide ion. Subsequent hydrogen abstraction from the N-terminus of the other peptide ion leads to water elimination. This is followed by radical recombination to form a peptide bond, thus resulting in linear coupling of two amino acid chains. We note that the sequential steps in the mechanism shown in Scheme 1 may actually be concerted. It is also possible that a covalent bond can be formed between the other N- and C-termini of the peptides. However, the coupling of the other N- and C-termini results in the same peptide sequence in this case.

Collisional activation of the water-loss product generates a series of b- and y-type ions that show complete sequence coverage of the newly synthesized GPGGGPGG octapeptide (Fig. 1e). The peaks above $m/z = 287$ (m/z value of $[GPGG+H]^+$) are evidence for the formation of the octapeptide ion ($[GPGGGPGG+H]^+$) by the coupling of two GPGG tetrapeptides. The CID analysis of the water-loss product confirms the formation of a covalent bond between two peptides upon VUV irradiation. In separate experiments the peptide GPGGGPGG has been synthesized using solid-phase synthesis techniques. Collisional activation of electrosprayed ions from the latter peptide yields a fragmentation pattern (see Supplementary Fig. 1) that is nearly indistinguishable from the octapeptide generated upon photoactivation of the complex.

3.2. Inter-molecular cross-linking between N- and C-terminal modified peptides

As illustrated with $[2GPGG+H]^+$ complex ions, the formation of a peptide bond occurs between N- and C-termini that are terminated by a primary amine and carboxylic acid, respectively. It is anticipated that the UV-induced amide bond formation is also possible between one amino acid side chain containing an amine group and another amino acid side chain containing a carboxyl group. This should result in a covalently cross-linked peptide or protein similar to those formed by enzymes such as transglutaminases [18]. The tetrapeptide VKMD has been chosen to examine the possibility for cross-linking between the Lys and Asp side chains. Additionally, blocking the N- and C-termini with acetyl (ac) and amido-4-trifluoromethylcoumarin (cu) groups, respectively, prevents the formation of a covalent bond between N- and C-termini, or side chains and termini. This circumvents the need to interpret extremely complex spectra. The C-terminal modification also introduces a chromophore that may increase the efficiency of UV-induced bond formation; current work is focused on determining the effect of this chromophore in the bond formation process as well as those of additional modifications.

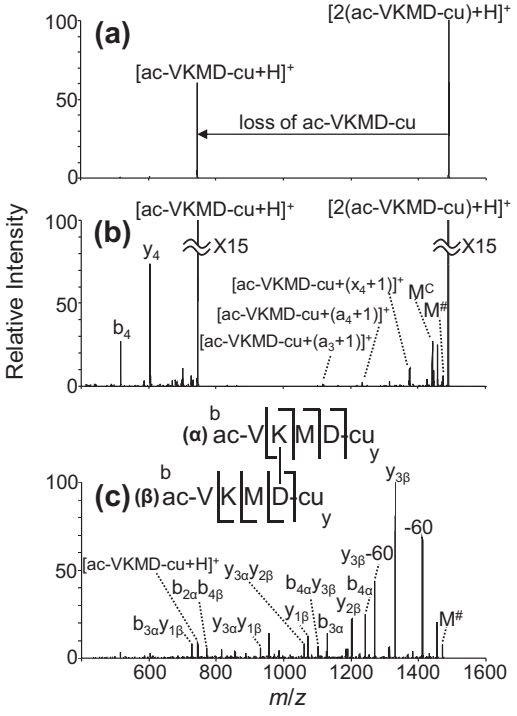
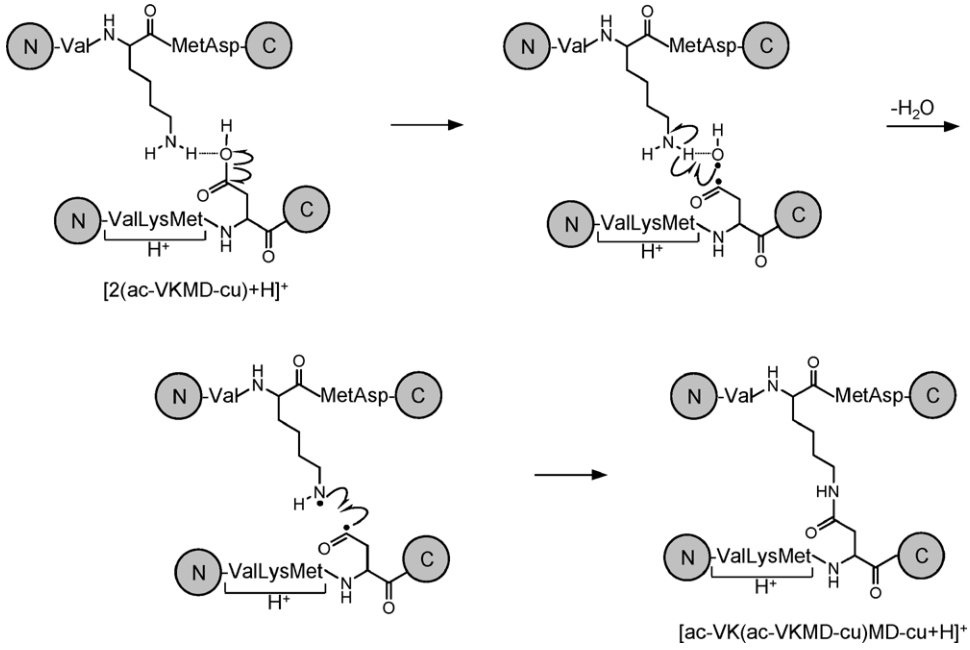


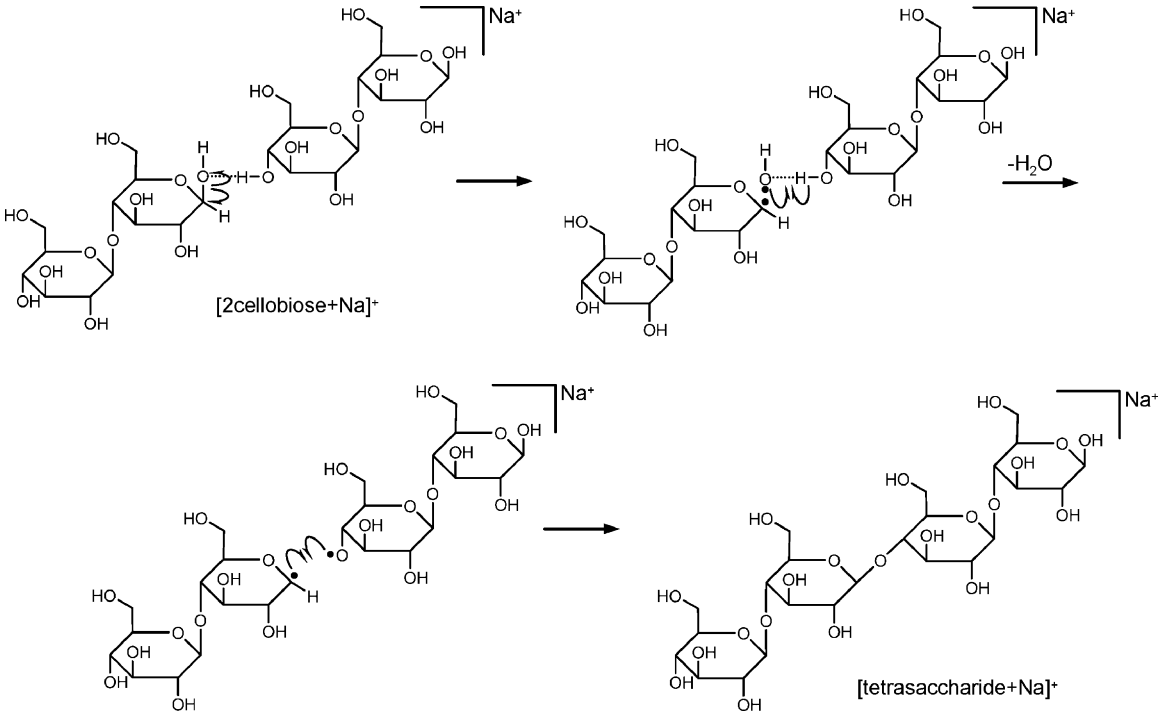
Fig. 2. Fragmentation spectra of $[2(ac\text{-}VKMD\text{-}cu)+H]^+$ obtained by (a) CID and (b) 157 nm irradiation. The superscripted C and # indicate the losses of CHO_2 and H_2O, respectively. The b-/a- and y-/x-type ions include N- and C-terminal blocking groups, respectively. (c) CID spectrum of the water-loss product ($M^{\#}$) in spectrum b. Peaks in spectrum c are assigned according to the inter-crosslinked peptide sequence.



Scheme 2. Proposed mechanism for formation of a cross-linked peptide ion between Lys and Asp amino acid residues of separate peptide ions obtained from the [2(ac-VKMD-cu) + H]$^+$ complex upon 157 nm irradiation. The circled N and C at the end of each peptide indicate N- and C-termini blocking groups, respectively.

Electrospraying the modified peptide leads to formation of the proton-bound [2(ac-VKMD-cu) + H]$^+$ dimer ion at m/z = 1489. Similar to the [2GPGG + H]$^+$ complex, collisional activation of the [2(ac-VKMD-cu) + H]$^+$ complex results in loss of the ac-VKMD-cu neutral, producing the [ac-VKMD-cu + H]$^+$ peak at m/z = 745 (Fig. 2a). The fragmentation spectrum confirms that the peak at m/z = 1489 corresponds to the noncovalent complex. Upon 157 nm irradiation, a small peak corresponding to the water-loss product ($M^{\#}$ in Fig. 2b) at m/z = 1471 is observed along with several noncovalent fragment complexes including [ac-VKMD-cu + (a_3 + 1)]$^+$, [ac-VKMD-cu + (a_4 + 1)]$^+$, [ac-VKMD-cu + (x_4 + 1)]$^+$, and [2(ac-VKMD-cu) − CHO_2 + H]$^+$ (M^c in Fig. 2b) at m/z = 1119, 1234, 1375, and 1444, respectively (Fig. 2b). Collisional activation of these fragment complexes results in their dissociation into peptide monomer and fragment ions (data not shown here), similar to those formed upon activation of the [2GPGG + H]$^+$ fragment complexes (Fig. 1c and d).

The assignment of CID fragments of the $M^{\#}$ ion verifies that two ac-VKMD-cu peptide monomers are cross-linked by connecting the side chains of two separate amino acid residues. As shown in Fig. 2c, the fragmentation spectrum of the $M^{\#}$ ion presents a complete set of b- and y-type ions involving both peptide chains



Scheme 3. Proposed mechanism for formation of a tetrasaccharide from the [2cellobiose + Na]$^+$ complex upon 157 nm irradiation.

of the cross-linked peptide ions. The collisional activation of the $M^{\#}$ ion produces primarily single-chain cleavage products as well as less abundant ions resulting from two peptide chain cleavages, which are typical fragments of cross-linked peptides. The nomenclature proposed by Young and co-workers is used to assign all CID fragments obtained from the UV-induced cross-linked peptide ion [19]. The fragment ions corresponding to the two peptides are designated with either α or β subscripts to indicate the peptide of origin. As expected, photoexcitation of the dimer complex leads to formation of a covalent, inter-molecular bond between a Lys residue of one peptide and an Asp residue of another. A possible mechanism for the cross-linking reaction is proposed in Scheme 2. The Norrish Type I cleavage leads to water elimination and formation of an amide bond between the amine from one peptide and carboxyl group of the other.

Finally, we note that the photoactivation step may yield an intramolecular isopeptide linkage. For example, upon photoexcitation of the ion complex, a low intensity species at $m/z = 727$ is observed. This ion could result from intramolecular bond formation between the Lys and Asp amino acid residues of the same peptide. Additionally, a water-loss peak associated with this fragment ion is also observed at $m/z = 709$.

3.3. Glycosidic bond formation between disaccharides

It is of interest to consider whether or not covalent bond formation between other biomolecules such as oligosaccharides can be produced by UV light. In contrast to the previous two examples, the oligosaccharides studied here do not have the carboxyl functional group where the Norrish Type I cleavage initiates. However, homolytic cleavage on a hydroxyl functional group of one oligosaccharide upon photoexcitation may give rise to water elimination by hydrogen abstraction from a hydroxyl group of the other oligosaccharide. Therefore, 157 nm irradiation of an oligosaccharide dimer has the potential to make linearly coupled saccharide polymers by formation of a covalent bond between the monomer units.

Singly sodiated $[2\text{cellobiose} + Na]^+$ dimer ions at $m/z = 707$ are observed by electrospraying cellobiose disaccharides. The long-lived complex ions have been subjected to collisional activation and mass spectrometric analysis to demonstrate the noncovalent nature of the complex (Fig. 3a). The resulting fragmentation spectrum displays the $[\text{cellobiose} + Na]^+$ monomer peak obtained from dissociation of the weak noncovalent bond. On the other hand, photoexcitation of the disaccharide complex generates several fragment complexes. The Domon-Costello nomenclature is used to assign the fragment ions of oligosaccharides [20] as shown in Fig. 3b; the peaks at $m/z = 528$, 545, 573, and 601 can be assigned to the $[\text{cellobiose} + B_1 + Na]^+$, $[\text{cellobiose} + C_1 + Na]^+$, $[\text{cellobiose} + {}^{1,5}X_1 + Na]^+$, and $[\text{cellobiose} + {}^{3,5}A_2 + Na]^+$ ions, respectively. Collisional activation experiments of the fragment complexes have confirmed that cellobiose and each respective fragment are noncovalently bound to each other (data not shown here).

In addition to the fragment complexes, a peak corresponding to the water-loss product ($M^{\#}$) at $m/z = 689$ is detected (Fig. 3b). Collisional activation of the $M^{\#}$ ion shows several diagnostic fragment ions that are comparable to those obtained from CID of singly sodiated cellotetraose $[\text{cellotetraose} + Na]^+$ ions (Fig. 3c and d). The results suggest that covalent bond formation occurs between cellobiose monomers upon 157 nm irradiation. A possible mechanism for coupling two oligosaccharides is illustrated in Scheme 3. As mentioned above, the first step involves the photolytic radical cleavage of the bond between the ring carbon and the hydroxyl oxygen. Then, the following step involves water elimination which is similar to that which occurs during the UV-induced amide bond formation with peptides. The final step is radical recombination to form the glycosidic bond between the cellobiose monomers.

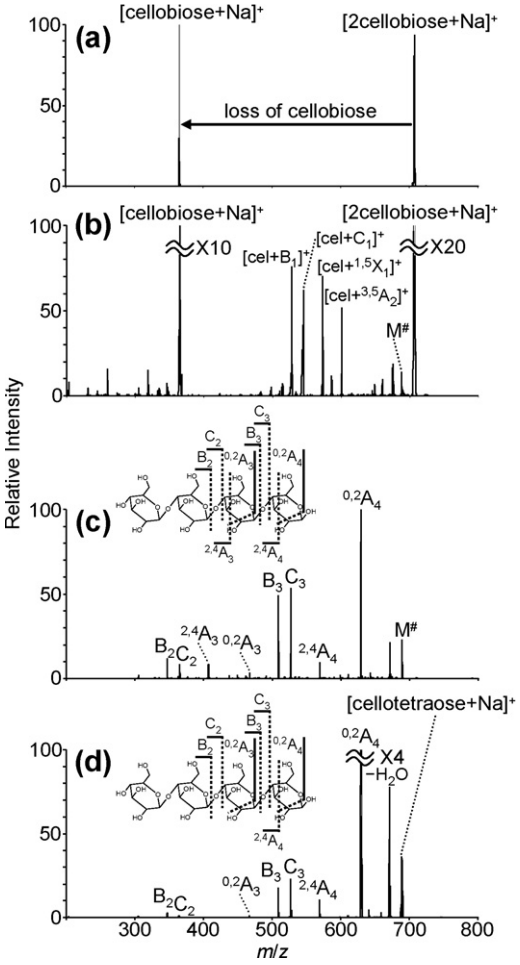


Fig. 3. Fragmentation spectra of $[2\text{cellobisoe} + Na]^+$ obtained by (a) CID and (b) 157 nm irradiation. The superscripted # indicates the loss of water. The $[\text{cel} + \text{fragment}]^+$ assignments correspond to singly sodiated noncovalent complexes between cellobiose and fragment ions. CID spectra of (c) the water-loss product ($M^{\#}$) in spectrum b and (d) $[\text{cellotetraose} + Na]^+$. The peaks in spectrum c are assigned according to the tetrasacchaide sequence, cellotetraose. All of the fragments are singly sodiated.

On the basis of the proposed mechanism, any hydroxyl group of cellobiose is available for the water elimination process. Thus, it is possible to form any linkage type between two cellobiose units. A close inspection of the fragmentation spectrum obtained upon collisional activation of the newly synthesized tetrasaccharide with that of cellotetraose reveals that there is an additional fragment at $m/z = 407$. This value corresponds to the ${}^{2,4}A_3$ ion (based on the molecular configuration of cellotetraose). We note that it is not present in the fragmentation spectrum of cellotetraose. Additionally, the relative intensities of ${}^{0,2}A_4$ fragment ions from the gas-phase synthesized tetrasaccharide ion are much lower than those obtained for cellotetraose under the same CID conditions. This suggests that glycosidic bonds between two cellobiose components are formed non-specifically upon photoexcitation. It is noted that modification of all hydroxyl groups such as permethylation with the exception of one should allow construction of longer saccharide polymers with specific glycosidic bonds.

4. Conclusions

Covalent bond formation between peptides or oligosaccharides can be achieved in the gas phase upon 157 nm irradiation of long-lived dimer complexes. The mechanism of UV-induced covalent bond formation involves a photolytic radical cleavage process as

its initial step. A subsequent water elimination step leads to formation of linearly coupled or cross-linked product ions. Collisional activation of newly synthesized product ions shows nearly full sequence coverage verifying covalent bond formation between two monomer units. This new approach to form a covalent bond upon 157 nm irradiation extends possible ways to generate modified biomolecular ions in the gas phase.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2012.02.015.

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