Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 8, pp. 979-982, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00152-7

DIRECT CHARACTERIZATION OF SOLID PHASE RESIN-BOUND MOLECULES BY MASS SPECTROMETRY

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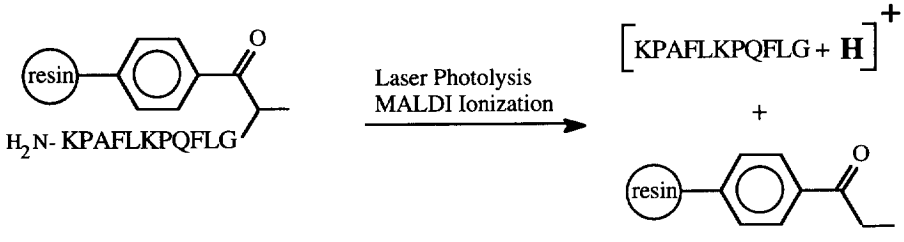
Abstract: In this work we demonstrate that analytes, covalently linked to a polymeric support through a photolabile linker, can be directly analyzed by matrix-assisted laser desorption/ionization (MALDI). Mass spectral analysis is performed in a single step requiring no pretreatment of the sample to induce cleavage from the support. Our results show that the UV laser light in the MALDI experiment can be used to simultaneously promote an analyte's photolytic cleavage from a solid support and its gas phase ionization for subsequent mass spectral analysis. In this manner, MALDI facilitates the dissociation and identification of resin-bound analytes in a single analytical procedure without the need for any prior chemical treatment. Copyright © 1996 Elsevier Science Ltd

Introduction

Mass spectrometry is playing an increasingly important role in the molecular characterization of combinatorial libraries, natural products, and biopolymers[1]. The recent development of soft ionization techniques such as matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) have significantly extended the application of mass spectrometry to a wide variety of compounds previously inaccessible because of their low volatility and thermal lability. Several reports[2-6] have also shown that mass spectrometry can be very useful in characterizing compounds from solid polymeric supports. These reports illustrated that compounds covalently bound to a single polymeric bead (~50 μm in diameter) or set of beads were amenable to mass spectral analysis subsequent to their chemical cleavage from the resin. Preliminary results[7] in another report suggested that MALDI could be used to analyze selected Fmoc-protected amino acids that were bound to a solid-phase resin through a photolabile link. In this report we demonstrate that peptides covalently linked to a polymeric support through a photolabile linker can be directly analyzed by MALDI mass spectrometry. We also show that the technique is suitable for following chemical reactions on the solid phase.



Scheme 1. Schematic representation of the products generated upon laser photolysis and MALDI ionization of a peptide covalently attached to a solid phase resin through a photolabile α-methylphenacyl-ester linker.

The approach outlined in Scheme 1 permits the characterization of resin-bound analytes in a single step that requires no pretreatment of the sample to induce cleavage from the support. The MALDI technique facilitates the vaporization and ionization of intact analytes by isolating analyte molecules in an appropriate matrix (usually a small organic compound) and irradiating the sample/matrix mixture with a UV laser. Our primary goal in this work was to determine if the MALDI experiment could be used to simultaneously promote an analyte's photolytic cleavage from a solid support and its gas phase ionization for subsequent mass spectral analysis.

Materials and Methods

The peptide of sequence KPAFLKPQFLG was manually synthesized from protected amino acids in a stepwise fashion using *in situ* neutralization/HBTU activation protocols for Boc chemistry[8]. The synthesis was initiated on Br-Wang resin (obtained from Novabiochem, San Diego, CA) that consists of a brominated α-methylphenacyl linker attached to a polystryrene-1% divinyl benzene support[9,10]. The first amino acid was activated as the Cs salt before it was coupled to the resin. During synthesis, the side chain amino groups of the two lysine residues were protected using a base labile Fmoc group. Prior to mass spectral analysis, resin samples were treated with 20% (v/v) piperdine in DMF in order to remove the Fmoc protecting groups, and then washed with 50/50 (v/v) dichloromethane/methanol.

For MALDI analysis, ~1 mg of the deprotected peptide resin was added to 5 mL of ethanol. A 2 μL ethanol/bead suspension containing ~50 beads was then deposited on the MALDI sample plate, prior to the addition of 2 μL of a saturated ethanol solution of the matrix, α-cyano-4-hydroxycinnamic acid. Samples were dried at room temperature and then analyzed on a PerSeptive Voyager Elite MALDI mass spectrometer or a prototype Ciphergen Biosystems mass spectrometer. Both instruments were equipped with a nitrogen laser which radiates at 337 nm.

It is important to note that our sample preparation involved the addition of 5 ml of ethanol to the resin-bound peptide. When the peptide resin was resuspended in smaller volumes (0.01 - 1.0 mL) of solvent, we detected free peptide in the MALDI analysis of the supernatent. Presumably, trace amounts of peptide (< 1 nmol or < 1% of the resin bound material) were present as a result of photolytic cleavage by room light. In this work we wanted to establish whether or not MALDI affects both the cleavage and ionization of resin-bound peptides. Therefore, it was important to suspend the peptide resin in a large solvent volume (~5 mL) in order to sufficiently dilute the residual free peptide in the sample to undetectable levels.

Results and Discussion

The results in Figure 1 show that MALDI analysis of the unprotected, resin-bound peptide yielded a characteristic $[M+H]^+$ signal at *m/z* 1247 which is consistent with the expected mass of the free peptide (MW = 1245.5 Da). The mass spectrum shown in Figure 1A was acquired from over 30 laser pulses on a single bead and the mass spectrum in Figure 1B was acquired from a single bead with one laser pulse (3 nanoseconds in duration). The results in Figure 1B indicate that photolytic cleavage and ionization of material from the bead occurred during the laser pulse, as this resin sample was not exposed to laser light prior to analysis. Using the

visualization system on the Voyager Elite instrument, we were also able to irradiate very specific regions of the sample. Peptide signal was only observed when individual matrix/bead deposits were irradiated on the sample plate. No peptide signal was observed upon irradiation of matrix crystals not associated with resin beads, suggesting that there was little or no free peptide in the sample prior to MALDI analysis. These same observations were made even after repeated MALDI sample preparations on the same set of beads, indicating that there was no appreciable cleavage of the peptide from the resin during our MALDI sample preparation. Also as expected, in the absence of matrix analysis of the resin-bound peptide no signal was produced.

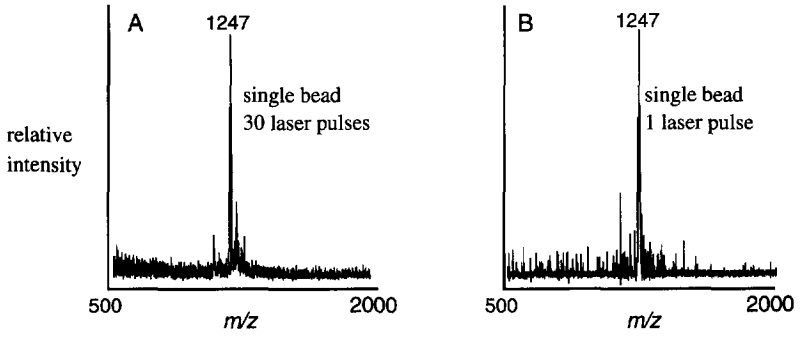


Figure 1. The direct MALDI analysis of the unprotected peptide KPAFLKPQFLG (MW 1245.5 Da) covalently attached to a polystyrene resin through a photolabile α-methylphenacyl-ester linker. The mass spectrum in (A) was summed over 30 laser pulses acquired from a single bead and the spectrum in (B) was acquired from a single laser pulse on a single bead. Both spectra were recorded in the positive-ion mode using α-cyano-4-hydroxycinnamic acid as the matrix. A strong signal for the singly protonated peptide was detected in each case.

Treatment of the resin (before and after MALDI analysis) with 20% (v/v) hydrazine in methanol gave the corresponding peptide hydrazide (MH^+ = 1261), as confirmed by MALDI and ESI mass spectrometry (data not shown). Our ability to recover the peptide in a chemical cleavage step from resin samples subjected to MALDI analysis indicates that the sample was not completely photolyzed in the MALDI experiment. This is not surprising as only a small amount of material (< femtomoles) is typically consumed during MALDI analysis[11].

The rapid, one-step MALDI procedure for the direct analysis of resin-bound molecules described above is ideally suited for studying chemical reactions on the solid phase. As a demonstration of the technique's utility, we followed the time course of the coupling reaction of a Boc-Arg(Tos) residue (preactivated as the hydroxybenzotriazole ester)[8] to the protected 11 amino acid peptide described above. Interestingly, the Fmoc protected peptide was not amenable to MALDI analysis. The absence of appropriate protonation sites in this sample likely prevents ionization during the MALDI process. Therefore, prior to analysis, resin samples were treated with 20% (v/v) piperdine in DMF in order to remove the Fmoc protecting groups. Resin samples taken at 0, 1, and 6 minute time points were subjected to MALDI analysis, the results of which are shown in Figure 2. The data was generated using the Prototype Ciphergen Biosystems mass spectrometer with 3,5-Dimethoxy-4-hydroxycinnamic acid as the matrix. Our results show that the reaction proceeded quickly and coupling appeared nearly complete in just six minutes.

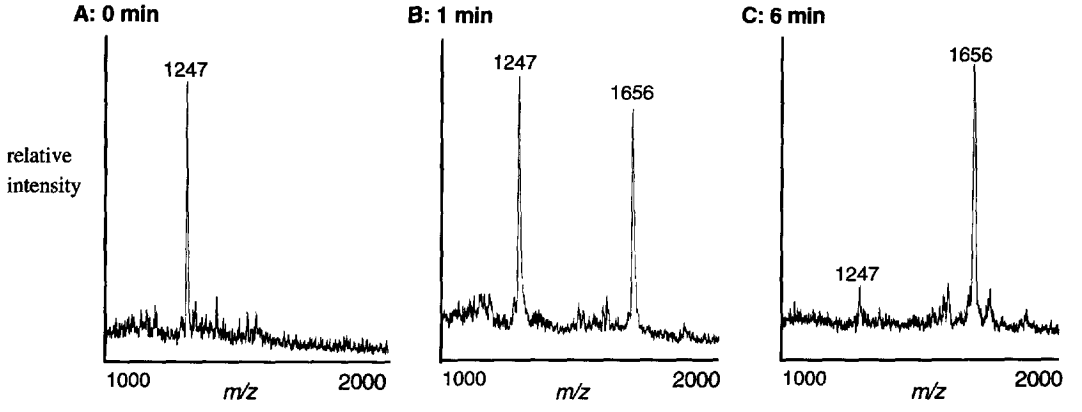


Figure 2. MALDI mass spectra showing the time course of reaction for the coupling of Boc-Arg(Tos) (MW = 428.5 Da) to the protected resin-bound peptide of sequence KPAFLKPQFLG. MALDI analysis was performed directly on peptide-resin samples after deprotection of the lysine side chain amino groups. Resin samples were taken after the reaction was allowed to proceed for (A) 0 , (B) 1, and (C) 6 minutes.

The direct analysis of resin-bound molecules by MALDI has several important advantages over other procedures which require an additional cleavage step prior to mass spectral characterization. Most importantly, the procedure can be used to monitor chemical reactions on the solid phase in real time, in much the same way that thin-layer chromatography is used to monitor reactions in solution. The capacity to perform MALDI directly on the solid phase also provides for more efficient sample management since the resin-bound sample can be easily recovered for subsequent manipulations following MALDI analysis. Analytes that are amenable to MALDI ionization should prove suitable for routine analysis by this procedure. The use of ionization tags may also facilitate the analysis of other compounds, such as protected peptides, that are not easily ionized.

Acknowledgments. G.S. and M.C.F. acknowledge many helpful discussions with Dr. William McBride and Professor Stephen B.H. Kent. M.C.F. was the recipient of an Amgen postdoctoral fellowship.

References

1. Siuzdak, G. *Proc. Natl. Acad. Sci. U.S.A* **1994**, 91, 11290.
2. Brummel, C. L.; Lee, I. N.; Zhou, Y.; Benkovic, S. J.; Winograd N. *Science*, **1994**, 264, 399.
3. Egner, B. J.; Langley, G. J., Bradley, M. *J. Org. Chem.* **1995**, 60, 2652.
4. Egner, B. J.; Cardno, M., Bradley, M. *J. Chem. Soc.-Chem. Comm.*, **1995**, 21, 2163.
5. Haskins, N. J.; Hunter, D. J.; Organ, A. J.; Rahman, S.; Thom, C. *Rapid Comm. Mass Spectrom.* **1995**, 9, 1437.
6. Brummel, C. L.; Vickerman, J. C.; Carr, S. A.; Hemling, M. E; Roberts, G. D.; Johnson, W.; Weinstock, J.; Gaitonopoulos, D.; Benkovic, S. J.; and Winograd, N. *Anal. Chem.* **1996**, 68, 237.
7. Wagner, D. S.; Brown, B. B.; Geysen, H. M. *Abstract of Posters,* 43rd American Society of Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, 1995, abstract 488.
8. Schnölzer, M.; Alewood, P.; Jones A.; Alewood, D.; Kent, S. B. *Int. J. Pep. Prot. Res.* **1992**, 40,180.
9. Wang, S. S. *J. Org. Chem.* **1976**, 41, 3258.
10. Sheehan, J. C.; Umezawa, K. *J. Org. Chem.* **1973**, 38, 3771.
11. Cerpapoljak, A.; Jenkins, A.; Duncan, M. W. *Rapid Comm. Mass Spectrom.* **1995**, 9, 233.

(*Received in USA* 15 *March* 1996; *accepted* 18 *March* 1996)