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Iodine-assisted matrix-assisted laser desorption/ionisation

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Dedicated to Dr. Yannik Hoppilliard on the occasion of her 60th birthday.

Abstract

Addition of iodine to the matrix has been found to lead to lower thresholds for ion formation in matrix-assisted laser desorption/ionisation (MALDI) of peptides and other compounds. Intensities of ions from the matrix have been suppressed through the addition of iodine to the matrix; intensities of ions corresponding to addition of sodium or potassium to the analyte have been diminished. These effects have been observed with a number of matrices, including 2,5-dihydroxybenzoic acid, dithranol and α-cyano-4-hydroxy-cinnamic acid. (Int J Mass Spectrom 219 (2002) 697–701)

Keywords: Proteomics; MALDI; Matrix

1. Introduction

Matrix-assisted laser desorption/ionisation (MALDI) using organic and other matrices [1,2] has been used to form gaseous ions from non-volatile, thermally labile compounds [3,4,5,6,7,8]. There is a need to increase the reliability of interpretation, reproducibility of measurement and sensitivity of MALDI [9,10,11,12]. The quality of spectra obtained depends in a complex and sensitive fashion on aspects of sample preparation [13].

MALDI has assumed particular importance for the characterisation of mixtures of peptides in the context of proteomics [14].

Matrix suppression [15,16] in MALDI is potentially valuable due to removal of interference peaks from spectra and avoidance of detector saturation and related effects. Matrix suppression as reported by Chan et al. [4] and Knochenmuss et al. [15] has been observed with analytes of moderate size (<20 kDa) mixed with commonly used matrices at relatively high concentrations (molar ratios of 1:100). Formation of Na^+ and K^+ adducts in MALDI is generally not advantageous with peptides, but invaluable with other compounds notably polymers [6,7]. Billeci and Stults [17] have reported that the use of carbohydrates leads to a reduction in Na^+ adduction to peptides. Asara and Allison [18] have found that ammonium salts added to matrices reduces intensities of the Na^+-adduction peaks with phosphopeptides, while enhancing detection of the protonated species. Similar effects of ammonium salts have been reported with oligonucleotides [19,20].

2. Method

Solvents were analytical grade (Sigma-Aldrich, Dorset, UK). 2,5-Dihydroxybenzoic (DHB) acid and

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α-cyano-4-hydroxy-cinnamic acid were purchased from Sigma-Aldrich. Bradykinin fragments 2–9 MW 904 (B1901), substance P MW 1347.6 (S6883), luteinising hormone releasing hormone MW 1182.3 (L7134), renin substrate tetradecapeptide MW 1759.0 (R8129), $[Arg]^8$-vasopressin MW 1084.2 (V9879) and somatostatin MW 1637.9 (S9129) were purchased from Sigma-Aldrich.

Peptides were dissolved in a 1:1 water/acetonitrile solution at an initial concentration of 1 mg/mL before dilution to a concentration of 7 μM. The standard peptide mixture (total peptide concentration 7 μM, was made by mixing equal volumes of six peptides. The following procedure was typical when iodine was added. α-Cyano-4-hydroxy-cinnamic acid 0.1 M in dimethyl formamide (DMF) with 0.1% trifluoroacetic acid (TFA) and 10% I_2 (0.1 M in DMF) was a standard matrix. An aliquot 0.5 μL of the matrix solution was pipetted onto the slide and dried for 5 min in a stream of air. A total of 0.3–0.4 μL of analyte were pipetted onto the matrix layer, and allowed to dry before measurement.

For the experiments on iodine concentration, substance P was dissolved in 1:1 methanol/water. Matrix 2,5-DHB (0.1 M) was dissolved in acetone, 1:1 methanol/water or DMF. The volumes applied during the substance P experiments were always 0.5 μL. Iodine was mixed with the matrix and dried. Once the iodine/matrix mixture was dry, substance P was applied and dried.

Kratos Kompact III time-of-flight mass spectrometer was used in linear positive-ion mode at 25 kV accelerating potential with a 3 ns nitrogen laser.

3. Results and discussion

The effects of addition of iodine were investigated with synthetic polymers, oligosacharides and peptides. Results on peptides are reported. Experiments were performed at a number of laser power settings in the presence of iodine and in the absence of iodine. When the laser power setting on the instrument was at 0, maximum attenuation was obtained; laser power setting 180 gave minimum attenuation. The relationship between instrument laser settings and transmission for the Kompact III was described using the following formula [21]

$$\%T = \frac{100}{10^{[2.0-(1.9\,L/180)]}} \quad (1)$$

L is the laser power setting and $\%T$ is the percentage of the laser fluence transmitted. Fig. 1a shows a MALDI spectrum of substance P using DHB as matrix with 10% iodine present. This spectrum was obtained at laser energy close to the threshold for analyte ion production. Fig. 1b shows the spectrum of substance P without iodine at the same laser power. Both spectra are the average of 100 laser shots. Fig. 1b displays a significantly lower ion intensity, which reflects a higher threshold for ion production when iodine was absent. The peak intensities in the absence of iodine increased significantly on raising the laser power.

MALDI spectra of the mixture of six peptides were measured with laser power settings L of 91, 93, 99 and 103. Fig. 2 shows the spectra at $L = 91$, which was the lowest laser setting at which all the peptides were observed in the absence of iodine. Strong peptides signals were observed with iodine well below $L = 91$. Below and at $L = 93$, all peptides exhibited higher intensities in the presence of iodine.

There are two points to be drawn from Fig. 2. One is that in the presence of iodine there were no matrix peaks in the spectrum (Fig. 2a). The second is that in the presence of iodine the intensities of sodiated and potassiated species were diminished relative to the protonated species, compared to the relative intensities in the absence of iodine.

Different ratios of iodine to matrix were investigated ranging from 0 to 90% iodine. The highest intensities for substance P ($[M + H]^+$) were observed at 10% iodine. The effects of iodine addition were observed with different solvent systems.

The decision to investigate iodine as a matrix additive was guided by two initial assumptions. The first was that the additive should sublime. Iodine does sublime, but did not evaporate completely in vacuum. The effects of iodine were still present after an hour

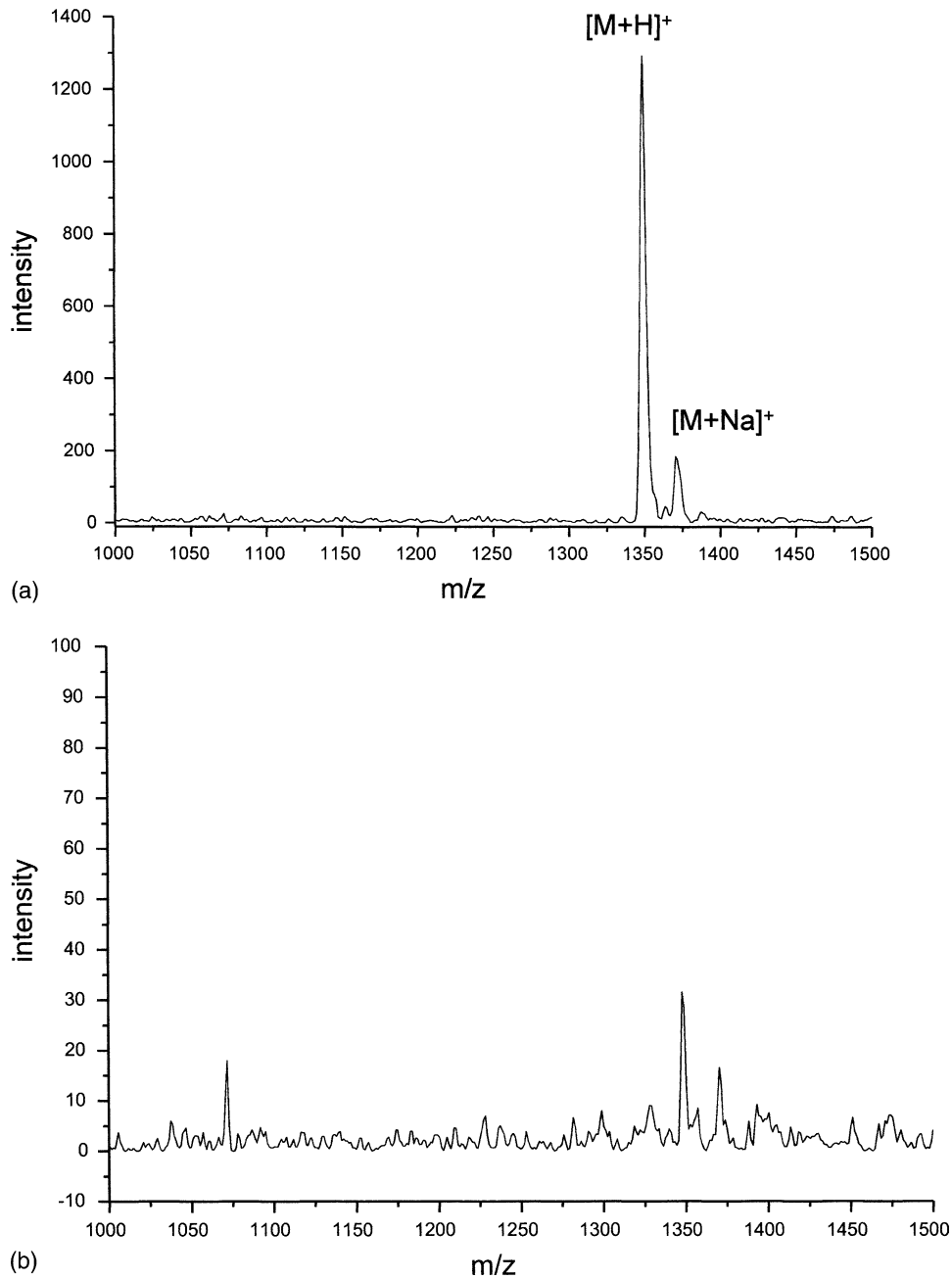

Fig. 1. Positive MALDI spectra of substance P using 2,5-dihydroxybenzoic acid as a matrix. (a) With iodine, (b) without iodine. Laser power the same in each case.

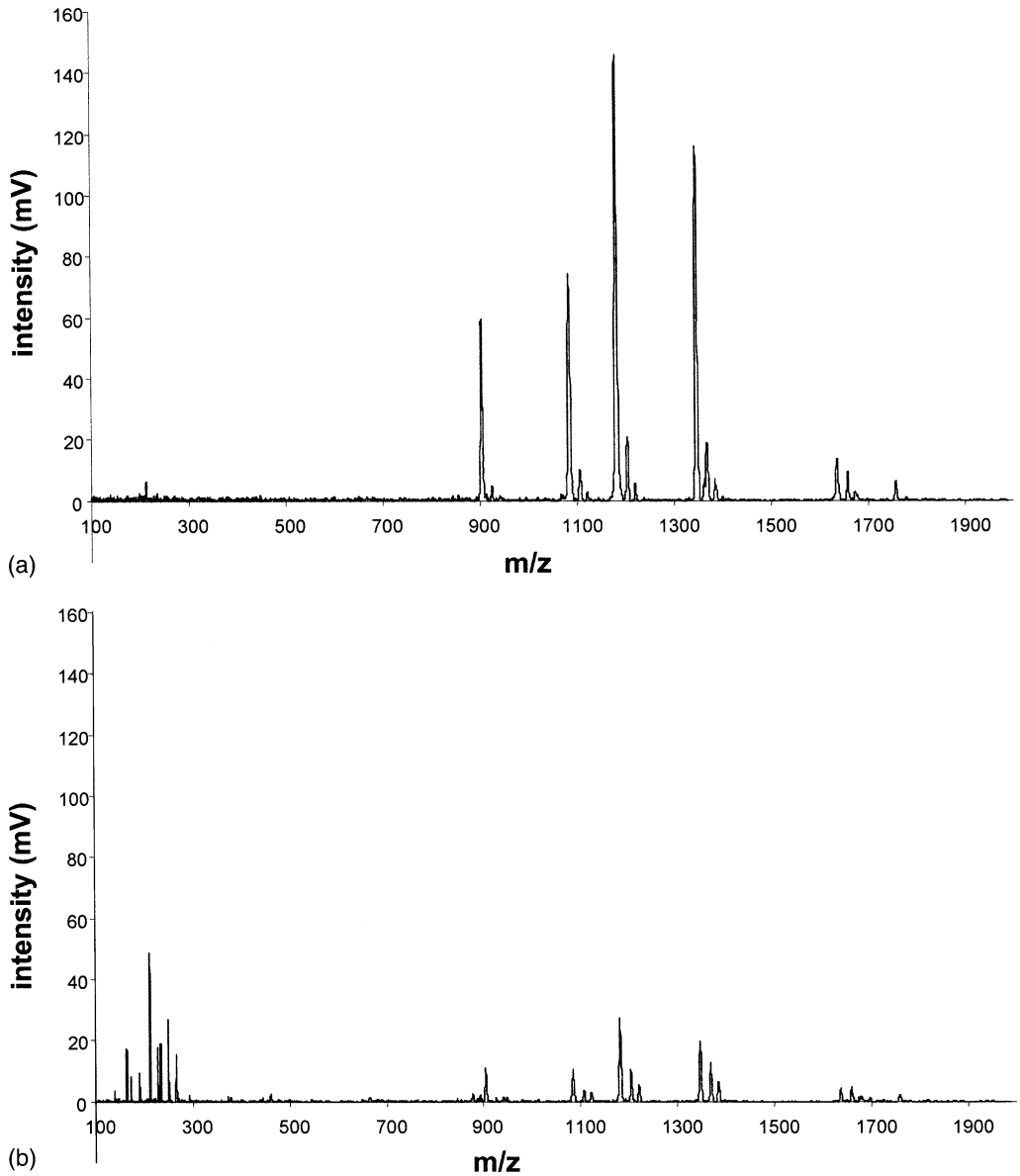

Fig. 2. Positive MALDI spectra of equimolar solutions of bradykinin fragments 2–9, [Arg]^8-vasopressin, luteinising hormone releasing hormone, rennin substrate tetradecapeptide, substance P and somatostatin. α-Cyano-4-hydroxycinnamic acid and 0.1% trifluoroacetic acid (TFA) as a matrix. Molar ratio analyte:matrix 10^{-4}:1 (a) with iodine, (b) without iodine. Laser power the same in each case ($L = 91$).

in vacuum. The second was that the additive should introduce radical species. Iodine did dissociate under the conditions of MALDI. A prominent radical ion peak I^- was observed in the negative-ion mode.

The practical reason why, when measuring the mixtures of peptides in protein digests, addition of iodine could be advantageous would stem from the fact that lower laser powers could be used. On the basis

of admittedly preliminary experiments, the major peaks in the MALDI spectrum of a protein digest would be more likely to represent protonated peptides at the lower laser powers with iodine present, making the comparison with the situation in the absence of iodine.

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