Short communications

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New type of matrix for matrix-assisted laser desorption mass spectrometry of polysaccharides and proteins

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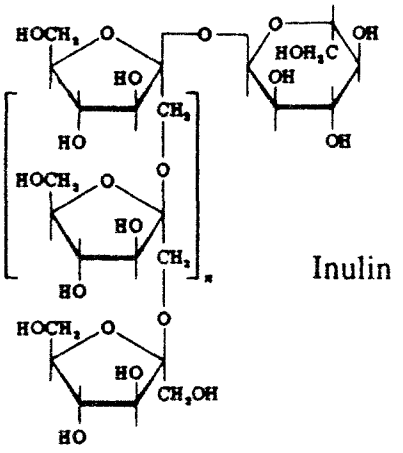
Abstract. 3-Aminoquinoline is introduced as matrix for matrix-assisted laser desorption mass spectrometry of polysaccharides and proteins. The first MALD mass spectrum of the polysaccharide inulin is reported. 3-Aminoquinoline seems to be more effective than the commonly used acidic matrix 2,5-dihydroxybenzoic acid, assisting in the desorption and ionisation of inulin. A higher sensitivity, sharper peaks and a lower background were observed. 3-Aminoquinoline is also quite effective when measuring the protein myoglobin.

Introduction

Matrix-assisted laser desorption mass spectrometry (MALD-MS) [1] is already a valuable tool for the analysis of peptides and proteins [2]. First results with other biopolymers such as lignins [3], oligo- and polysaccharides [4] and oligonucleotides [5, 6] have been reported. The effect of the matrix compound [2] absorbing the energy from the laser beam was assigned to a reduction of internal excitations and of the initial kinetic energy of the analyte molecules and to a support of ionisation in the course of the desorption process. In addition, the matrix compound has to be able to separate single analyte molecules from each other, thereby limiting aggregation which would otherwise prevent molecular ion formation (matrix isolation) [2].

For example, in the case of polar biopolymers such as polysaccharides and proteins, respectively, the matrix has to break up the intermolecular hydrogen bonds between the polymer chains. That can best be effected if the matrix is able to form hydrogen bonds to the analyte. Thus, aromatic acids such as 2,5-dihydroxybenzoic acid (DHB) were commonly used as matrices assisting efficiently in the desorption and ionisation of biomolecules [7].

Recently, we tested systematically a variety of low molecular weight and volatile compounds as matrices in ^{252}Cf-plasma desorption mass spectrometry (PDMS) measurements of oligosaccharides. Some heteroaromatic amines, i.e. 3-aminoquinoline (3-AQ), and phthaleins, i.e. fluorescein, proved to be especially suited to enhance the quasimolecular ion intensity of oligosaccharides [8]. Furthermore, we have shown that matrices which enhance the quasimolecular ion intensity of oligosaccharides in PDMS are also suitable MALD matrices when they absorb at the wavelength of the irradiating laser beam [9]. In this contribution, the new matrix 3-aminoquinoline (3-AQ) is introduced and applied to the polysaccharide inulin and to the protein myoglobin. The first MALD mass spectrum of inulin is presented.



Inulins are linear D-fructose polymers with 1,2-glycosidic linkages (fructane) and with a degree of polymerisation (DP) of up to 35–40, which varies according to the plant species and the life cycle. Recently, a mass spectrum of dahlia inulin was reported using direct chemical ionisation mass spectrometry [10].

Experimental

MALD-MS. The mass spectrometer used for the analysis of inulin was a reflectron-type time-of-flight (TOF) instrument (Finnigan MAT Vision 2000) with a 337 nm nitrogen laser.

The spectrum of myoglobin was obtained with a linear time-of-flight mass spectrometer originally built for PDMS and modified for applying laser induced desorption. The stop-MCPs were replaced by a detector unit which is similar to those applied in other laboratories. It is a venetian blind secondary electron multiplier (EMI 9642 2B) with an additional dynode supplied with a separate negative potential (−3 kV to −20 kV). A cylindrical tube on ground potential is placed in front of the dynode to shield the drift region from electric fields. The SEM anode is directly coupled to a fast amplifier (input impedance 50 Ω, rise time less than 500 ps) for current to voltage conversion. The focused and attenuated light pulses (15 ns FWHM) of a Q-switched frequency tripled (355 nm) Nd:YAG laser (Hyperyag from JK) hit the sample in the spectrometer at an angle of 7°. Single shot spectra are recorded by a 125 megasample transient recorder (LeCroy 9400, DSO) controlled by an IBM PC/AT computer. The DSO trigger is generated by the signal of a fast photodiode excited by a laser beam reflex. The photodiode is connected to a constant fraction discriminator for time jitter reduction and for amplitude discrimination. The spectrum of myoglobin was accumulated from 30 single spectra. A local variation of the laser focus on the sample turned out to be unnecessary due to the sample preparation applied. But a selection of single shots must be made mainly due to fluctuations of the laser pulse amplitude.

Sample preparation. The matrices 3-AQ and DHB were dissolved at a concentration of 10 g/L in 10% (v/v) methanol-water solution. Analytes (1 g/L) were prepared in doubly distilled water and then mixed with the matrix solution (1 : 1). For prepar-

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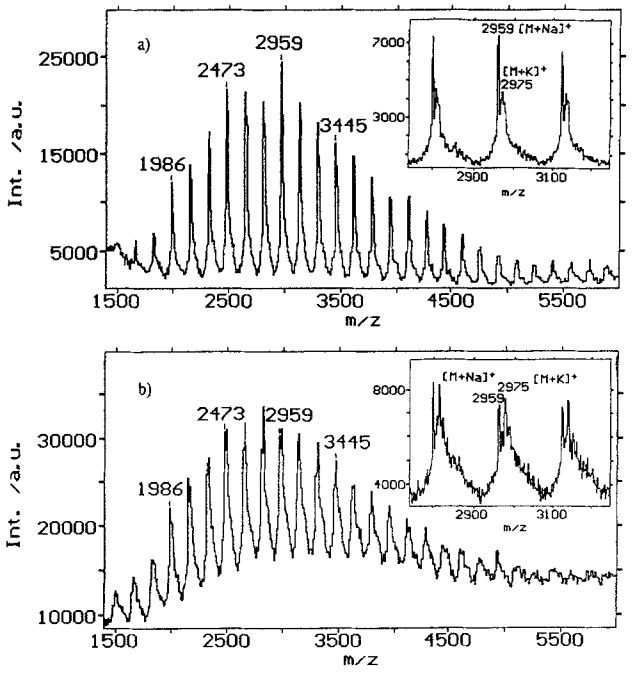

Fig. 1. Matrix-assisted laser desorption mass spectrum of chicory inulin using *a*) 3-aminoquinoline and *b*) 2,5-dihydroxybenzoic acid as matrix. The spectra are accumulated from *a*) 27 and *b*) 50 single shots. Intensity in arbitrary units, molecular weight M_r in Da (m/z)

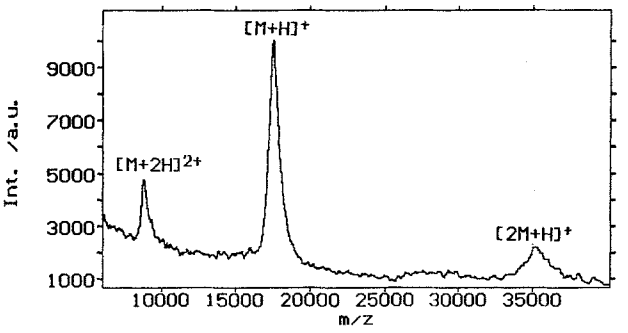

Fig. 2. MALD mass spectrum of myoglobin (horse skeletal muscle) using 3-aminoquinoline as matrix. The spectrum is accumulated from 30 single shots

ing the inulin sample, 1 µL of this mixture was placed on the target and the solvent was removed in a gentle stream of air. For preparing the myoglobin sample, 10 µL of this mixture were sprayed on the target by a nebulizer which we often use to produce nearly homogenous layers [9].

Results and discussion

Figure 1a gives the MALD mass spectrum of chicory inulin (Sigma) using 3-AQ as matrix. Two series of monosodium and monopotassium oligofructosan quasimolecular ions (Δm = 162 u) were observed. The molecular weights of the oligomers range from 1400 to 6000 Da, equivalent to a DP from 9 to 37. Figure 1b shows the MALD mass spectrum of the same inulin using DHB as matrix. This matrix introduced by Karas and Hillenkamp [7] was successfully applied for the measurement of proteins, oligosaccharides [4] and lignins [3]. A comparison of Fig. 1a and 1b reveals that the quality of the inulin mass spectrum is higher with the new matrix 3-AQ than with DHB:

1. The spectra given in Fig. 1a and 1b were accumulated from 27 and 50 single shots, respectively, indicating a higher sensitivity when using 3-AQ as matrix.

2. The oligomer ion peaks are sharper and the background is reduced when using 3-AQ instead of DHB as matrix.

3. The relative intensities of the sodium and potassium attached ions are almost equal when using DHB as matrix whereas 3-AQ as matrix gives predominantly sodium attached ions (see insets in Fig. 1a and 1b), thus reflecting the different concentrations of sodium and potassium in the matrices.

The new basic matrix 3-AQ was also successfully applied to the protein myoglobin. Myoglobin was already measured using sinapinic acid or DHB as matrix [11, 12]. The MALD mass spectrum of myoglobin from horse skeletal muscle (Sigma) obtained with 3-AQ as matrix is given in Fig. 2. The quasimolecular ion was observed at about 16,980 Da with high intensity beside the dimeric and the double charged quasimolecular ions at 34,000 Da and at 8,500 Da, respectively. 30 laser shots were sufficient to obtain the spectrum of Fig. 2. Thus, it could be shown that 3-AQ is quite effective as matrix assisting the desorption and ionisation of myoglobin.

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