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Fragmentation of insulin in the MALDI method

ABSTRACT

Results of observations of fragmentation processes of insulin ions by using the Matrix Assisted Laser Desorption Ionization (MALDI) method and the reflectron time of flight mass spectrometer are described. On the basis of the mass spectra the fragmentation channels of insulin are proposed. For higher used accelerating voltages fragmentation processes are lower and the detection of insulin is more precise. These results can be very useful for the detection of insulin in biomedical samples.

1. INTRODUCTION

Mass spectrometers are universal instruments used for the analysis of molecules. Practically, time of flight mass spectrometers are the only ones that have unlimited range of masses measured. This advantage is very important for observations of heavy biomolecules. When a molecule absorbs a quantum of energy higher than its ionisation energy, it makes a transition to an electronically highly excited state, which is either in the ionisation and/or dissociation continuum, or metastable. Excitation into the ionisation continuum will generally yield an ion, since the electron escape rate is many orders of magnitude higher than the rate of potentially competing processes such as dissociation or fluorescence.

Metastable states M* have a number of possible pathways to decay

$M^* \rightarrow M + h\nu$ (fluorescence), (1)
$M^* \rightarrow A + B$ (predissociation), (2)
$M^* \rightarrow A^+ + B$ (ion pair formation), (3)
$M^* \rightarrow M^+ + e$ (autoionization). (4)

All these processes have their own time scale [1, 2]. Under normal mass spectrometric conditions all decomposition reactions are necessarily unimolecular, since the mean free path of ions is much longer than their actual trajectories through that instrument. All described above ionization and fragmentation processes take place also in the Matrix Assisted Laser Desorption and Ionization (MALDI) method [3–5]. In this method ions are formed as a result of directing a pulsed laser beam onto a sample dissolved in the matrix (Fig. 1). The matrix material absorbs laser radiation and intermediates in the energy transfer to the studied substance. Lasting few nanoseconds laser pulse initiates desorption and ionization of sample's molecules. Ions created in this way are accelerated to a fixed energy in an electrical field and directed through the analyser to a detector of mass spectrometer, the time of flight mass spectrometer in our case. For an estimate of the range of reactions rates that is probed by a mass spectrometer it is useful to consider the time, which an ion spends in the different parts of the instrument.

The residence time of ions in the ion source of a mass spectrometer is in order of 1 μs. Thus, fragment peaks in the mass spectrum correspond to ions that decompose within the first microsecond after ionization. On the other hand, the molecular ion peak corresponds to ions that have a life time longer than about tens of μs, which is the total flight time from ionization region to the detector. Ions with adequately decomposition rates are lost when they decompose in the analysing field regions, or they can be observed as "metastable" ions in the spectrum if they fall apart in the field – free region of mass spectrometer.

Fragmentation that occurs after ionisation is very important in the identification of molecules. The molecular ion peak and its isotope peaks give information about the molecular weight and number of defined atoms, while the fragmentation gives information about specific groups of the molecule.

The fragmentation processes of molecular ions can be minimalized by shortness of their time of flight between the ion source and detector. It can be reached by using the higher voltage in the accelerating area of mass spectrometer.

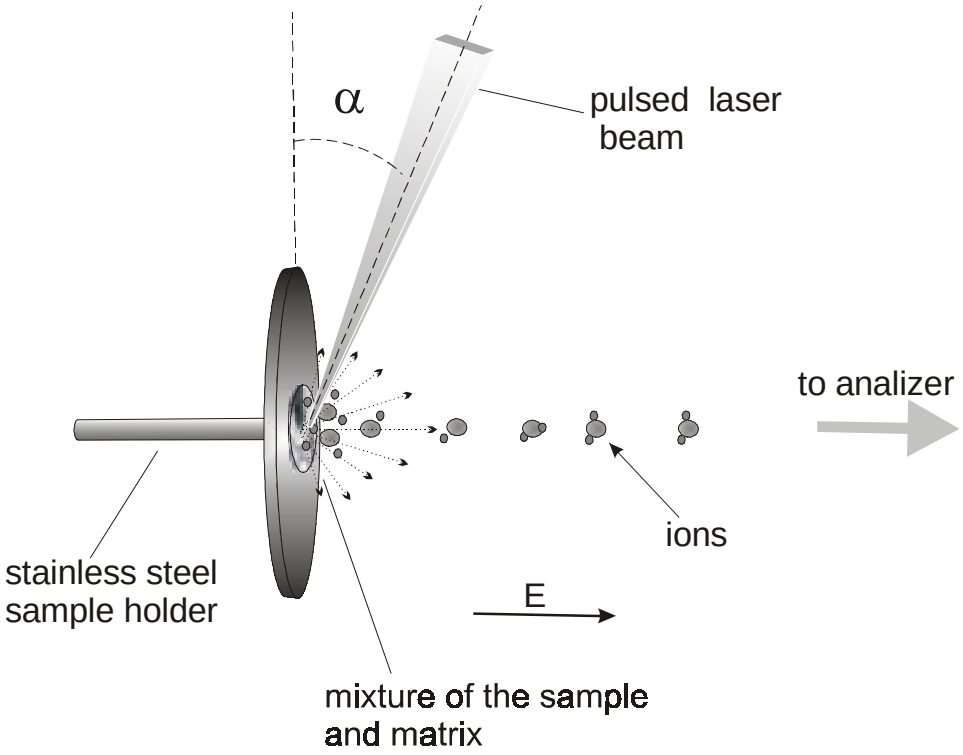


Fig. 1. Laser desorption sample holder in MALDI source (E – electric field)

In this work we present results of observations of fragmentation processes of molecular insulin ions. These investigations were made by using the MALDI method and the reflectron time of flight mass spectrometer.

2. EXPERIMENTAL AND RESULTS

A schematic diagram of the apparatus used in the presented investigations is given in Figure 2 [6–10]. Ions are created during the 5 ns pulse laser ablation of the sample and matrix mixture placed on the stainless steel holder. The created ions are then accelerated to a fixed energy in an electrical field and directed through the field free region and reflectron to the detector. The idea of separations of ions of different mass to charge ratio, m/z, results from the following relation:

$$\frac{m}{z} = 2\frac{U}{L^2} \cdot t^2 \tag{5}$$

where U is an accelerating voltage, L is a length of a flight way, t is the time of flight of ions from the ion source to the detector. This time for ions of the same m/z ratio and accelerated by the established voltage U is the same, too.

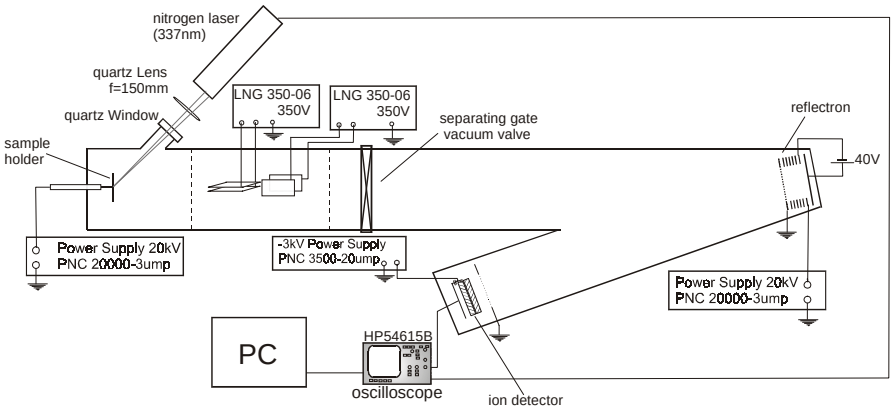


Fig. 2. Schematic diagram of the reflectron time of flight mass spectrometer used in the presented investigations

The nitrogen laser (LN 300C, Laser Photonics) used here has a wavelength of 337 nm, an output pulse length of 5 ns, maximum pulse energy specified as 250 μJ and a rectangular output shape of approximately 4 mm × 9 mm. The angle of incidence of the laser beam, which was focused in the plane of the sample, is 30° (see Fig. 1). The greatest intensities for the biomolecules investigated here were obtained using a slightly defocused laser beam. In this case the calculated dimensions of the defocused laser beam spot were approximately 400 μm × 900 μm and the power density was ~14 $MW \cdot cm^{-2}$. The ions were detected using a two channel plate (Hamamatsu) ion detector operated at 2.1 kV. The all spectra presented here were acquired as an average of 256 laser shots. Acquisition and averaging were performed using a Hewlett Packard HP54615B, 500 MHz (1Gsamples/s) oscilloscope. The spectra were then transferred to PC for processing.

In the presented investigations 2,5-dihydroxybenzoic acid (154 Da) as a matrix and Insulin from Bovine Pancreas, both from Sigma Aldrich were used. A mixture of 10 μL (0.6×10^{-6} M) of the matrix dissolved in the distilled water and 70% aqueous acetonitryle and 5 μL (0.8×10^{-9} M)

of insulin dissolved in distilled water was placed on the sample holder. To get uniform crystallization of this mixture we dried it in the air at room temperature. For the observation of influence of time of flight of ions on the fragmentation of insulin ions, accelerating voltages changed from 3 kV up to 15 kV, 0.5 kV step, were applied.

Insulin (5734 Da) consists of two main peptides chains connected by two sulfur bridges (see Fig. 3). Each chain is composed of aminoacids connected by the peptide bond.

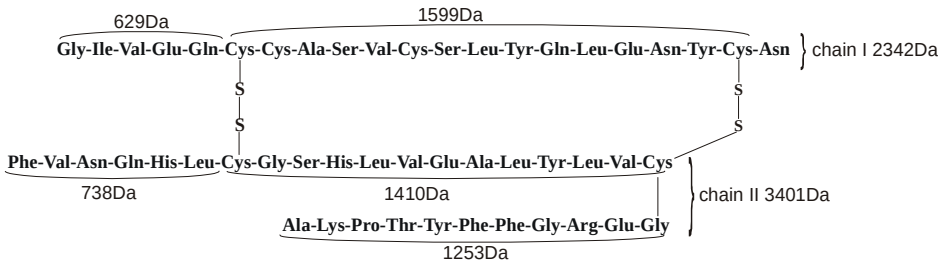
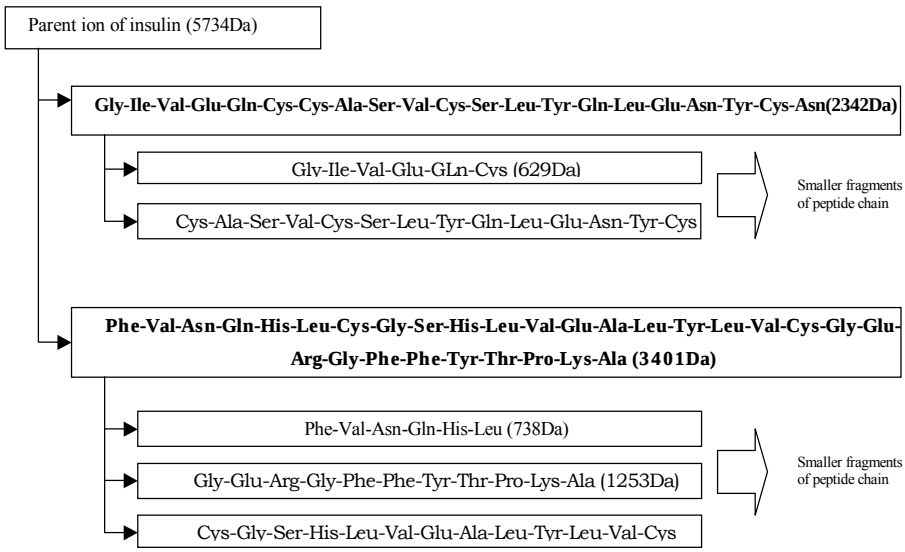


Fig. 3. Chemical structure of insulin

Figure 4 presents the mass spectrum of insulin obtained as an average of 256 laser shots. On this spectrum parent ions (insulin 5734 Da) and several identified fragment ions are marked. Fragmentation channels of insulin proposed by us are following:



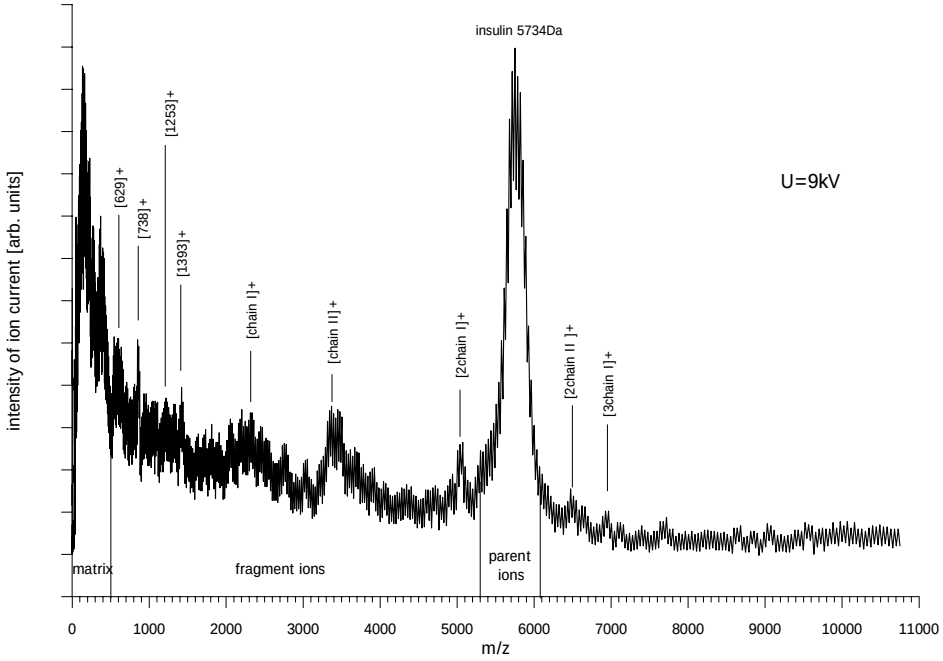


Fig. 4. Positive ion mass spectrum of insulin (M = 5734 Da) with a matrix of 2,5-dihydroxybenzoic acid (154 Da) obtained at 9kV accelerating voltage. The spectrum is an average of 256 laser shots.

The influence of the time of flight of ions on the fragmentation processes is showed on the mass spectra in Figure 5. The spectra were obtained for three different accelerating voltages: a) 3 kV, b) 9 kV and c) 15 kV. According to used voltages the time of flight of insulin ions is: a) 229 μs, b) 131 μs and c) 103 μs. A highest fragmentation and lowest intensity of insulin ion current for the longest time of flight is observed. For this case any interpretation of fragment ions and detection of insulin is very difficult. For higher accelerating voltages (short time of flight, adequately) we observe lower fragmentation and the high intensity of insulin peak. As results, the use of higher accelerating voltage is very important from the detection of insulin point of view.

Summarized results of our investigation are collected in the Figure 6. Figure 6a shows the relative intensities of: (a) insulin ion current (parent ion current/sum of fragment and parent ion current) and (b) fragment ions current (fragment ions current/ sum of fragment and parent ion current) versus the accelerating voltage (see Fig. 4). As results from these figures the influence of time of flight of ions from the ion source to the detector on

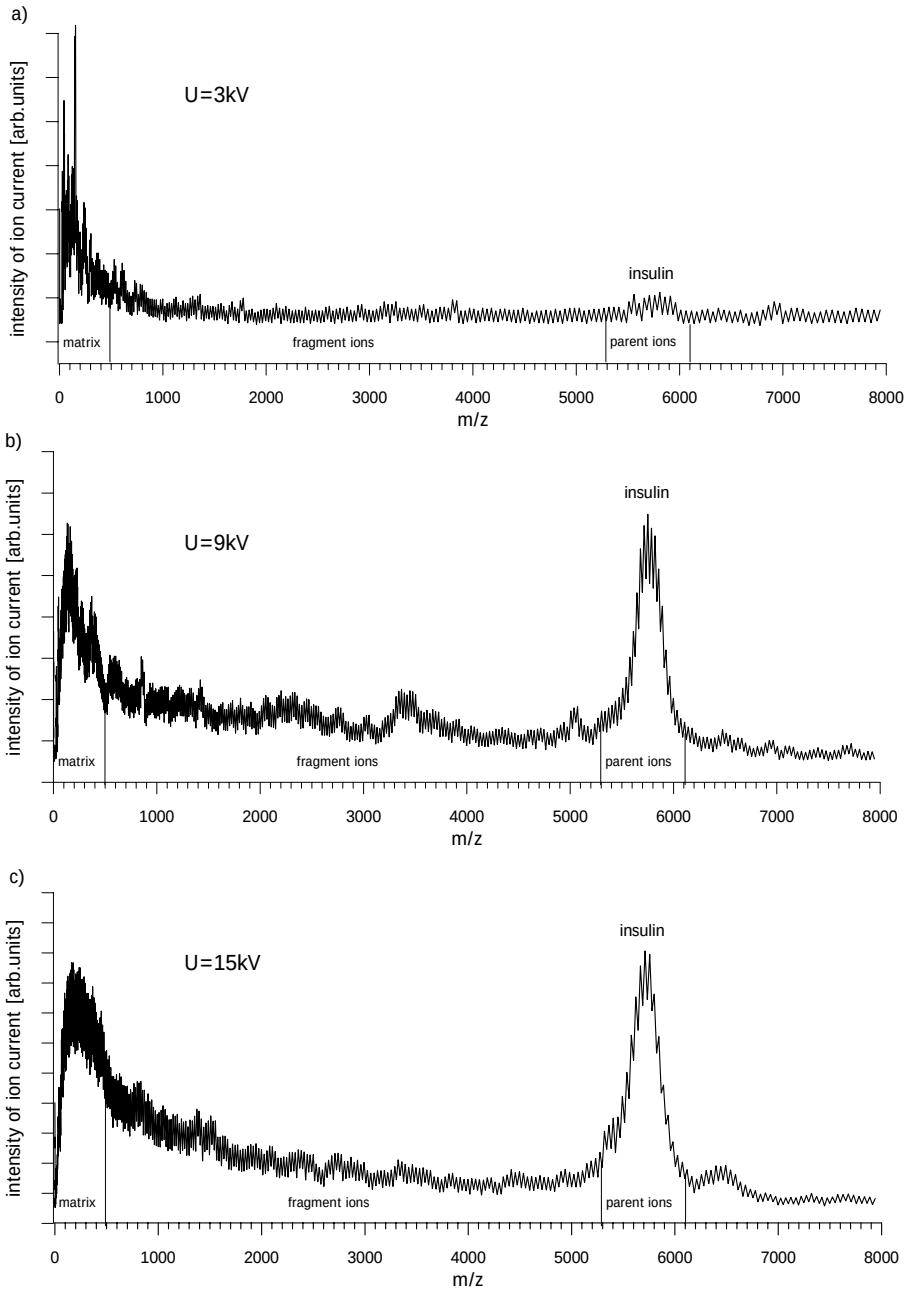


Fig. 5. Positive MALDI ion mass spectrum of insulin obtained at accelerating voltages: a) 3 kV, b) 9 kV and c) 15 kV. Each spectrum is an average of 256 laser shots

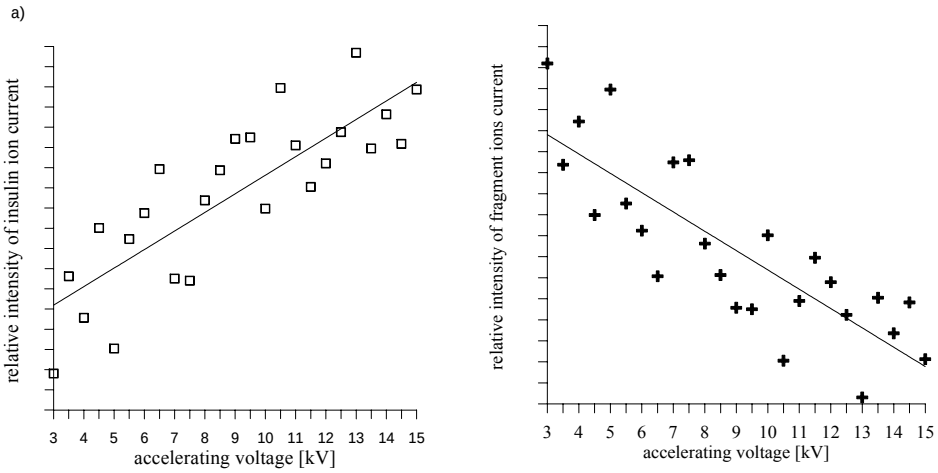


Fig. 7. Relative intensities of: a) insulin ion current (parent ion current/sum of fragment and parent ion current), b) fragment ions current (fragment ions current/ sum of fragment and parent ion current) versus the accelerating voltage

the fragmentation process is evident. In our investigation a higher accelerating voltage (>15 kV) could not be applied.

3. CONCLUSION

In this work we present results of observations of fragmentation processes of molecular insulin ions. These investigations were made by using the MALDI method and the reflectron time of flight mass spectrometer. The influence of the time of flight of ion on the fragmentation proc-

esses as well as a possibility of insulin detection is evident. Generally, for higher used accelerating voltages fragmentation is lower and detection of insulin is more precise. These results can be very useful for the detection of insulin in biomedical samples.

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