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A New Technique for Decomposition of Selected Ions in Molecule Ion Reactor Coupled with Ortho-Time-of-flight Mass Spectrometry

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A molecule ion reactor (MIR), i.e. a gas filled radio-frequency only quadrupole with a longitudinal electrical field (RFQLEF), is used as an atmospheric pressure ionization interface for an orthogonal time-of-flight mass spectrometer (O-TOFMS). A new phenomenon of selective ion 'heating' in a MIR near Mathieu's instability threshold was found and confirmed by computer simulation. The 'heating' in collisions with buffer gas molecules leads to ion decomposition. In the case of multicharged ions, fragments with an *m/z* value higher than that of the parent ion have a stable motion and can be analysed by an O-TOFMS. Fragmentation of bradykinin and β-endorphin molecular ions having a selected charge state is demonstrated. The spectra show clear 'ladder' structure. The phenomenon may be used as an alternative to tandem mass spectrometry (MS/MS) for molecule structure analysis. © 1997 by John Wiley & Sons, Ltd.

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Tandem mass spectrometry (MS/MS) with electrosprayed ions is being increasingly applied to determine molecule structure, for example for the high-sensitivity sequencing of short peptides.[1] In this method collisionally induced dissociation of selected ions yields information about the composition and structure of these ions. As an alternative we suggest here to use a molecule–ion reactor (MIR), based on a gas filled radio frequency quadrupole (RFQ) with a superimposed longitudinal electrical field, to investigate the composition and structure of selectivity 'heated' ions. The molecule–ion reactor described previously[2,3] has two modes of operation. In the first mode the motion of parent and fragment ions is chosen to be stable. The inhomogeneous RF electric field forces the ion to oscillate around the quadrupole axis while the collisionally induced fragmentation of the ion is controlled by the strength of the longitudinal electric field. In the second mode the amplitude and frequency of the RF electric field are chosen to be near Mathieu's instability threshold for the parent ion. This leads to a resonant increase of the ion velocity and thus to a collisional 'heating' and fragmentation of the selected parent ion. A combination of both methods can be used also. The present study explores this fragmentation technique and the possibility of using it for a structure analysis of short peptides.

DESIGN OF THE MIR

A molecule–ion reactor, shown in Fig. 1, is a part of an electrospray ionization interface. Electrosprayed ions pass through a gas curtain and through a nozzle (dia. 0.15 mm) into the MIR chamber and through a skimmer (dia. 0.35 mm) to the O-TOF mass analyser. The MIR itself is an RF-only quadrupole with a longitudinal electric field. Each of the four quadrupole rods consists of a set of metal rings (dia. 4 mm, length: 2.1 mm) separated by thin insulators. Longitudinal and RF voltages are applied through series of resistors and capacitors shown in Fig. 1. The full length of the quadrupole is 25 mm and the distance between opposite rods is $2\rho = 3.6$ mm. The pressure in the MIR chamber is maintained at 0.1–4 mbar.

EXPERIMENTAL RESULTS

Experiments were performed using the home-built MIR-TOFMS. This system has been described in detail elsewhere.[3,4] Our TOF mass analyzer provides a routine mass resolving power ~10 000 full width at half maximum (FWHM) and an accuracy of the mass measurement of 10 ppm. As an illustration of the new method for a fragmentation of selected ions two examples are presented, bradykinin (Sigma) and β-endorphin (Beckman), with the monoisotopic molecular masses 1059.651 Da and 3462.822 Da, respectively. These peptides were dissolved in methanol at a concentration of 10^{-5} M; the β-endorphin solution contained 2% acetic acid. A fused silica capillary with i.d. of 0.05 mm was used in the electrospray ion source with a flow rate of 0.18 μL/min. The electrospray voltage between the capillary and gas curtain electrode was about 3 kV. Pressure in the MIR chamber was 0.93 mbar at room temperature. Dry air was used for the gas curtain and as the MIR buffer gas. Ion masses and charge states in mass spectra were analysed by measuring an accurate *m/z* value for monoisotopic peaks and by an *m/z* difference between neighbouring peaks in the isotopic distribution.

In mode 1 the fragmentation of the bradykinin ions was performed using a constant RF amplitude, $U_{RF} = 85$ V, and frequency $\nu_{RF} = 1.001$ MHz. A few

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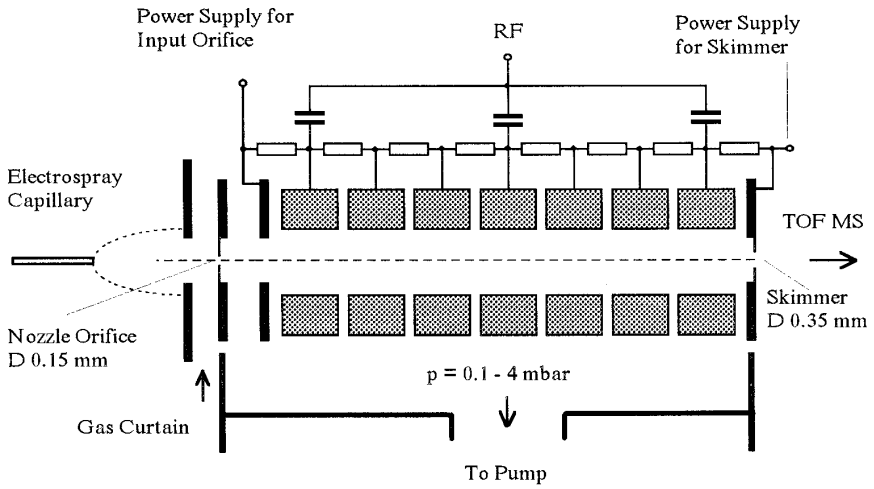

Figure 1. Schematic view of MIR atmospheric pressure interface.

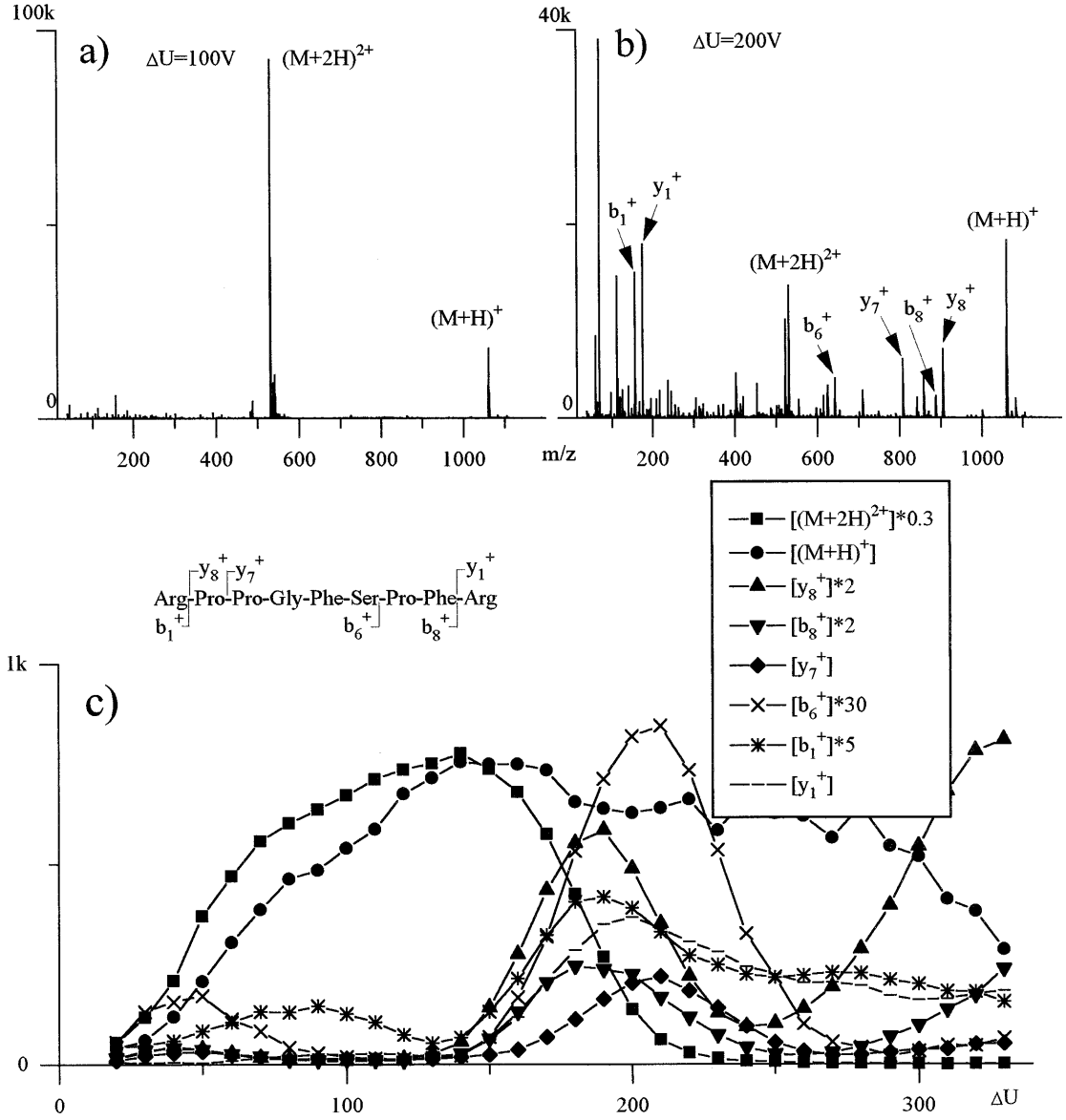

Figure 2. Mass spectra of bradykinin for longitudinal potential differences (a) ΔU = 100 V and (b) ΔU = 200 V, in the MIR. Also shown are mass fragmentograms for several ions as functions of ΔU, (c). Note that the intensity of the doubly charged bradykinin ions (m/z = 530.8) decreases drastically for $\Delta U \geq 200$ V, and that at the same time the intensities of the fragments increase for $\Delta U \geq 200$ V.

dependencies of ion current intensities on the longitudinal MIR-voltage (ΔU), for the ions of interest (mass fragmentograms), plus two mass spectra obtained at different ΔU values are shown in Fig. 2. The mass fragmentograms were constructed from a series of mass spectra obtained by ΔU variation. There is no fragmentation in the mass spectrum (Fig. 2(a)) observed at low ΔU = 100 V, and intense peaks of singly and doubly charged bradykinin ions are observed. The mass spectrum (Fig. 2(b)) recorded at higher ΔU = 200 V is of another kind. Here the peaks of parent bradykinin ions and their fragments are indicated. Note, that in mode 1 fragment ions with *m/z* in the entire recorded range are observed. It is seen from the mass fragmentograms (Fig. 2(c)) that the increase of intensity of the fragment ions follows the appropriate decrease of intensity of the parent ions.

In mode 2 the fragmentation of the parent doubly charged bradykinin ion was performed using constant ΔU = 44 V, and frequency of RF voltage ν_{RF} = 501.6 kHz, by the variation of the amplitude of the RF voltage U_{RF}. A few mass fragmentograms and two mass spectra observed using the different RF voltages are presented in Fig. 3. These mass fragmentograms were constructed from the series of mass spectra recorded with variation of RF voltage. Note that in mode 2 the decrease of intensities of parent ion peaks and appearance of the appropriate fragment ion peaks in the mass spectra show a sharp threshold behavior. There is no fragmentation in the mass spectrum of bradykinin (Fig. 3(a)), observed at low RF voltage. One can see intense peaks of the parent bradykinin ions in this spectrum. One can see intense peaks of the parent bradykinin ions in this spectrum. In the mass spectrum (Fig. 3(b)) observed at higher RF amplitude, a number of fragments resulting from the decomposition of the doubly charged bradykinin ion $[M + 2H]^{2+}$ is observed. One can see the sharp increase of peaks of the fragment ions with an appropriate sharp decrease of the molecular ion peak $[M + 2H]^{2+}$. The peculiar feature of the mass spectra recorded in mode 2 is absence of fragment ions with *m/z* smaller than that of the parent ion $[M + 2H]^{2+}$. These ions are rejected due to the unstable character of their motion in RFQ.

As an illustration of the MIR-TOFMS performance, the mass spectrum of bradykinin fragments obtained in mode 2 using the RF voltage U_{RF} = 82 V, which was shown in Fig. 3(b), is presented in detail in Fig. 4. Besides the clearly identified fragment ions peaks of bradykinin there are some contamination ions in this mass spectrum. It is easy to determine the charge states and thus the masses of these ions by the difference in

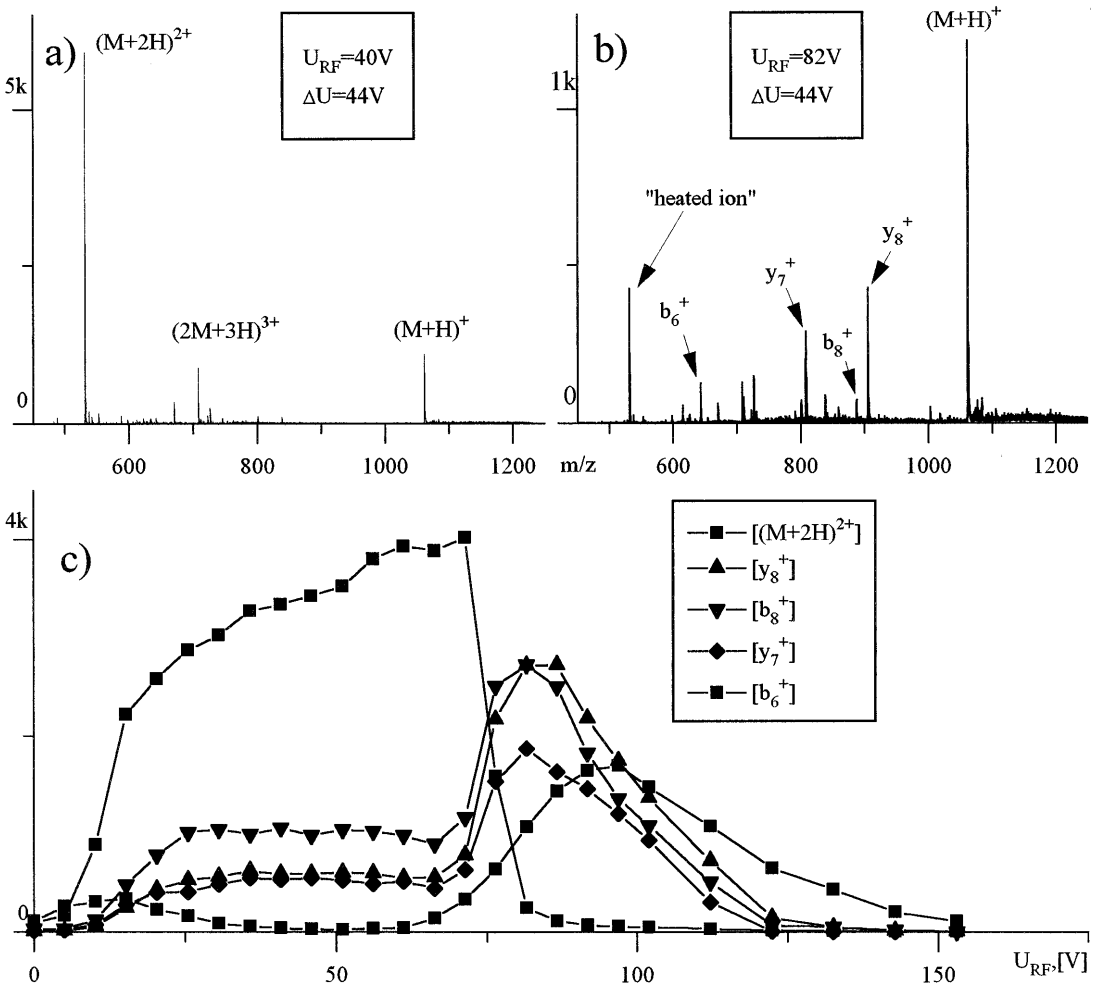

Figure 3. Mass spectra of bradykinin for RF voltages (a) U_{RF} = 40 V or (b) U_{RF} = 82 V, in the MIR at RF frequency 501.6 kHz. Also shown are mass fragmentograms for several ions as functions of U_{RF} (c). Note that the intensity of the doubly charged bradykinin ions (*m/z* = 530.8 u) decreases drastically for $U_{RF} \geq 75$ V and that at the same time the intensities of the fragments increase for $U_{RF} \geq 75$ V.

m/z values of neighboring peaks in isotopic distribution.

We have examined the efficiency of the fragmentation in mode 2 for larger peptides. β-endorphin, which has monoisotopic molecular mass 3462.822 Da, was chosen as the example. The primary structure of this peptide, and a few characteristic fragments, are shown in Fig. 5. A series of 19 mass spectra was recorded with constant ΔU = 90 V and ν_{RF} = 437 kHz, with variation of the RF voltage amplitude U_{RF}. Four mass spectra recorded using different RF voltages are shown in Fig. 6. Note that β-endorphin parent ions which have different charge states start to decompose at different RF voltages, each producing its own mass spectra of fragment ions. One can see the agreement between the decrease of the intensity of parent β-endorphin ion peaks and the appearance of the fragments in the mass spectra. As an illustration of the fragmentation of $[M + 4H]^{4+}$, $[M + 3H]^{3+}$ and $[M + 2H]^{2+}$ parent ions, several mass fragmentograms in Fig. 7 are presented. One can see the agreement between the decrease of intensity of these parent ion peaks and the consequent appearance of their fragments y_8^{1+}, y_{12}^{1+}, and y_{22}^{1+}, respectively. The two mass spectra obtained using the different RF voltages, corresponding to the fragmentation of the $[M + 4H]^{4+}$ and $[M + 3H]^{3+}$ ions, are shown in Fig. 8(a),(b). To simplify the data analyses, and to increase the signal-to-noise ratio, we have changed the time resolution parameter in our data acquisition program and as a result decreased slightly the effective

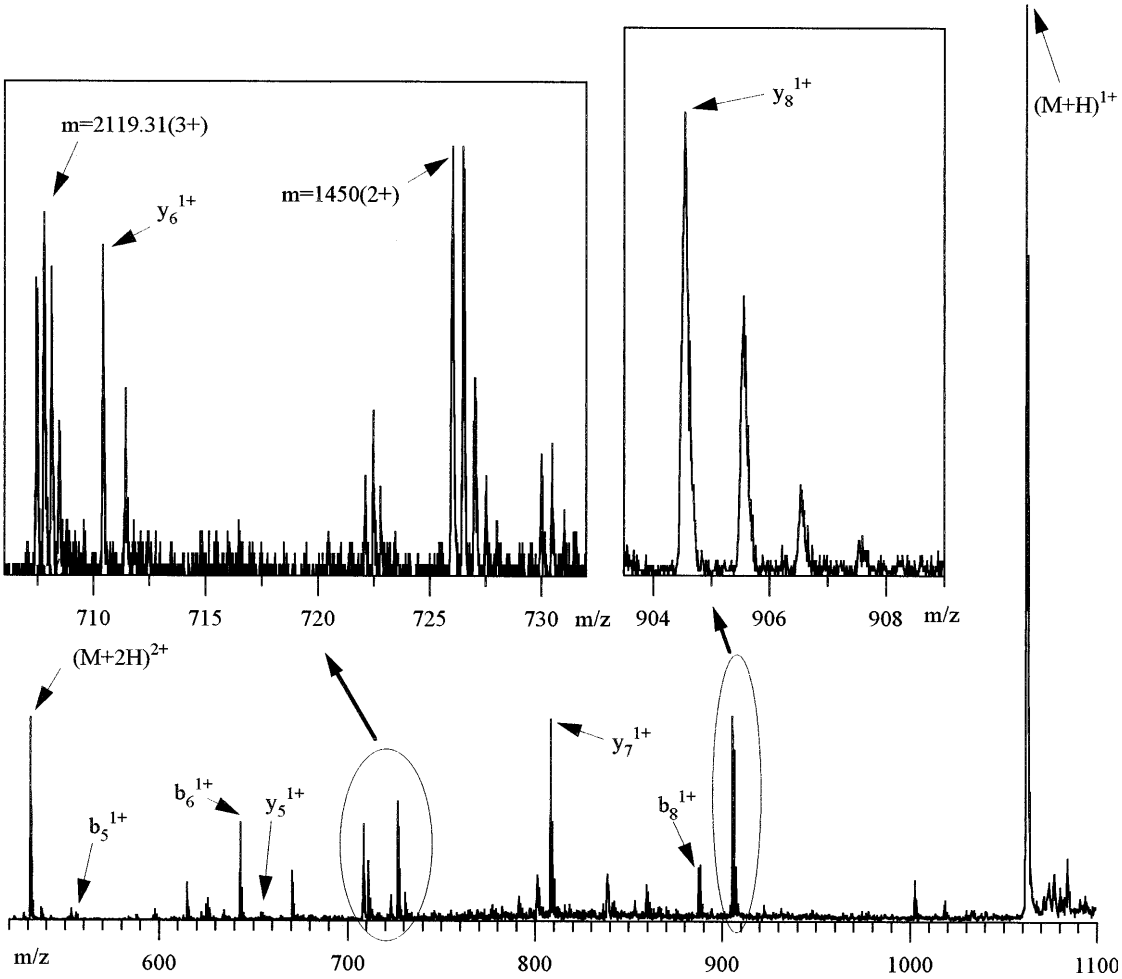

Figure 4. The detailed mass spectrum of bradykinin recorded at RF voltage U_{RF} = 82 V and longitudinal MIR voltage ΔU = 44 V. The fragments formed by peptide bond cleavages and some contaminant ions are shown.

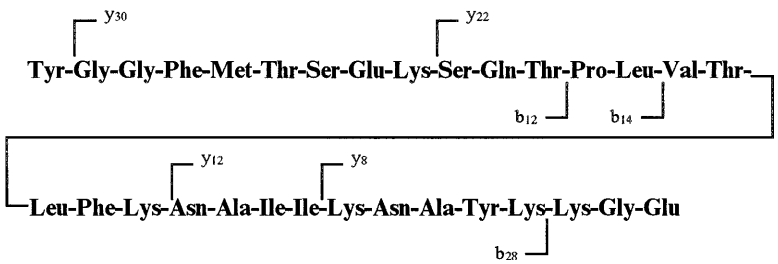

Figure 5. The primary structure of β-endorphin. Some characteristic fragments due to peptide bond cleavages are indicated.

resolving power of MIR-TOFMS. This may be seen from the detailed view of several parts of mass spectra in Fig. 8(a),(b). Note, that most of the fragments in the mass spectra are formed by the peptide bond cleavage. It is remarkable that these mass spectra show clear 'ladder' structure.

COMPUTER SIMULATION OF ION MOTION IN THE MIR

To quantitatively estimate the effect of the combined action of the radial RF and the longitudinal DC fields on the ion velocity we have used a computer simulation. This model is based on the calculation[5] of ion trajectories in an RF-only collisional mutliple ion guide, by using the Langevin equation for the velocity u of a particle undergoing frictional and thermal action in a medium:

$$du/dt = -u/\tau + A + F_{RF}\cdot sin(\omega\cdot t) + F_l. \quad (1)$$

Here A is the random thermal force, F_{RF} the quadrupolar electrical force of angular frequency ω, and F_l the longitudinal electrical force; all forces are mass normalized. The velocity relaxation time τ takes into

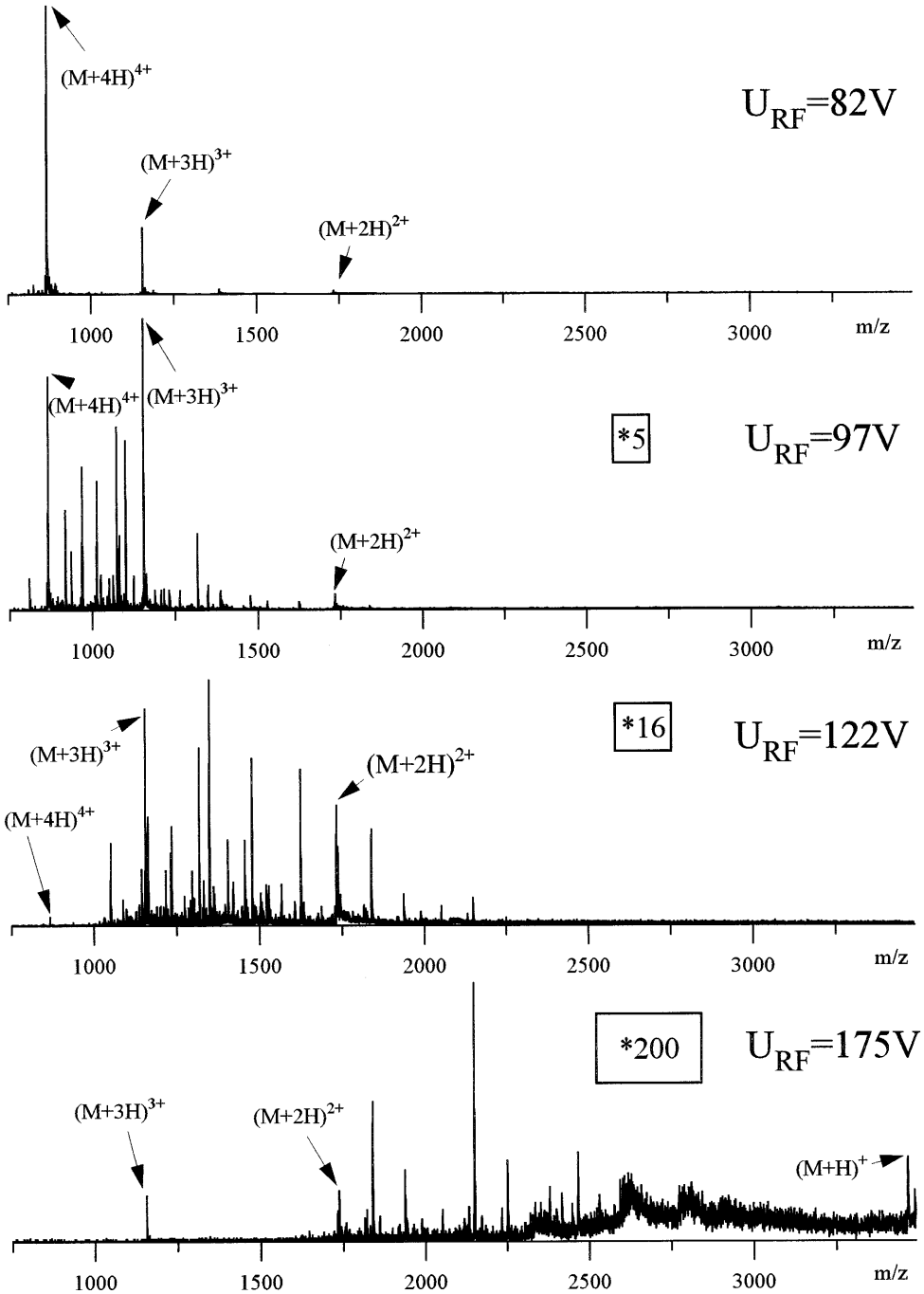

Figure 6. The mass spectra of β-endorphin (10^{-5} M solution in MeOH with 2% acetic acid), for four RF voltages. There is no fragmentation in the first mass spectrum (U_{RF} = 82 V), in the second spectrum (U_{RF} = 97 V) one can see the decomposition of the quadruply charged ion $[M + 4H]^{4+}$, in the third the triply charged ion decomposes. The doubly charged ion decomposition is shown in the fourth mass spectrum.

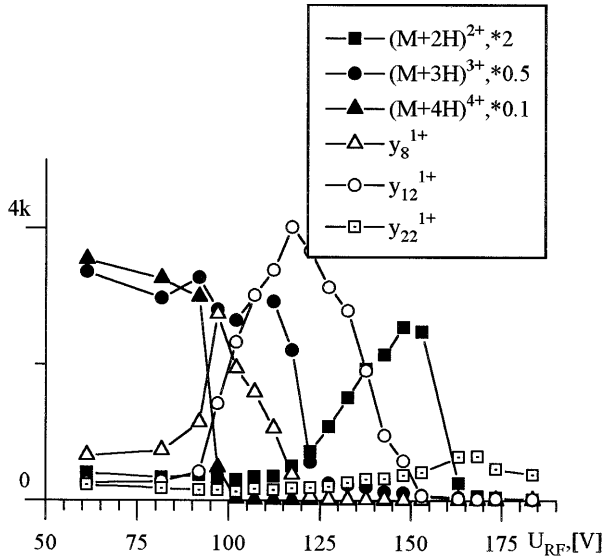

Figure 7. The mass fragmentograms constructed from the series of mass spectra of β-endorphin obtained with variation of the RF voltage. One can see that different fragments may be produced from parent ions having specific charge states.

account the frictional effect of a medium. The effective RF potential in an RF-only quadrupole was estimated[5] on the basis of Eqn (1). In the limit of vacuum motion when the frictional and thermal terms become negligible, the equation collapses to the famous Mathieu equation[6] applied to, RF-only quadrupoles. In this case the ion motion is stable as long as the Mathieu stability parameter $Q_M = (4qU_{RF})/(M\omega^2\rho^2)$ is lower than the threshold value $Q_{th} = 0.91$. Here U_{RF} is RF voltage, M/q the ion mass-to-charge ratio and $\rho = 0.18$ cm is the radius of the RF quadrupole aperture. The influence of a medium becomes significant with $\omega\cdot\tau \sim 1$. Both the RF effective potential and the stability threshold are shifted.[5,7,8] Dependence of the low mass threshold on experimental conditions was investigated experimentally and compared with results of the theory.[7,8] It was shown that for a sufficiently high buffer gas pressure, when $\omega\cdot\tau \sim 1$, the instability of the ion motion for low mass-to-charge ratio m/q is damped by the friction force, so that the Mathieu stability parameter Q_{th} corresponding to the instability offset may reach values far above the vacuum stability limit of 0.91.

The stability threshold RF voltage measured experimentally for doubly charged bradykinin ion at RF frequency 501.6 kHz is $U_{th} = 76.5$ V, which corre-

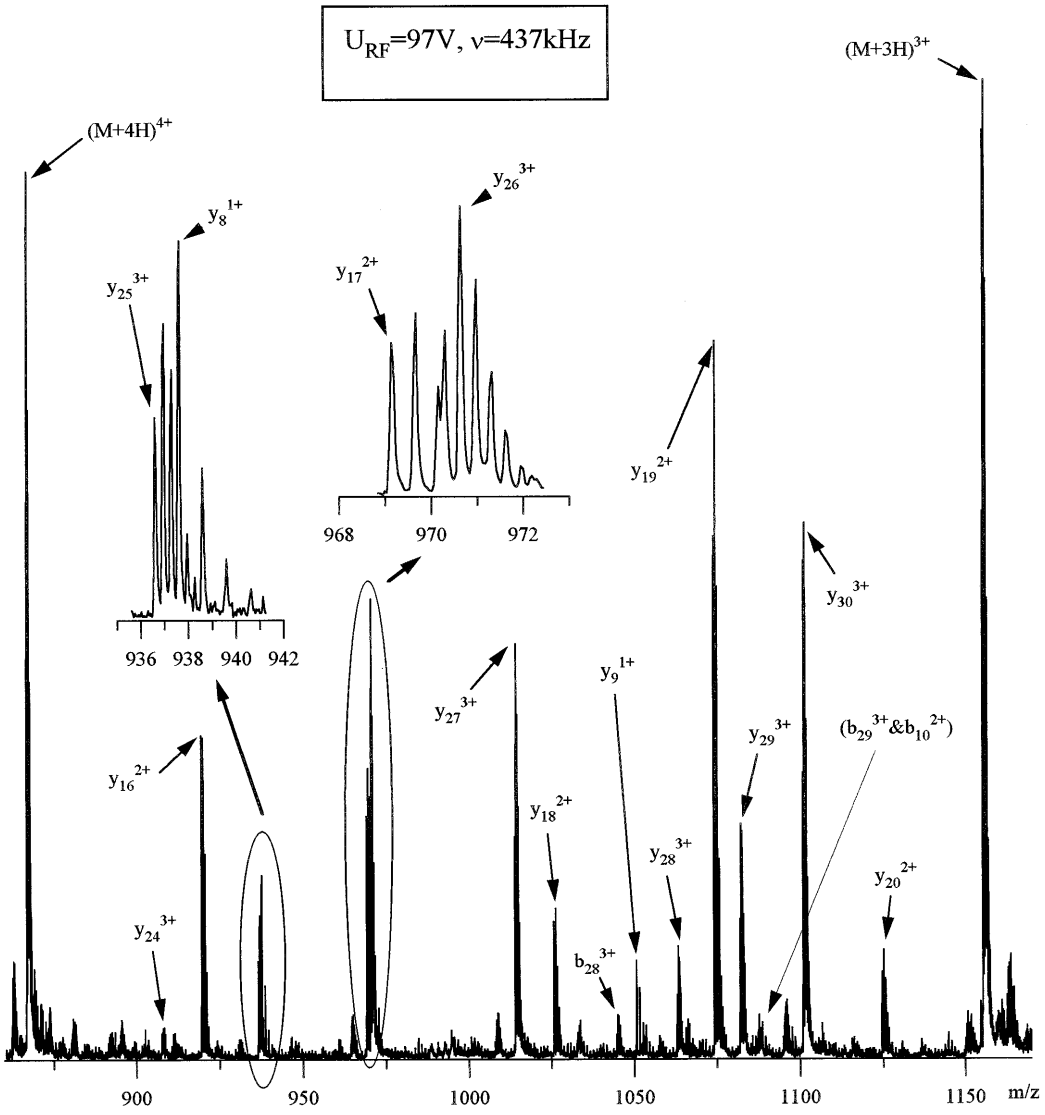

Figure 8. Continued on next page.

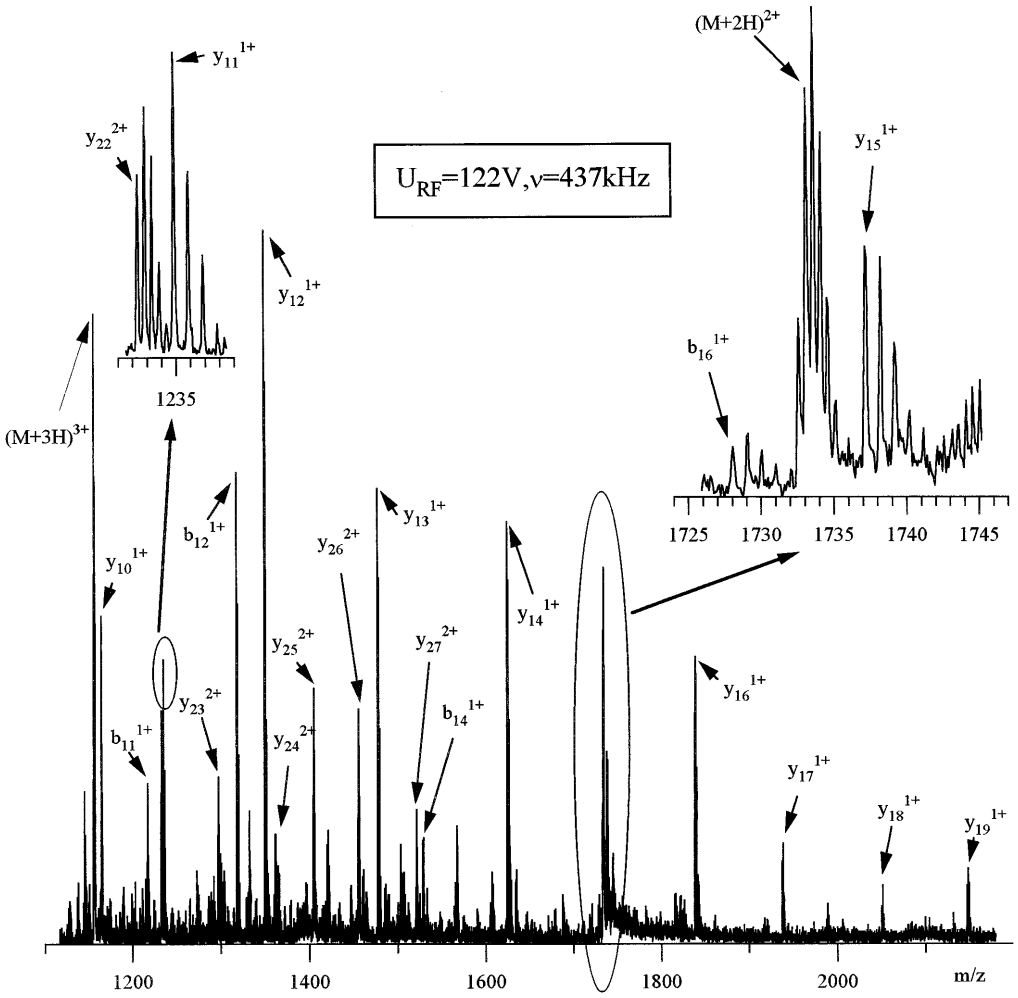

Figure 8. The mass spectra of β-endorphin obtained at two different RF voltages and RF frequency 437 kHz. The fragmentation of (a) the $[M + 4H]^{4+}$ and (b) $[M + 3H]^{3+}$, resulted in the clear 'ladder' structures of the mass spectra.

sponds to Q_{th} = 1.72, considerably higher than the vacuum value. This indicates a noticeable influence of the frictional term $-u/\tau$ on the ion motion. We have used the theoretical[7,8] dependence $Q_{th}(\omega\cdot\tau)$ to define a corresponding value of the $\omega\cdot\tau$ = 1.42 or τ = 0.45 μs. This τ value was used for numerical simulation of the bradykinin ions' motion.

Results of the computer model are presented in Fig. 9. In Fig. 9(a) the average path along the quadrupole axis Z_{ave} of the primary doubly charged bradykinin ions in RFQ is shown as function of U_{RF}. As long as the RF voltage stays lower than the stability threshold U_{th} = 76.5 V, all ions reach the exit of the quadrupole and Z_{ave} equals the quadrupole length of 2.5 cm.

The value of interest for the ion decomposition study is the maximal internal temperature of an ion, T_i, that can be achieved in the MIR reactor. Assuming the formation of a collisional ion–molecule complex, and that the molecule after decay of the complex has a temperature corresponding to the ion internal temperature, we obtain an equation for internal temperature of the ion T_i moving in a gas under electric field influence:[2,4]

$$\frac{dT_i}{dt} = \frac{T_{ss} - T_i}{\tau_h} \qquad (2)$$

where the steady state ion internal temperature:

$$T_{ss} = T_g + \frac{(m/M)\cdot K_{ion}}{C_g} \qquad (3)$$

Here T_g, m, K_{ion} and C_g are the buffer gas temperature, molecule mass, the ion kinetic energy and molecular heat capacity of the gas, respectively. There are two characteristic times that describe how the system reaches a steady state. The first is the velocity relaxation time τ, already mentioned. The second time τ_h describes the internal temperature relaxation of the ions. We may estimate τ_h as the time betwen collisions multiplied by the ratio of heat capacities of the ion and a buffer gas. For the case of bradykinin ion and N_2 buffer gas we get:

$$\tau_h \approx 2\tau$$

and we may estimate the internal temperature of an ion as a function of the ion kinetic energy K_{ion} convoluted with the exponent $exp(-t/\tau_h)$ in accordance with the above equations.

Maxima of the total convoluted ion kinetic energy were calculated for 100 computed trajectories, and then averaged. Circles in Fig. 9(b) present the values of the

averaged maxima as a functionof U_{RF}. Triangles in Fig. 9(b) show dispersion of the convoluted kinetic energy. It is clearly seen that a sharp jump in the total kinetic energy exists near the instability threshold, and the maximum kinetic energy of the ions can reach a value near $\sim 0.2qU_{th}$. This energy is of the order of magnitude of the energy used for collisional ions, decomposition in MS/MS. For the described experimental conditions (mode 2) we estimate from Eqn. (3) and Fig. 9(b) that the internal temperature of the doubly charged bradykinin ion reaches $T_i \simeq 4300$ K, assuming that decomposition does not take place. ($C_g = 5/2k$ is used for the estimate, where k is the boltzmann constant).

The fragment ions with M/q ratio above the stability threshold will not undergo RF heating, and moreover their internal energy will be efficiently dissipated by collisions with buffer gas molecules. Thus further decomposition of fragments should be prevented. This may explain the 'ladder' fragmentation structure observed here for bradykinin and β-endorphin.

CONCLUSIONS

The MIR designed as an RF-only quadrupole with a longitudinal electric field allows operation at pressures ~ 1 mbar, typical for a gas dynamic interface of an electospray ion source. The MIR working pressure is considerably higher, by 2–3 orders of magnitude, than the pressure used in a conventional collision induced dissociation (CID) cell for MS/MS. Large numbers of collisions in a process of ion heating by RF or longitudinal electric field allows creation of soft fragmentation conditions. In the present study we have considered fragmentation by the longitudinal electric field (mode 1) and the RF quadrupolar field (mode 2). Use of both modes allow us to obtain complete fragmentation mass spectra of peptides studied. However, we have observed that it is easier to reach fragmentation conditions using the RF heating. The theoretical investigation performed shows that RF heating of ions results in internal ion temperature values corresponding to CID energies of several tens of eV. The RF heating takes place for ions having m/z ratios lower than the low mass Mathieu stability limit, thus allowing selective fragmentation. We have demonstrated that fragment spectra resulted from decomposition of selected charge states of the peptide molecular ions. The high resolving power and mass measurement accuracy of our O-TOFMS facilitate identification of all fragments in the mass spectra.

The selective RF heating (mode 2) can be used for molecule structure investigation, including peptide sequencing, as an alternative to classical CID MS/MS techniques. The MIR combines functions of a CID cell and an API interface, hence we may expect a higher sensitivity from our approach in comparison with MS/MS which uses an additional mass filter and gas dynamic interfaces. The disadvantage of mode 2 is rejection of light ions, so the fragmentation mode 1 is used to observe such species.

Finally, while this work was being prepared for publication, a paper by Javahery and Thomson appeared[9] which describes a somewhat similar segmented RF-only quadrupole collision cell, which was used to measure mobilities and collisional cross-sections of ions.

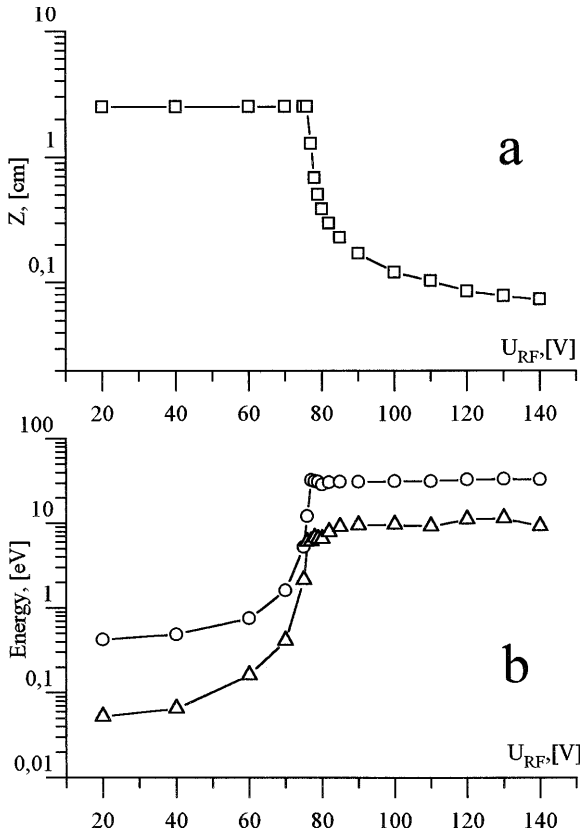

Figure 9. The calculated average path (a), averaged maximum of the ion kinetic energy convoluted with exponent $exp(-t/\tau_h)$ (b, circles), and its dispersion (triangles), of the doubly charged bradykinin ions in the collisoinal RF only quadrupole as function of the RF voltage U_{RF}.

Acknowledgements

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