High Sensitivity in CID Mass Spectrometry, Structure Analysis of Pyrolysis Fragments

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An apparatus is described for Collision Induced Dissociation Mass Spectrometry, where essential increase in sensitivity in the detection of the fragment spectrum is obtained by simultaneous detection. Decrease in resolution due to kinetic energy release on dissociation is prevented by post-acceleration of the fragment ions.

The instrument was used to investigate the structure of the mass $m/z = 59$ compound in the pyrolysis products of *Mycobacterium* cells. This mass is a key mass in the pyrolysis mass spectrometric identification of dangerous mycobacterial strains.

Introduction

In the last decade new techniques have been developed in mass spectrometry, directed to the study of the secondary ion spectrum arising after either unimolecular or collision induced dissociation of complex ions. Investigation of the dissociation products provides information on the structure of the precursor ionic complex.

Most instruments used for this purpose are reconstructed double focusing mass spectrometers with either normal or reversed geometry and usually provided with a collision cell in the first or in the second field free regions [1–4].

Recently, a new type of instrument has been introduced consisting of three consecutive quadrupole mass filters [5]. The first quadrupole selects the precursor ion. The second quadrupole serves as a collision cell. It is operated with merely the high frequency field, so no mass selection occurs but the field provides a high transmission efficiency for all masses. In the last quadrupole finally the fragment spectrum is scanned. This instrument has the peculiarity that the energy of the ions varies largely along the ion orbits, so the collision energy is not well defined.

All these instruments have in common that the fragment ions are recorded in a scanning mode. This has two main consequences: in the first place a very large fraction of the ions is not detected on scanning, but also there is a certain minimal time, necessary to obtain a complete spectrum.

Especially in CID-mass spectrometry the sensitivity in measuring the fragment spectrum is of great importance as each individual precursor ion results in the detection of only one fragment ion and therefore only contributes to one fragment mass peak.

A considerable advantage of our non-scanning instrument is the simultaneous collection of fundamentally all ions produced in the collision cell. Moreover our procedure enables us to investigate even the fastest processes.

In the first stage of our instrument the ion under investigation is selected by a magnetic sector field. After passage through the collision cell the fragment ion beam is analysed by a second magnet and the spectrum is detected simultaneously. Three electric and one magnetic quadrupole lenses are incorporated in the second stage for rotation of the focal plane, variation of the dispersion and additional focusing of the ion beams.

Apparatus

As a detailed description of the apparatus, schematically shown in Fig. 1, has already been given by Louter *et al.* [6], we will give only a brief resume in this article.

The instrument consists of two parts: a selection part where the desired precursor ion is selected, and an analysing part, where the fragment ion spectrum is recorded.

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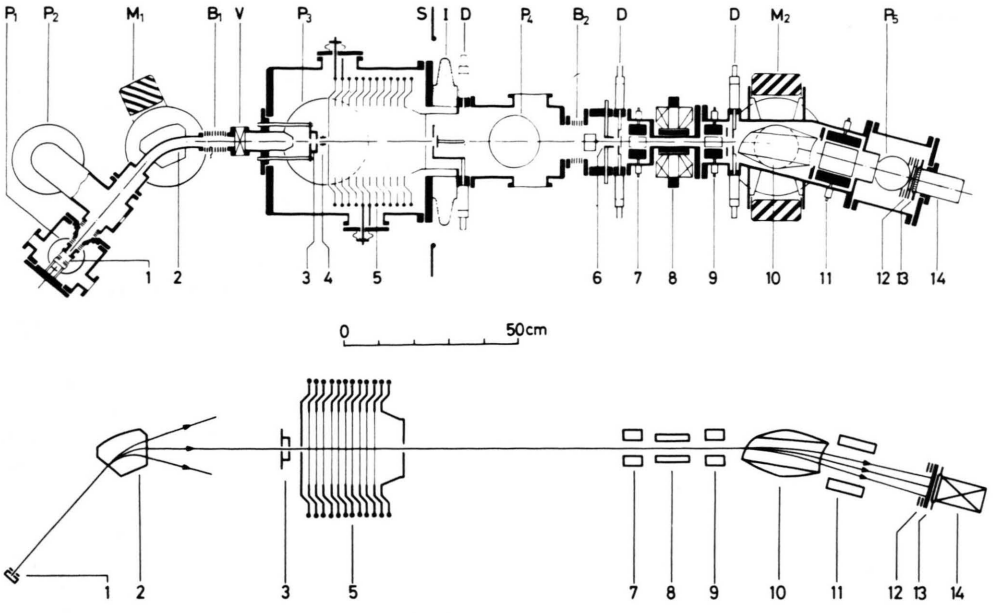

Fig. 1. Above: top view of the apparatus. Under: simplified picture, showing the main ion optical functions. 1. ion source, 2. selection magnet, 3. collision gas cell, 4, 6. deflection plates, 5. post-acceleration slits, 7, 9, 11. electrostatic quadrupoles, 8. magnetic quadrupole, 10. analysing magnet, 12. CEMA, 13. phosphor screen, 14. fibre optics slab. P1, 2, 3, 4, 5 oil diffusion pumps, M1, 2. sector magnet yokes, B1, 2. bellows, V. valve, S. high voltage shield, I. high voltage insulator, D. adjustable diaphragms.

The selection stage consists of a conventional magnetic sector mass spectrometer. Ions are produced in an electron impact source and accelerated from 500 up to 10000 eV. The radius of the main orbit in the analyser magnet is 150 mm, the deflection angle is 50 °. The maximum magnetic field strength is 1.5 T, the resolution of the first stage is 600.

At the site of the detector slit a collision cell is mounted. Its length is 10 mm, both entry and exit slit widths can be adjusted independently up to 2.00 mm. This possibility to adjust the slit widths permits to find a compromise between the sometimes contradictory requirements for beam intensity and resolution of the first stage, transmission through the gas cell and target gas loss.

The fragmentation of the ions in the collision cell is attended with an energy spread of the fragment ions due to the kinetic energy release. In order to reduce the relative energy spread the fragment ions are post-accelerated. The post-acceleration section consists of twelve equidistant electrodes, which have a maximum total potential difference of 30 kV. By an appropriate potential distribution over these electrodes the ion beam can be confined to optimize the transmission through the pole gap of the second magnet over the full mass range.

A consequence of the introduction of the post-acceleration is, that part of the instrument has to be well insulated from the rest of the instrument as well as from ground. Either the first stage (inlet system, source and selection magnet) should be raised on + 30 kV or the second stage (analysing magnet with quadrupole lenses and detector) should be put on − 30 kV. Both possibilities are incorporated.

Obviously post-acceleration reduces the relative energy spread of ions of equal mass, but also the relative energy differences of ions of different masses. In an instrument where the analysis of the fragment beam is performed by using an electric sector field, the mass dispersion will decrease in exactly the same way and post-acceleration has no effect at all on the resolution. If, however, a magnetic field is applied as an analyser, the mass resolution $\Delta m/m$ tends to the usual value of $2\,\Delta p/p$ for conventional single focusing mass spectrometers. So only for instruments with magnetic analyser the resolution improves on post-acceleration.

The main reason for application of a magnet is that this permits the simultaneous detection of a large part of the spectrum [7]. The possibility to improve the resolution by post-acceleration is an additional advantage.

The analysing magnet was designed in such a way that a mass range with a 4:1 ratio between highest and lowest masses could pass. As the height of the ion beam due to the angular spread on fragmentation in the collision cell may amount up to 15 mm, a large pole gap is desired. This reduces the maximum field strength in the magnet to about 0.9 T. With full acceleration of 10 kV and post-acceleration of 30 kV an ion of mass 550 would describe an orbit with $R = 750$ mm. In case this mass describes the main orbit the whole mass range from $m/z = 250$ up to 1000 should pass the magnet. Obviously the large difference of their radii requires a small deflection angle. A 15 ° deflection for the main orbit has been chosen.

The focal action of such a small angle sector-magnet is only weak and in order to avoid very large focal distances additional focusing is necessary. This is effectuated by a series of four quadrupole lenses, which also perform a rotation of the focal plane in order to get coincidence with the detector plane and which, moreover, can produce a variation of the dispersion. For details we refer to the previous article [6].

The detector has in principle been described by Tuithof *et al.* [7]. A double channelplate of 75 mm diameter and 25 μm pore size followed by a phosphor screen is used. (Channeltron Electron Multiplier Array (CEMA), type 3075-P 20, Galileo Electro-optics Corp. Mass, USA). The line spectrum is imaged onto a photodiode array (1024 channels, 0.43 mm high, 25 mm long, detector model 1410, controller model 1218, Princeton Applied Research Corp., Calif.) by an 85 mm camera objective ($f/D = 1.2$).

In this way a detection sensitivity of one ion per 10 seconds at a S/N ratio of 1 can be obtained [6]. The mass resolution depends mainly on slit width, post-acceleration voltage, mass ratio of daughter and precursor ion and the ratio magnification/dispersion of the last quadrupole. The CID mass resolution of fragments without dissociation energy is better than 1000. For details about the mass resolution of fragments with a certain amount of energy spread due to kinetic energy release we refer to the article of Tuithof [8].

Application

The experimental set up as described in the foregoing part allows the structure elucidation of various ions representing a single mass peak in a spectrum and is in particular developed for measurement of very short-lasting ion pulses, *e. g.* those obtained on flash pyrolysis (Curie point pyrolysis [9] or laser desorption mass spectrometry [10]). In the following section an example is described to demonstrate the applicability of the system. It concerns one of the mass peaks in pyrolysis mass spectra of complete bacteria.

The identification of *Mycobacterium* strains by pyrolysis-low voltage EI mass spectrometry is already several years subject of investigation in our group [9, 11–13]. The main purpose is the identification of so-called Tuberculosis complex *Mycobacterium* strains. The species belonging to this group, viz. *M. bovis*, *M. bovis BCG*, and *M. tuberculosis* can cause severe diseases (tuberculosis) in man and animal. A fast and fully automated system for recognition of such dangerous strains within series of clinical *Mycobacterium* isolates was developed by Meuzelaar *et al.* [9] and Wieten *et al.* [11–13] in our group. A discrimination between pyrolysis mass spectra of Tuberculosis complex strains and other, less pathogenic strains has been made by computer, using multivariate spectrum analysis. The procedure is based on the occurrence of significant differences in the relative intensities of a small set of characteristic "key" masses, one of them being $m/z = 59$. In order to trace back the biochemical origin of such inter-species differences the chemical structures contributing to such a key mass have to be determined.

$M/z = 59$ in the pyrolysis mass spectra of bacteria can have several origins. Two probable fragment molecules with mass 59, liberated by thermal fragmentation of such complex biological matter are acetamide and trimethylamine. Acetamide can be derived mainly from N-acetylaminosugar moieties like N-acetylhexosamines [14] and N-acetylneuraminic acids [15] present as building blocks in polysaccharides and glycoconjugates, and, to a lesser extent also from proteins [14, 16]. Trimethylamine is a very characteristic pyrolysis product from choline and choline conjugates (*e. g.* phosphatidyl choline [17]). Another compound possibly contributing to $m/z = 59$ is 1-propylamine which could be formed

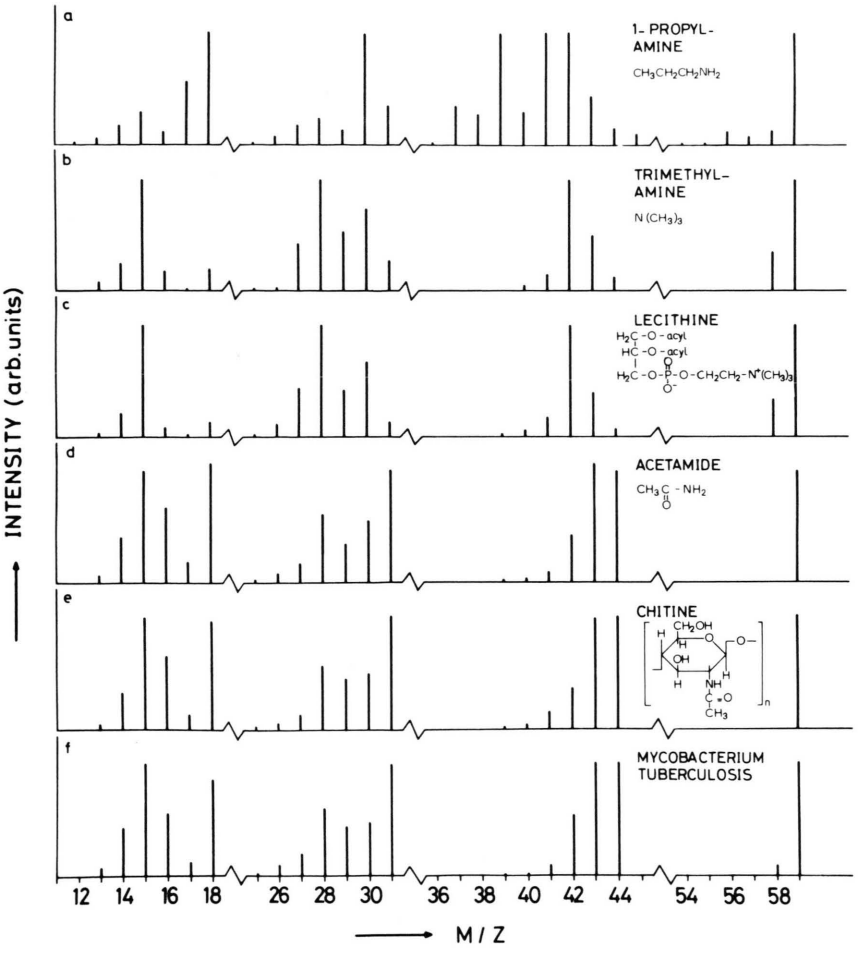

Fig. 2. CID spectra of the precursor ion at $m/z = 59$ in the pyrolysis mass spectrum of lyophilized cells of *Mycobacterium tuberculosis* as compared with reference CID spectra of the molecular ion ($m/z = 59$) of 1-propylamine, trimethylamine, and acetamide, and of the 59^+ ions in the pyrolysis mass spectra of lecithine and chitine. Instrumental conditions: energy of the ionising electrons 70 eV, energy of the precursor ions, 4 keV; collision gas, Ne; pressure inside the collision cell, $\sim 10^{-1}$ torr; post-acceleration, 20 keV; pyrolysis temp. 320 °C, total pyrolysis time, 2 min. No correction was made for spatial sensitivity variations of the detector. The reproducibility of the peak intensities within a mass group (see Fig. 3) therefore is typically better than 10%.

as a pyrolysis product from amino-compounds like proteins, (non-protein) amino acids or polyamines.

In Fig. 2 a, b and d the collision induced dissociation spectra are given of the $M^{+\cdot}$-ions of 1-propylamine, trimethylamine and acetamide, respectively. The spectra of the isomeric amines show strong differences. In the spectrum of 1-propylamine intense peaks at $m/z = 43$, 41 and 39 probably represent the alkyl fragments $C_3H_7^+$, $C_3H_5^+$ and $C_3H_3^+$. The intense peak at $m/z = 42$ can be assigned to the fragment $H_3C{-}CH{=}CH_2^{+\cdot}$ and the peak at $m/z = 30$ may represent the fragment $H_2C{=}NH_2^+$. The formation of NH_4^+ ($m/z = 18$) from this primary amine is evident.

The spectrum of the tertiary amine also shows intense peaks at $m/z = 43$ and 42 which in this case may represent the fragments $[H_3C{-}N{=}CH_2]^{+\cdot}$ and $[H_2C{=}N{=}CH_2]^+$, respectively. For $m/z = 30$, the same explanation can be given as mentioned for 1-propylamine; the peak at $m/z = 28$ may be CH_2N^+. Both amines show the production of M-H fragments ($m/z = 58$) in significant amounts.

The CID spectrum of the 59^+-ion in the pyrolysis mass spectrum of the reference compound lecithin

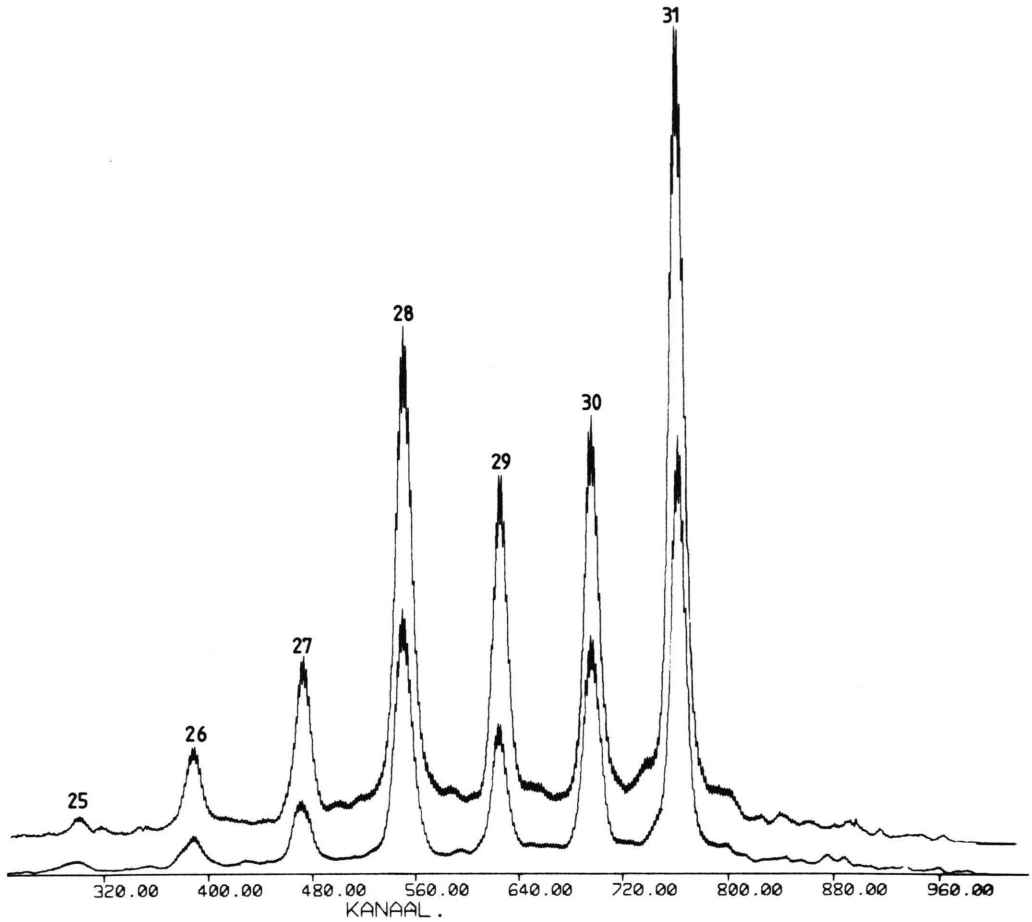

Fig. 3. Comparison of the $m/z = 25-31$ region of the CID spectra of mass 59 of the acetamide (below) and of the pyrolysate of *Mycobacterium tuberculosis* cells (above). Instrumental conditions: see Fig. 2. The total ion current leaving the collision cell was about 4×10^{-14} Amps., the measuring time about 3.6 s.

(phosphatidyl choline, Fig. 2 c) shows a very close correspondence with the spectrum in Fig. 2 b, indicating that the fragment with mass 59, formed on pyrolysis of this phospholipid is exclusively trimethylamine.

The CID spectrum of the $M^{+\cdot}$-ion of acetamide (Fig. 2 d) differs strongly from the spectra obtained for both amines *e. g.* in that it shows no M-H formation, whereas high intensities are found at $m/z = 44$, 43 and 31 (probably representing the fragments $O{=}C{=}NH_2^+$, $H_3C{-}C{\equiv}O^+$ and $H_3C{-}NH_2^{+\cdot}$, respectively). Again the peak at $m/z = 18$ has to be attributed to NH_4^+. The high intensities at $m/z = 31$, 30 and 29 as compared with 70 eV EI spectra of acetamide are remarkable.

An identical CID spectrum was obtained from the 59^+-ion in the pyrolysis mass spectrum of the macromolecular reference compound chitine (Fig. 2 e), a linear polymer of $\beta(1 \rightarrow 4)$ linked N-acetylglucosamine residues, so that the formation of acetamide on pyrolysis of this polymer is evident.

Fig. 2 f shows the CID mass spectrum obtained from the 59^+-ion in the pyrolysis mass spectrum of complete, lyophylized cells of *Mycobacterium tuberculosis.* Comparison of this spectrum with those of the reference substances (Fig. 2 a, b and d) clearly indicates that this characteristic pyrolysis fragment is almost exclusively acetamide. The small peak at $m/z = 58$ (see Fig. 2) may point to a very small contribution of some alkylamine with mass 59. In Fig. 3

the $m/z = 25-31$ part of the CID spectrum of acetamide as produced actually by the instrument is compared with the corresponding mass range in the CID spectrum of 59^+ in the pyrolysate of the *Mycobacterium* strain.

This finding implicates that in the identification by pyrolysis mass spectrometry of *Mycobacterium* strains of the Tuberculosis complex the relative amount of acetamide, which should be considered as derived mainly from cell wall N-acetylaminosugar components, is an important parameter.

In this study the possibility of structure elucidation of pyrolysis fragments of complex bioorganic matter by CID mass spectrometry is demonstrated. The CID spectra of isobaric as well as of isomeric ions show very significant differences. Our instrument, equipped with post-acceleration and simultaneous ion detection offers the possibility of very sensitive measuring CID spectra of ions produced on very short lasting processes.

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