Negative Field Desorption Mass Spectrometry of Nucleotides

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Summary: Negative ion field desorption mass spectrometry (NFD MS) has been applied to some nucleotides. Polyethylene glycol (PEG 4000) was used as a matrix to facilitate the ion formation at low field strengths and temperatures. Molecular ions of mononucleotides could be obtained with no or a low level of fragmentation. In contrast, NFD mass spectra of dinucleotides and a mononucleotide triphosphate did not exhibit molecular ions but only fragment ions.

Introduction

Several mass spectrometric methods have been developed for the ionization of thermally labile and non-volatile biological compounds. Among these are electrospray (ES) [1], thermospray (TS) [2], fast atom bombardment (FAB) [3] and field desorption (FD) [4] mass spectrometry. In field desorption mass spectrometry (FD MS) the sample is deposited on a so-called emitter, a thin wire typically covered with carbon microneedles, and exposed to a high external field. Heating of the wire leads to the emission of mass spectral ions. The negative ion mode of field desorption mass spectrometry (NFD MS) has been developed as a matrix method [5,6]. The matrix facilitates the ion emission at field strengths below the onset of a destructive electron emission. A further effect of the matrix is a lowering of the emitter temperature at which ion emission occurs i.e. a reduction of the thermal stress to sample molecules [7].

Nucleotides are the building units of the nucleic acids. Removing the phosphate group of a nucleotide, for example, by hydrolysis of the C-O

bond, a nucleoside remains. Thus nucleotides are phosphates of the corresponding nucleosides. Each nucleoside is composed of a sugar (pentose) and a base (purine or pyrimidine).

NFD mass spectra of the nucleosides thymidine and adenosine have already been reported [8]. It has also been shown that small amounts of LiCl are in favour of aboundant $(M-H)^-$ ion while larger amounts give $(M+Cl)^-$ molecular ions [8]. Nucleotides have been extensively studied by FAB MS [9] and FD MS [10,11]. Mass spectra of mono- and dinucleotides have been obtained by FD MS. The first NFD mass spectra of nucleotides are reported here.

Experimental

Mass Spectrometer

The experiments were performed with a 60° single focusing magnetic mass spectrometer (modified Atlas CH_3) equipped with a self-constructed FI/FD ion source. The vacuum in the ion source was about 10^{-4} hPa. For 4 keV ions the mass range of the spectrometer was about m/z 1200. The ion current was recorded by a channeltron secondary electron multiplier. Mass spectra were obtained by a magnetic field scan. The mass resolution m/Δm for FD and NFD spectra was less than 500.

FD ion source

The FD ion source allowing the positioning of the emitter by micromanipulators and a replacement of the emitter by a push rod and vacuum lock system was used. The emitter cathode potential was typically -4 kV while the counter electrode was at ground potential. The emitter wire/counter electrode distance was about 7 mm. This larger distance was chosen to avoid field electron emission at -4 kV. During the measurements the emitter wire was heated resistively by a DC power supply.

Emitters

In our experiments bare 10 μm W wires were used as field cathodes. They were spotwelded on an emitter holder with a free space of about 4 mm between the two supporing stainless steel pins.

Sample preparation

In order to facilitate the desolvation of ions at low extraction field strenghts and to prevent field electron emission polyethylene glycol of a mean molecular weight of 4000 amu (PEG 4000) was added to the sample solution. A small amount of this mixture was loaded onto the emitter wire either by the dipping or by the syringe technique [4].

Results

Mononucleotide-monophosphates

NFD mass spectra of three nucleotides are shown in Fig. 1-3. The molecular ion of the cylic nucleotide (Fig. 1) is rather easily obtained because it is emitted in rather broad temperature range i.e. between 13 and 27 mA emitter heating current. A similar behaviour was found for the nucleotide in Fig. 2. However, with this compound fragment ions are desorbed above mA. In contrast to this behaviour the guanosine-5' monophosphate disodium salt yielded only molecular ions in a very narrow temperature range i.e between 12 and 13 mA, and for short time of a few minutes. The fragment ions are probably due to thermal reactions on the heated emitter surface.

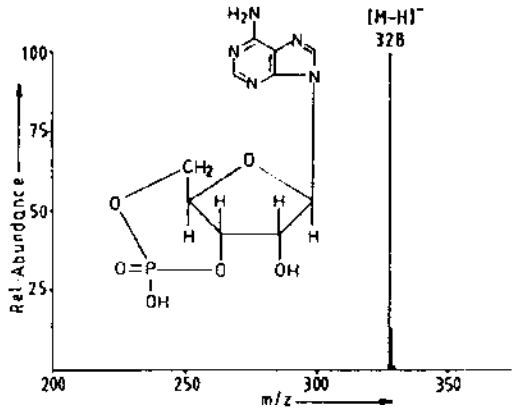

Fig.1: NFD mass spectrum of adenosine-3',5'-cyclic-monophosphoric acid (mol.wt. = 329) mixed with water and PFG 4000 (1:2:2). Emitter heating current range 13-27 mA.

Dinucleotides and mononucleotide triphosphates

Dinucleotides and mononucleotide triphosphates gave no molecular ions in NFD MS but only

fragment ions. The NFD spectrum of the latter is shown in Fig. 4. The ion emission range was between 11- 22 mA. Both fragment ions of Fig. 4 were rather intense and lasted for a long time (10 min). Mononucleotide disphosphate were not examined.

Discussion

Mononucleotide monophosphoric acids desorb in a wide range of temperature. They are not fragmented at low temperatures. NFD mass

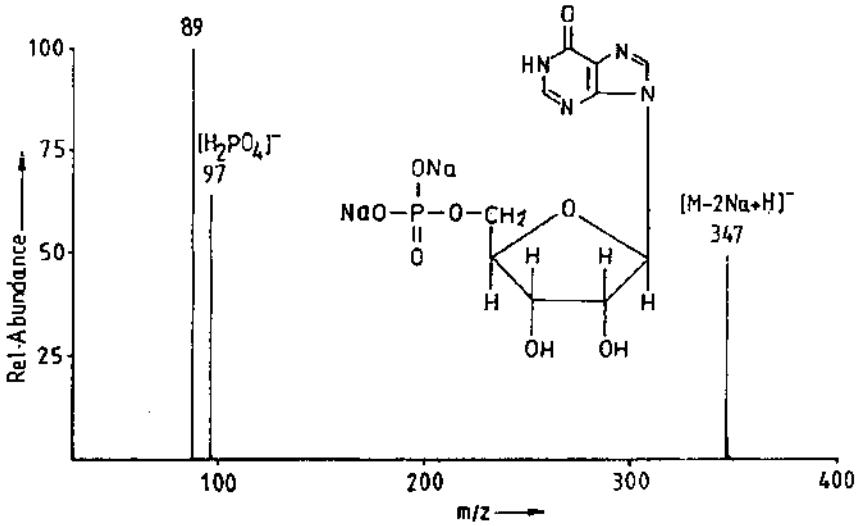

Fig.2: NFD mass spectrum of inosine-5'monophosphate disodium salt (mol.wt. = 392) mixed with water and PEG 4000 (1:2:2). Emitter heating current 20mA.

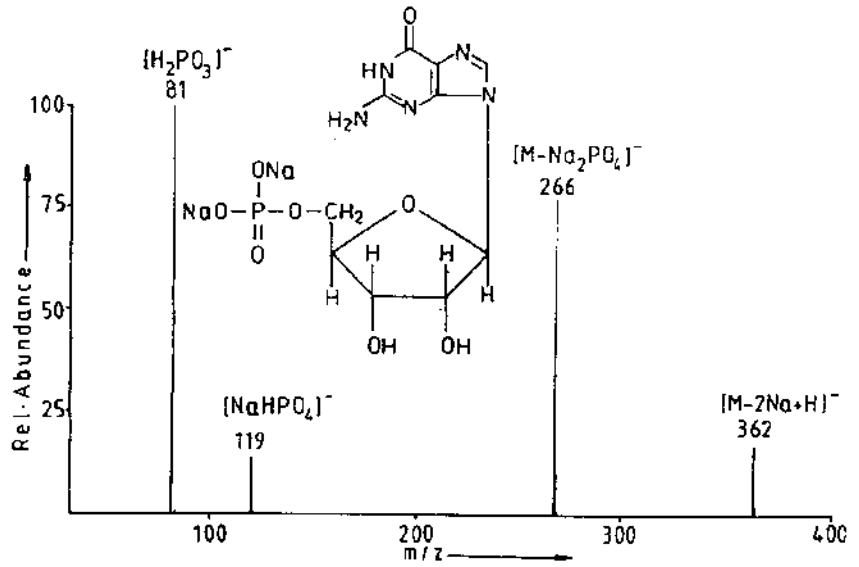

Fig. 3: NFD mass spectrum of guanosin-5'monophosphate disodium salt (mol.wt. = 407) mixed with water. Emitter heating current 12mA.

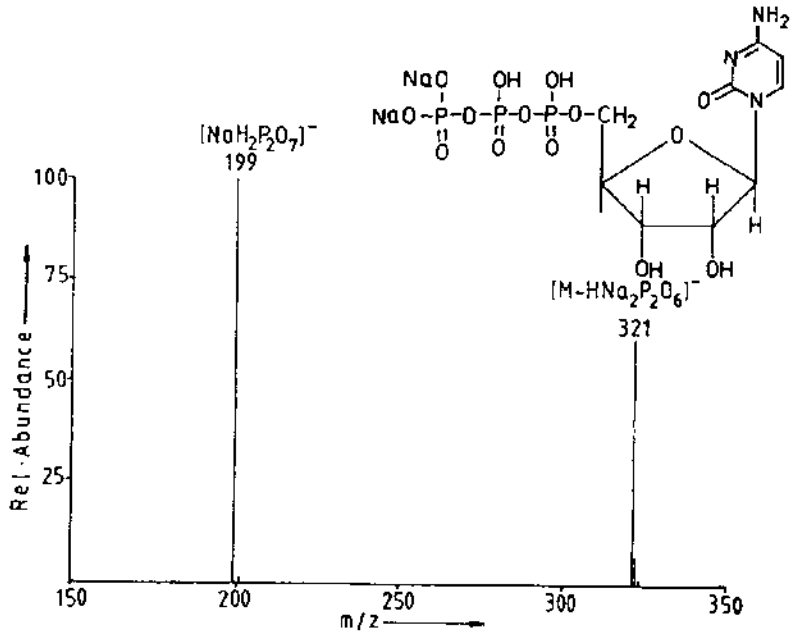

Fig.4: NFD mass spectrum of cytidine-5'triphosphate disodium salt (mol.wt. = 526) mixed with water and PEG (1:2:2) Emitter heating current 20mA.

spectra of their salts are more difficut to obtain because of the short desorption time in a narrow temperature range. PO-C cleavage leads to abundant fragment ions. In Fig. 2 the ion at m/z = 89 forms the base peak. It probably arises from a fragmentation of PEG at elevated temperatures and can be explained by the formation of ethoxyethanol in a degradation reaction which by proton abstraction is converted to the ion of m/z = 89 on the field desorption emitter. Since this peak is missing in other spectra the nucleotides might be involved in the degradation of PEG. In contrast to FD MS molecular ions of dinucleotides could not be obtained by NFD MS. Molecular ions of triphosphates have also not been obtained by FD MS yet. These limitations might be overcome in part by the development of new sample preparation techniques in the future.

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