Sequence specific fragments in the field desorption mass spectra of dinucleoside phosphates*

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ABSTRACT

In field desorption mass spectrometry of ribodinucleoside phosphates the formation of nucleoside cyclophosphates can be used to determine the base sequence.

INTRODUCTION

As recently reported¹⁾ the complete high resolution spectra of unprotected dinucleoside phosphates could be obtained by field desorption mass spectrometry²⁾. In all cases these spectra contained the protonated molecular ions and - depending on the conditions of desorption applied - structural information about the building blocks of the dinucleoside phosphates, i.e. their nucleobases, nucleosides, and mononucleotides. In addition the corresponding cyclophosphates were formed by a thermally/field induced process (Figure 1).

By comparison of the spectra of CpA and GpU it could be seen that the formation of cyclophosphate preferentially takes place via the nucleotide of the 5'-end (position 1). If this effect were also to be observed in the case of the inverse phosphates then a differentiation between isomeric ribodinucleoside phosphates should be possible by mass spectrometry.

At first the experimental proof of this assumption could not be obtained since the investigated ApC contained salt impurities. Thus a rather complete cationization³⁾ of the molecule was the result and consequently the necessary formation of fragments was suppressed.

Here we would like to report on the investigation of the formation of nucleoside cyclophosphates for the case of five inverse pairs of free ribodinucleoside phosphate acids.

DISCUSSION

As shown in Table 1 the formation of cyclophosphates is independent of the

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Figure 1. Field desorption mass spectrum of CpA. Emission-controlled FD-MS. Photographic detection, exposure time 8 min.

polarity of the investigated dinucleoside phosphates and only slightly dependent on the condition used (see Experimental). In all cases the formation of cyclophosphate preferentially takes place by the nucleotide in position 1 (5'-end).

In the first instance this behaviour could be explained purely statistically, for cleavage of the 5'-ester bond the nucleotide in position 1 can react to the 5'-OH under formation of a six membered ring as well as to the 2'-OH under formation of a five membered ring. The nucleotide in position 2 (3'-end), after cleavage of the 3'-ester bond, has only the possibility of a ring closure to the 3'-OH group (Scheme 1).

More probable and in agreement with chemical results⁴⁾ is that P-O cleavage of the dinucleoside phosphate and cyclisation preferentially takes place by neighboring group participation of the vicinal 2'-OH group of the nucleoside in position 1. The cyclisation to the 5'-OH group is much less favoured. Only the nucleoside in position 1 can react to the favoured 2',3'-cyclophos-

	ApC	CpA	ApG	GpA	ApU	UpA	CpU	UpC	GpU	UpG
base 1	++++	++++	+++	++++	+++++	++++	+++++	++	+++++	++++
base 2	+++++	++++	+++++	+++++	+	+++++	++	+++++	+++	++
nucleoside 1	+	+++		+	+++	+	++	+		
nucleoside 2	+++	++++		+	+++++	+++++	+++++	+++	++++	+
cyclophosphate 1	+	+++++	++	+	+++++	++++	+++++	+++	++++	++
cyclophosphate 2		++			+++	++	++	+	+	
nucleotide 1		+			+	+	+	+		++
nucleotide 2		++			++++	+	+	+	+++	

Table 1.

The major types of ions observed in the FD mass spectra of ribodinucleoside phosphates (mass range m/e 100 to m/e 400). The relative intensities of the signals are given in five degrees of blackening of the photoplate: Very high rel. int. = +++++, high rel. int. = +++++, medium rel. int. = +++, low rel. int. = ++, and weak rel. int. = +

Scheme 1. Sequence-specific formation of nucleoside cyclophosphates in FD-MS

phate. Consistently a signal for the nucleoside 2 can be observed which is more intense than the peak due to the nucleoside 1 in all cases observed.

As Biemann could show⁵⁾ the formation of sequence specific ions by electron impact fragmentation of pertrimethylsilyl ribodinucleoside phosphate results from the preferred rupture of the 5'-ester bond. Probably this is not the determining process for the field induced fragmentation and formation of the cyclophosphates under FD conditions. As can be seen from the spectra of those ribodinucleoside phosphates CpA, ApU, and GpU which show spectra with fragments of high intensity a predominant formation of cyclophosphate is accompanied by a relative intensive signal for the formation of the mononucleotide in position 2. While the primarily formed mononucleotide of the 5'-end reacts in a second step to the 2',3'-cyclophosphate, the nucleotide of the 3'-end gives rise to only low yields of the corresponding 3',5'-cyclophosphate (Table 1).

Thus the first step to accomplish sequence information of unprotected oligonucleotides by mass spectrometry has been made and future applications of field desorption mass spectrometry to large subunits of nucleic acids are one of the most promising areas of this technique.

EXPERIMENTAL

The high resolution field desorption mass spectra were recorded on a modified CEC 21-110 B double focusing spectrometer using vacuum evaporated AgBr plates (Ionomet) for photographic detection. Field anodes employed were 10 μ m tungsten wires activated at high temperature⁶⁾. The length of the microneedles was about 25-30 μ m on average, their morphology and distribution similar to these shown in reference 6). The field desorption emitters could only be used for one field desorption measurement of the dinucleoside phosphates. The ionization efficiency was sufficient for obtaining the reference peaks in the field ionization mode but did not allow repeated field desorption measurements. The accelerating voltages were varied between + 6 kV and + 10 kV for the field anode and - 2 kV and - 6 kV for the slotted cathode plate. The observed total ion currents measured at the cathode plate were between 5×10^{-9} A and 2×10^{-7} A. The solvents were water, methanol and DMSO. The samples were admitted by use of the syringe technique²⁾. In all cases the substances were desorbed in an emission controlled time-/temperature program¹⁾. The time required for one FD analysis including sample transfer onto the FD emitter, desorption of the sample and exposure of the reference compound was one hour on average. The sample amount consumed in one measurement was about 1 μ g. The resolution achieved was ~ 10000 (10% valley definition). The error in the accurate mass measurements was about 10 ppm for peaks of medium and low intensity (+++, ++).

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