Mass spectral studies of probe pyrolysis products of intact oligoribonucleotides

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ABSTRACT

Pyrolysis of underivatized homogeneous oligoribonucleotides in the source of a mass spectrometer leads to production of simple mass spectra which resemble the spectra of the bases themselves. It is proposed that pyrolysis occurs by hydrogen transfer to the base moiety followed by elimination of the neutral base. Ionization by electron impact gives rise to the observed spectra. Mixed oligoribonucleotides pyrolyze readily to give spectra of adenine and uracil, but evidence for the presence of guanine and cytosine in mixed polymers and native RNA's is difficult to obtain presumably because of their low vapor pressure. The method may be useful for detection of modified bases in tRNA and for studies of temperature effects on RNA pyrolysis.

INTRODUCTION

The capabilities of mass spectrometry in biochemistry are now well established. Recommendations for the technique include high sensitivity (in the picogram range), detailed structural information content, and rapidity of analysis. A major problem is often encountered because of the requirement to vaporize the sample prior to ionization. Nucleic acids are particularly challenging because of their low vapor pressure. Direct sample introduction of the intact molecule into the mass spectrometer source can be accomplished successfully only for the bases and certain nucleosides. By employing a variety of micro derivatization procedures, spectra can be obtained readily for the bases, nucleosides, and mono and dinucleotides.

Because it is advantageous to forego the derivatization step to avoid sample loss and the large masses encountered for the modified molecule, underivatized oligodeoxyribonucleotides have been submitted to mass spectral analysis. Using this approach, sample introduction is accomplished by pyrolysis on the direct introduction probe to form more volatile subunits which are then ionized and mass analyzed. The strategy has been effective in sequencing and detecting minor nucleotides in DNA.
Another approach makes use of pyrolysis/high resolution field desorption mass spectrometry or of Curie point pyrolysis followed by mass spectral analysis of the pyrolysate. Whereas the latter approach only yields information on the sugar moiety, thus permitting DNA and RNA to be distinguished, the former method looks more promising because all the bases in a native DNA were detected.

There are few reports of the direct mass spectrometric analysis of oligoribonucleotides. Wiebers and Shapiro point out that oligoribonucleotides are nearly inert under the conditions they employed for the direct analysis of deoxyribonucleotides. Nevertheless, we have begun a systematic investigation of RNA's by high resolution mass spectrometry using the direct inlet pyrolysis technique and electron impact ionization. This is a preliminary report of studies which demonstrate the potential of the method for rapidly determining the occurrence of the various bases. Furthermore, the rapid acquisition of mass spectra as a function of temperature results in a "thermogram" of the RNA. Temperature effects on the pyrolysis may yield useful information concerning the tertiary structure of the RNA.

EXPERIMENTAL

Mass spectra were obtained on an AEI MS-5076 ultra high resolution, double focussing mass spectrometer equipped with an INCOS Model 2000 data system. Samples were admitted to the mass spectrometer on the direct probe inlet and pyrolyzed by slow heating from ambient up to as high as 300°C in certain cases. The source temperature was 200°C and the ionizing energy was 70eV. Low resolution spectra were repeatably obtained in 3 sec/decade of mass scan, and the spectra reported in this paper are the average of at least 20 scans over the pyrolysis period. High resolution scans were taken at R=10,000 using a 10 sec/decade scan with PFK as an internal mass calibrant.

The various RNA samples were obtained as commercial samples from Miles Laboratories, Inc., and in general, were used without any pretreatment. To insure that monomeric species initially present in the polymer were not contributing to the observed spectra, poly (A) was precipitated from ethanol/water ten times. The mass spectra of the pyrolysis products of this sample were identical to untreated material.

RESULTS AND DISCUSSION

Homogeneous Polymers. The mass spectra obtained for direct probe pyrolysis of the four homogeneous RNA polymers are shown in Figure 1. As discussed in the Experimental section, each spectrum is an integrated view of the
pyrolysis obtained by summing and renormalizing at least 20 spectra acquired between 150 and 275°C. These integrated spectra are nearly identical with the previously published spectra of the bases themselves.\(^1,2\) This suggests that pyrolysis promotes a hydrogen transfer from a sugar moiety to the base followed by elimination of the neutral base. Vaporization and electron impact ionization produce the observed spectra.

Further support for this hypothesis comes from exact mass measurements, at a resolving power of 10,000, which were obtained in a separate experiment. The elemental compositions corresponding to the exact masses of all ions were assigned; selected assignments are given in Table I.

For poly(U), the onset for ion current is approximately 130°C although sufficient ion intensity is not obtained until 150°C. The base peak at nominal mass 112 is the molecular ion, \([C_4H_4N_3O_2]^+\) which gives the expected losses of HNCO and H_2NCO to form the ions \(m/e\) 69 and 68 respectively.

Likewise, poly(A) gives a very simple spectrum. The molecular ion, \(m/e\) 135--\([C_5H_5N_3]^+\), undergoes fragmentation by loss of HCN, \(C_2H_2N\), \(C_2H_3N_2\) and \(C_2H_3N_3\) to yield the ions at \(m/e\) 108, 95, 81, and 66 respectively. The temperature characteristics for pyrolysis of poly(A) are almost identical to poly(U).

The integrated spectrum for poly(C) is somewhat more complex. Although the onset for ion production is approximately 130°C, a relatively higher temperature is required before a significant ion current can be observed. In addition to the molecular ion at \(m/e\) 111, \([C_3H_3N_3O]^+\), intense ions at higher mass are observed. The peak \(m/e\) 112 is a doublet consisting of the C-13 isotopic species of \(m/e\) 111 and \([C_3H_3N_3O]^+\). The latter signal may arise because of contamination by uracil and this is supported by the high resolution scans at \(m/e\) 68 and 69 which reveal the presence of \(C_2H_2N^+\) and \([C_3H_3NO]^+\) respectively. It is tempting to assign the peak at \(m/e\) 135 to adenine; however, the exact mass measurement is in accord with the composition \([C_6H_5N_3O]^+\). This ion may correspond to cytosine attached to an acetylenic moiety and could form by dehydration of the ribose followed by elimination of a two carbon fragment attached to the base. Elimination of a three-carbon fragment plus base may account for the ions at \(m/e\) 148 and 149. These complicating features in the spectrum of cytidine are not found in adenosine and uridine. Presumably, the slightly higher temperature required for poly(C) leads to more extensive pyrolysis than is observed for poly(A) and poly(U).

The pyrolysis of poly(G) occurs under the most stringent conditions of temperature. Very little ion current is found until a temperature of 250°C is
Figure 1. Pyrolysis Mass Spectra of four homogeneous RNA Polymers. Ionizing Energy = 70eV. See text for experimental Conditions.
reached, and this is in accord with the low vapor pressure of guanosine. The integrated spectrum is dominated by the molecular ion of guanine at m/e 151, \([\text{C}_5\text{H}_5\text{N}_5\text{O}]^+\), and its fragmentation products: loss of \(\text{NH}_2\) (m/e 135), \(\text{NH}_3\) (m/e 134), \(\text{C}_2\text{H}_2\text{N}_2\) (m/e 110), \(\text{CH}_2\text{N}_2\) (m/e 109), and \(\text{CHNO}\) (m/e 108). However, a significant fraction of the ion current is carried by other low mass ions as well as two higher mass peaks. Similar to poly(C), the signals at m/e 175 and 187 may correspond to guanine attached to two and three carbon fragments from the ribose moiety.

Mixed Polymers. To test the generality of the method, two synthetic mixed RNA's were investigated: poly(A,U,U) and poly(A,G,U). Their spectra are presented in Figure 2. The integrated view of the pyrolysis of poly

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<th>Elemental Composition</th>
<th>Rel. Error 1</th>
<th>m/e</th>
<th>Elemental Composition</th>
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1) Expressed as parts-per-million.
(A,U,U) shows high intensity peaks for uracil (m/e 112, 69, 68) and adenine (m/e 135, 108, 95, 81). The ratio of the relative abundances of the molecular ions of adenine and uracil is approximately 2:1. Clearly, mass spectrometry is an excellent technique not only for identification, but also for a semi-quantitative estimate of A,U-base composition in these biopolymers.

The "thermogram" for pyrolysis of poly(A,U,U) was obtained by plotting the intensities of m/e 135 and 108 for adenine and m/e 112 and 69 (uracil) as a function of scan number (temperature). We find adenine appearing at approximately 150° and uracil at approximately 175°C.

For poly(A,G,U), adenine and uracil are present in equal amounts, and this is reflected in the nearly 1:1 ratio for m/e 135 and 112 in the integrated spectrum (see Figure 2). However, the ions characteristic of guanine are of very low abundance even at the highest temperatures (approximately 300°C). These results indicate the inherent difficulty in freeing guanine in the pyrolysis or the high temperature required for vaporization of guanine. The fact that the situation is not improved at high temperature (the spectrum is still dominated by adenine and uracil) suggests that guanine must remain in the nonvolatile pyrolysis residues. As in poly(A,U,U), the appearance of uracil is first observed at approximately 175°, but adenine also appears at 175° rather than at 150° as was observed for poly(A,U,U). Furthermore, maximum intensity for adenine occurs at a higher temperature than for uracil, whereas for poly(A,U,U), the uracil intensity maximizes prior to adenine.

The third intact RNA studied in this series is a natural product, Qb-Viral RNA. Its integrated spectrum, shown also in Figure 2, was taken from 150 to 240°C. Although the presence of adenine and uracil is readily detected in a ratio of approximately 1:1, very little cytosine can be seen (m/e 111) and there is no evidence for guanine at these temperatures. Further temperature increments produce a more complicated spectrum with large intensity peaks at m/e 112, 135, 149 and 167. Unfortunately, the presence of cytosine and guanine is still not revealed: the relative intensities of m/e 111 and 151 are less than 10% and 1% respectively.

CONCLUSIONS
The identity of homogeneous RNA's [poly (A), (U), (G) and (C)] can be established readily by mass spectrometry. For each of these polymers, the mass spectra integrated over a temperature range of 130 to 275°C resemble closely the mass spectra of the bases themselves. These observations suggest a clean pyrolysis which occurs by hydrogen transfer to the base followed by
Figure 2. Pyrolysis Mass Spectra of poly (A, U, U), poly (A, G, U), and Qβ-Viral RNA. See text for experimental conditions.

elimination and vaporization of the base. Unlike direct probe pyrolysis of intact DNA's which yield complicated spectra containing not only the base but various higher molecular weight fragments, the spectra of the RNA's are simple. Of course, this means that the pyrolysis products contain no sequence information. The direct probe pyrolysis has a definite advantage over Curie point pyrolysis because it is indicative of the bases present, particularly adenine and uracil.

The limitation of the direct probe method, however, is revealed in the studies of various mixed RNA's. Here the presence of adenine and uracil is readily documented allowing an assessment of their relative composition. Unfortunately, cytosine and especially guanine are difficult to detect, which may be ascribed to a slow rate for the pyrolysis-induced base elimination or to the lower vapor pressure of these bases. Improved methods of sample handling may resolve the problems, and additional studies are contemplated to overcome the limitations discussed above. Possible approaches include the use of inert supports for the sample and derivatization of the sample prior to mass spectral analysis.
Another problem encountered in this study is the unexpected observation of m/e 135 in the pyrolysis of poly(C). Without the capability of making exact mass measurements, this signal could be interpreted mistakenly to indicate the presence of adenine. Accordingly, high resolution mass spectrometry is recommended, at least for the initial studies.

The incentive for further study is provided by two potential applications of the method. First, a high proportion of modified bases occurs for transfer RNA. One strategy for structure elucidation of these bases is to hydrolyze the tRNA, separate and derivatize the nucleosides, and then obtain the mass spectra. An excellent review of this methodology has recently been published by McCloskey and Nishimura. Direct pyrolysis may prove to be more expeditious. Secondly, we have noted interesting "thermograms" produced by rapid acquisition of mass spectra during pyrolysis. Although we are uncertain of their meaning at this time, the exciting potential for assessing tertiary structure still exists. Further research is planned to test these ideas.

ACKNOWLEDGEMENT

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REFERENCES