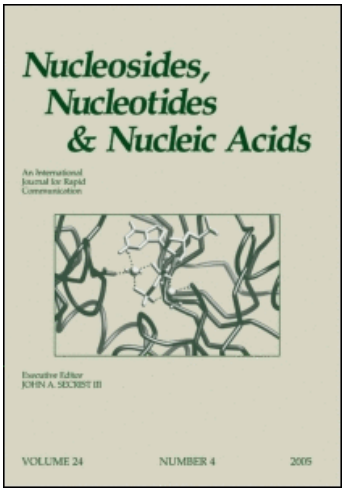

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Mass Spectrometry: Applications in the Analysis of Nucleosides, Nucleotides and Oligonucleotides

Karl H. Schram[a]
[a] College of Pharmacy, University of Arizona, Tucson, AZ

To cite this Article Schram, Karl H.(1990) 'Mass Spectrometry: Applications in the Analysis of Nucleosides, Nucleotides and Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 9: 3, 311 — 317
To link to this Article: DOI: 10.1080/07328319008045142
URL: http://dx.doi.org/10.1080/07328319008045142

NUCLEOSIDES & NUCLEOTIDES, 9(3), 311-317 (1990)

MASS SPECTROMETRY: APPLICATIONS IN THE ANALYSIS OF NUCLEOSIDES, NUCLEOTIDES AND OLIGONUCLEOTIDES

Karl H. Schram, College of Pharmacy, University of Arizona, Tucson, AZ 85721

INTRODUCTION

The past ten years have been an exciting time in mass spectrometry as a number of important instrumental developments have revolutionized the field, including the analysis of nucleic acid components.[1,2] The focus of this talk will be on the impact that new ionization methods, e.g., plasma desorption(PD) and fast atom bombardment(FAB), and new magnet technology (expanded mass range and scan speed capability) have had on the analysis of nucleosides and nucleotides. Results from the speaker's laboratory will be used to illustrate the significance of capillary GC/MS techniques for the separation and analysis of complex mixtures of nucleosides derived from a biological source. In addition, some approaches being developed to overcome current limitations in the FAB analysis of nucleosides and nucleotides will be described. Unfortunately, time does not permit a discussion of other new areas of interest, i.e., LC/MS[3] and MS/MS.[4]

APPLICATIONS

GC/MS Analysis of Urinary Nucleosides

Interest in the identification and quantitation of modified nucleosides in urine is based on the potential of these compounds to serve as biological markers of cancer.[5] The major objectives of this project are 1) to confirm structural assignments made on the basis of HPLC retention times and UV data and 2) to identify nucleosides of unknown structure.

The first example involves the identification of an unknown nucleoside present in substantial amounts in HPLC fraction 1 from pooled normal urine (PNU1) thought to contain only 1-methyladenosine (m^1A). Identification of this component[6] as 5,6-dihydrouridine (D) was established by comparison of the mass spectrum and retention time with a known reference sample. This is a straight-forward illustration of the application of GC/MS to the identification of an unknown nucleoside since a sample of the reference material was readily available.

A second nucleoside of considerable interest has also been identified in the urine of a lung cancer patient. Based on the presence of sugar fragments[7] which are identical in m/z value to sugar-related ions in the spectrum of 5'-methylthioadenosine (MTA) and a tentative identification of the aglycone as guanine, the unknown is tentatively assigned the structure 5'-methylthioguanosine (MTG). If this assignment is correct, the presence of MTG in the urine of a lung cancer patient is of considerable

importance because 1) MTG has been identified only in the urine of the lung cancer patient and MTG may, thus, be a specific biological marker of lung cancer, 2) MTG has not been reported as a natural or synthetic compound and 3) since MTA plays an important role in cell growth regulation and differentiation,[8] MTG may also have a significant role in cellular processes. Synthesis of a reference sample and studies of the biochemical role of MTG are in progress.

These two examples illustrate the importance of rapid scan speeds which permit the coupling of capillary GC columns to a mass spectrometer for the separation and identification of complex nucleosides mixtures isolated from a biological matrix. However, a number of hypermodified nucleosides and most nucleotides are not amenable to gas-phase analysis. The introduction of FAB[9] has thus been of fundamental importance in the analysis of thermally labile and/or highly polar nucleic acid components.

Fast Atom Bombardment Mass Spectrometry of Nucleosides and Nucleotides.

A comparison of the FAB and EI mass spectra of a nucleoside reveal some significant differences. Firstly, the FAB mass spectrum is relatively simple. The most important feature of the FAB mass spectrum of a nucleoside[4,10] is the presence of an unambiguous MH^+ ion, thereby permitting molecular weight assignments of nucleosides that cannot be analyzed by EI or CI. Molecular weight information, along with the appearance of a strong BH_2^+ ion, allows determination of the mass of the sugar

residue. Secondly, because of the simplicity of the FAB spectrum, a net loss of structural information is apparent relative to the EI data.

Three problems identified with the FAB mass spectrometry of free nucleosides include: 1) a loss in structural information relative to the EI spectrum,[11] 2) the presence of artifact and matrix-related ions and 3) low sensitivity. Solutions to these problems have been investigated in our laboratory.

A significant increase in the number of structurally relevant fragment ions is observed when the FAB analysis is performed on the TMS derivative of the nucleoside.[12] While the extent of fragmentation is considerably enhanced (essentially all of the base- and sugar-related ion series[7] are observed), the molecular weight of the sample remains easily recognized by the production of an abundant MH^+ ion. Additional advantages, over conventional GC/MS analyses include: 1) the spectrum is obtained at room temperature, thereby permitting the analysis of thermally labile samples, and 2) the TMS functionality permits the same matrix to be used in the analysis of all nucleoside (and nucleotide) samples.

The problem of artifact ions in a FAB mass spectrum may be circumvented in a number of ways. Most commonly, a second matrix is used to shift the m/z values of the "background" peaks to new values. Sample-related ions will thus be "unmasked" and will become obvious because of their stationary nature relative to the matrix ions.

A second approach involves the use of mass spectrometry/mass spectrometry (MS/MS) techniques.[4] Selection of a sample-related ion using MS1 with subsequent generation of a mass spectrum in MS2 effectively purifies the sample ions and eliminates all non-sample-related ions. In addition, MS/MS combined with collisional activation (CA) is a very effective means of increasing fragmentation. Reports of the use of MS/MS CA for the analysis of nucleosides[4], nucleotides and small oligonucleotides[13] have been published recently. The major disadvantage of the MS/MS technique is the cost of the instrumentation and the level of technical expertise needed to operate such an instrument.

An alternative approach to the problem of artifact ions involves the use of a heatable FAB probe.[14] By melting the sample on the tip of the FAB probe, this technique allows the FAB sample to function as its own matrix. The "matrixless FAB" spectrum of adenosine displays a moderately intense MH^+ ion, permitting MW assignment, along with most of the structurally-important fragment ions observed in the EI spectrum. Most importantly, however, is that all ions in the FAB mass spectrum are sample-related and the presence of artifact and matrix ions is eliminated. The sensitivity and utility of this "matrixless FAB" technique are currently being investigated.

The approach we have developed[15] in our laboratory to overcome the lack of sensitivity of the FAB technique is based on the analysis of the TMS derivatives of nucleotides.

Two nucleotides of biomedical interest, tricyclic nucleotide 5'-monophosphate (TCNMP) and 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP), have been analyzed using FAB in the low nanogram range with detection in the negative ion mode.

The future of mass spectrometry in the analysis of nucleic acid components of all types, whether of synthetic or natural origin, is expected to be bright.

ACKNOWLEDGEMENTS

Support for portions of this research include funds from the National Cancer Institute (CA43068 and CA42309) and the Biotechnology Program (U of A) to KHS and a grant from Patriarch Takahito Miki and the Church of Perfect Liberty to KN.

Travel funds have in part been provided by a University of Arizona Foreign Travel Grant and the Symposium Organizing Committee.

Collaborators include: Urinary Nucleosides Project, Drs. Katsuyuki Nakano and Toshi Yasaka of the PL Comprehensive Research Institute in Osaka, Japan and Dr. Mark Reimer and Mr. Tom McClure of the University of Arizona; FAB Studies, Drs. Linda Wotring and Leroy Townsend and Ms. Katherine Borysko of the University of Michigan and Mr. Bill Hammargren, Ms. Qing-mei Weng and Ms. Debra Slowikowski of the University of Arizona. Slides were prepared by Lindsey Schram, Bill Hammargren and Tom McClure. I would like to dedicate this presentation to the memory of Debra Slowikowski.

REFERENCES

1. K.H. Schram, "Purines and Pyrimidines" in Clinical Biochemistry. Principles Methods Applications. Mass Spectrometry, A.M. Lawson, ed., Walter de Gruyter, Berlin, 1989, Chapter 10.

2. J.A. McCloskey, "Mass Spectrometry of Nucleic Acid Constituents and Related Compounds" in Mass Spectrometry in the Health and Life Sciences, A.L. Burlingame and N. Castagnoli, Jr., eds., Elsevier, Amsterdam, 1985, pp. 521-544.

3. C.C. Nelson and J.A. McCloskey, Adv. Mass Spectrom., **11**, 1296 (1989).

4. F.W. Crow, K.B. Tomer, M.L. Gross, J.A. McCloskey and D.E. Bergstrom, Anal. Biochem., **139**, 243 (1984).

5. F. Cimino, G.D. Birkmayer, J.V. Klavins, E. Pimentel and F. Salvatore, eds., Human Tumor Markers, Walter de Gruyter, Berlin, 1987.

6. M.L.J. Reimer, K.H. Schram, K. Nakano and T. Yasaka, Anal. Biochem., **181**, 302 (1989).