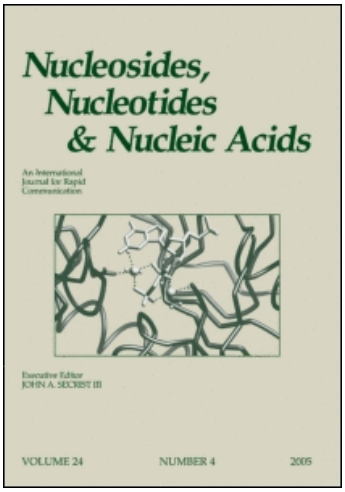

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Qualitative and Quantitative Analysis of Cyclic Nucleotides and Related Enzymes by Static and Dynamic Fast Atom Bombardment Mass Spectrometry

James I. Langridge[a]; A. Gareth Brenton[a]; Terry J. Walton[a]; Frank M. Harris[a]; Russell P. Newton[a]
[a] Biochemistry Group, School of Biological Sciences, and Mass Spectrometry Research Unit Department of Chemistry, University of Wales Swansea, Swansea, United Kingdom

To cite this Article Langridge, James I. , Brenton, A. Gareth , Walton, Terry J. , Harris, Frank M. and Newton, Russell P.(1995) 'Qualitative and Quantitative Analysis of Cyclic Nucleotides and Related Enzymes by Static and Dynamic Fast Atom Bombardment Mass Spectrometry', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 739 — 742
To link to this Article: DOI: 10.1080/15257779508012462
URL: http://dx.doi.org/10.1080/15257779508012462

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 739-742 (1995)

QUALITATIVE AND QUANTITATIVE ANALYSIS OF CYCLIC NUCLEOTIDES AND RELATED ENZYMES BY STATIC AND DYNAMIC FAST ATOM BOMBARDMENT MASS SPECTROMETRY

James I Langridge, A. Gareth Brenton, Terry J. Walton, Frank M. Harris and *Russell P. Newton.

Biochemistry Group, School of Biological Sciences, and Mass Spectrometry Research Unit, Department of Chemistry, University of Wales Swansea, Singleton Park, Swansea, SA2 8PP, United Kingdom

ABSTRACT Fast atom bombardment mass spectrometry in concert with collisionally-induced dissociation and mass-analysed kinetic energy spectrum scanning has enabled unequivocal identification of putative cyclic nucleotides in tissue extracts; quantitative analyses have enabled kinetic studies of cyclic nucleotide-related enzymes with multi-component monitoring, and dynamic spectrometric systems provide a potential means of continuous assay for these enzymes.

The advent of soft ionization methods has enabled the production of mass spectra containing significant molecular peaks from cyclic nucleotides without any need for derivatization, previously essential for mass spectrometric analyses of these compounds[1]. While such fast atom bombardment (FAB) mass spectra do not yield a great deal of structural information because of a low level of fragmentation, combination of FABMS with collisionally induced dissociation (CID) and mass-analysed ion kinetic energy spectrum (MIKES) scanning provides a form of tandem mass spectrometry yielding a high level of structural information, and has been successfully used to differentiate cyclic nucleotide isomers[2,3]; CID/MIKES analysis of the protonated molecule of 3',5'-cyclic nucleotides shows characteristic fragments at m/z values corresponding to protonated base, protonated base + 28 mass units and protonated base + 42 mass units, and unambiguous identification of putative cyclic nucleotides in tissue extracts has been possible by application of FABMS-CID/MIKES analysis. By this method several contentious issues have been resolved, including the demonstration of cyclic AMP and cyclic GMP in higher plants[4] and the natural occurrence of cyclic CMP[5]. Three other cyclic nucleotides have also been established as endogenous components of both mammalian and plant tissues, viz. cyclic-

UMP, -IMP and -dTMP[6,7], and in the absence of alternative methodology MS remains their only means of analysis. Cyclic CMP, -UMP and -IMP and their 2'-deoxy counterparts have now been identified as secreted products in the growth medium of *Corynebacterium diptherium*, elevated levels of 2'-deoxy cyclic AMP have been observed in frog brain, and a novel cyclic nucleotide, adenosine-2'-phospho-3',5'-cyclic diphosphate has been identified in the red seaweed *Porphyra umbilicalis*, in an extracted fraction which is the source of several natural herbal remedies.

FAB/MIKES analysis has also made a significant contribution to qualitative studies of cyclic nucleotide-related enzymes. For example while the enzymes responsible for cyclic AMP and cyclic GMP synthesis are well characterized, the analogous enzyme for the synthesis of cyclic CMP, cytidylyl cyclase, has only been finally identified after the elucidation, by FAB/MIKES analysis of the enzyme products, of four novel cyclic CMP analogues which are side products of the reaction[8]. Identification of these compounds has enabled the development of a specific assay for cytidylyl cyclase[9], capable of resolving the cyclic CMP product from them, and also a separatory step incorporated into a highly sensitive and specific radioimmunoassay for cyclic CMP[10]. Similarly FAB/MIKES analysis of the products of cyclic CMP hydrolysis by two phosphodiesterases has revealed that one produces only 5'-CMP as product, while the other produces a mixture of 5'- and 3'-CMP.

In addition to naturally occurring compounds, FAB/MIKES analysis has also proved invaluable in the identification of synthetic derivatives of cyclic nucleotides. For example to synthesize cell-permeating cyclic nucleotide derivatives, of value in artificially elevating intracellular cyclic nucleotide concentrations, it is necessary to introduce lipophilic side chains at specific sites on the parent molecule; contamination of the desired product with side products seriously compromises any resultant data, and the correct positions of substitution and retention of the 3',5'-cyclic phosphate group are vital. The FAB mass spectrum of the product, together with the FAB/MIKE spectrum of the protonated molecule establish the number of lipophilic substituents added and the integrity of the cyclic phosphate moiety, while the FAB/MIKE spectra of the fragments resulting from cleavage of the glycosidic bond indicate the positions of substitution[11]. Similarly structural verification by FAB/MIKE analysis of succinyl, succinyltyrosinyl and succinyliodotyrosinyl derivatives of cyclic nucleotides has proved invaluable in the

development and optimization of cyclic nucleotide radioimmunoassays[12], for which such derivatives are an essential component.

The application of FABMS to cyclic nucleotide-related enzymes has now been extended to quantitative analyses. While measurement of peak heights of protonated molecules will not alone produce reliable quantitative data, successful quantitation of enzyme activity has been achieved by two methods:-

(a) the MIKE spectra of $[MH]^+$ ions selected from the FAB mass spectrum of both substrate and product in an enzyme incubation sample is obtained, then secondary spectra are obtained after the sample has been spiked with known quantities of either substrate or product as internal standard[13,14].

(b) The peak heights of a number of sample-derived ions for both substrate and product are expressed relative to peak heights of matrix-derived ions, and quantitation is achieved by a computational proportionation of the sums of relative peak intensities[15].

Good correlation is obtained with the data from conventional radiometric assays, and the quantitative MS assays have the integral advantage of allowing multiple component monitoring. Thus with a protein kinase, for example, not only the phosphorylation rate but the binding and hydrolysis of several cyclic nucleotides in a single incubation can be followed simultaneously[15]. The use of continuous flow or dynamic FABMS, in which the contents of a reaction cell can be pumped directly into the spectrometer, provides the means of a continuous assay system, such that components can be added during the course of a reaction and both their effects upon the reaction and any changes in their molecular status can be monitored simultaneously [16]. Current applications of this technique in our laboratories include monitoring interactions between drugs used to treat schizophrenia and the enzyme adenylyl cyclase.

ACKNOWLEDGEMENT. The authors gratefully acknowledge financial support from the Wellcome Trust.

REFERENCES

1. Lawson, A.M., Stillwell, R.N., Tacker, M.M., Tsubogama, K. and McCloskey, J.A., *J. Amer. Chem. Soc.* **1973**, *93*, 1014.

2. Kingston, E.E., Beynon, J.H. and Newton, R.P., *Biomed. Mass Spectrom.* **1984**, *11*, 367.

3. Kingston, E.E., Beynon, J.H., Newton, R.P. and Liehr, J.G., *Biomed. Mass Spectrom* **1980**, *12*, 525.

4. Newton, R.P., Gibbs, M., Moyse, C.D., Wiebers, J.L. and Brown, E.G., *Phytochem.* **1980**, *19*, 1909.

5. Newton, R.P., Salih, S.G., Salvage, B.J. and Kingston, E.E., *Biochem. J.*, **1984**, *221*, 665.

6. Newton, R.P., Kingston, E.E., Hakeem, N.A., Salih, S.G., Beynon, J.H. and Moyse, C.D., *Biochem. J.*, **1986**, *236*, 431.

7. Newton, R.P., Chiatante, D., Ghosh, G., Brenton, A.G., Walton, T.J., Harris, F.M. and Brown, E.G., *Phytochem.* **1989**, *28*, 2243.

8. Newton, R.P., Hakeem, N.A., Salvage, B.J., Wassenaar, G. and Kingston, E.E., *Rapid Commun. Mass Spectrom.* **1988**, *2*, 118.

9. Newton, R.P., Salvage, B.J. and Hakeem, N.A., *Biochem. J.* **1990**, *265*, 581.

10. Newton, R.P., Evans, A.M., van Geyschem, J., Diffley, P.J., Hassan, H.G., Hakeem, N.A., Moyse, C.D., Cooke, R. and Salvage, B.J. *J.Immunoassay* **1994**, *in press*.

11. Newton, R.P., Walton, T.J., Basaif, S.A., Jenkins, A.M., Brenton, A.G., Ghosh, D. and Harris, F.M. *Org. Mass Spectrom.* **1989**, *24*, 679.

12. Newton, R.P., Evans, A.M., Brenton, A.G. Hassan, H.G., Harris, F.M. and Walton, T.J., *Org. Mass Spectrom.* **1993**, *28*, 899.

13. Newton, R.P., Walton, T.J., Brenton, A.G., Kingston, E.E. and Harris, F.M., *Rapid Commun. Mass Spectrom.* **1989**, *3*, 178.

14. Newton, R.P., Khan, J.A., Ghosh, D., Langridge, J., Brenton, A.G., Harris, F.M. and Walton, T.J., *Org. Mass Spectrom.* **1991**, *26*, 447.

15. Newton, R.P., Khan, J.A., Brenton, A.G., Langridge, J.I., Harris, F.M. and Walton, T.J., *Rapid Commun. Mass Spectrom.* **1992**, *6*, 601.

16. Langridge, J.I., Brenton, A.G., Walton, T.J., Harris, F.M. and Newton, R.P., *Rapid Commun. Mass Spectrom.* **1993**, *7*, 293.