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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF BLEOMYCIN A_2 AND B_2 AND THEIR METAL COMPLEXES

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SUMMARY: The new mass spectrometric technique of fast atom bombardment is applied to the analysis of bleomycins either separately or in mixtures. The spectra are reproducible and afford valuable analytical data and structurally significant fragmentation patterns on these important antibiotics. The procedures outlined have also enabled the characterisation of various metal complexes.

INTRODUCTION

The bleomycins are a family of complex glycopeptide antibiotics produced by various Streptomyces and have recently attracted considerable attention as clinically useful antitumour agents (1). It was established fairly early that the various bleomycins differ from one another only in the structure of the terminal amine entities. A wide range of semi-synthetic bleomycins can be prepared by chemical and biochemical methods as described in (2,3). At present, the principle bleomycin complex used clinically consists mainly of bleomycins A_2 and B_2. The absence of accurate molecular weight data complicated the structural elucidation of the basic glycopeptide skeleton. An initial

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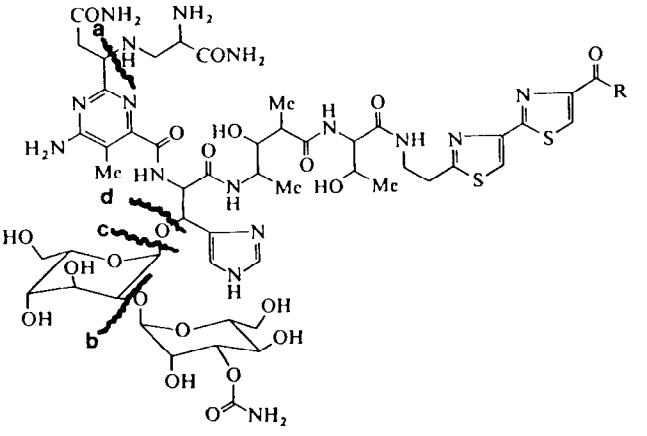

Fig. 1 Structures of bleomycins

R = OH bleomycinic acid, R = NH_2 bleomycin B_1,

R = $NH(CH_2)_3.\overset{+}{S}Me_2$ bleomycin A_2, R = $NH(CH_2)_4\ NH.\overset{NH}{\overset{\|}{C}}-NH_2$ bleomycin B_2

Principle fragmentation processes involving the loss of the sugar residues and side-chain are indicated.

formulation based on extensive degradative and spectroscopic studies incorporated a β-lactam ring, but this was subsequently modified to an open chain amide in the light of molecular weight data obtained from ^{252}Cf-plasma desorption mass spectrometric analyses (PDMS) on bleomycin B_1 (4). The structure (Fig. 1) was later confirmed by n.m.r. spectral data and x-ray crystallographic analysis (5) on other bleomycins and related compounds. More recently FD has been successfully applied to bleomycin B_2 and the related compounds phleomycin D_1 and E (6). However, both techniques for reasons of experimental difficulty and lack of structural information would seem to be of limited general applicability.

The considerable importance of this large group of antibiotics and the associated pharmacology, coupled with our interest in modified peptide antibiotics (7) led us to consider the application of the recently developed fast atom bombardment (FAB) technique (8) as a means of convenient mass spectrometric analysis. Studies

with other groups of thermally labile and highly polar peptides such as viomycin, micrococcin and vancomycin using this technique have provided not only molecular weight determinations which were unobtainable by other methods i.e. field desorption (FD), but also structurally useful fragmentation patterns with associated metastable data (9).

We now report our investigation using a FAB ion source for the analysis of bleomycin A_2 and B_2 and some of their metal ion complexes.

MATERIALS AND METHODS

Bleomycin complex (a mixture consisting primarily of A_2 and B_2) and bleomycin A_2 were obtained from Lundbeck Ltd. Chromatographic analysis was conducted as described (10,11). Fast atom bombardment mass spectrometry was carried out on a Vacuum Generators ZAB instrument with a modified ion source. The atom gun consists of a high efficiency charge-neutralised ion source with suitable collimation and electrostatic beam cleansing electrodes. The sample stage is a variable temperature platinum ribbon. 1 μg of sample was deposited together with 10 μℓ of glycerol as a sample support on to the stage. This type of sample preparation resulted in spectra which are stable for many minutes and permits the use of all normal mass spectrometric techniques on one sample.

RESULTS AND DISCUSSION

A typical positive ion FAB mass spectrum showing the molecular ion region of a bleomycin complex composed primarily of A_2 and B_2 is illustrated in Fig. 2. The pseudo-molecular ion $(M + H)^+$ is observed for B_2 at m/z 1425 and the sulphonium cation species from A_2 at m/z 1414 are in accord with the proposed structures of the principle components. Additionally A_2 shows a significant ion at m/z 1512 which corresponds to the $(M + H)^+$ species from A_2 sulphate. The significant ion at m/z 1400 was initially considered to be the pseudomolecular ion $(M + H)^+$ for demethyl A_2,

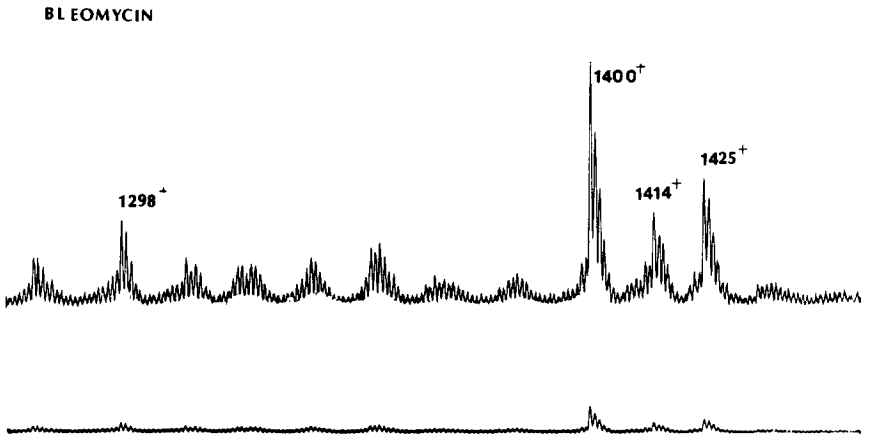

Fig. 2 Fast atom bombardment mass spectrum of bleomycin complex composed primarily of A_2 and B_2

as this is a known minor component of the complex. Bleomycin A_2, as well as sulphonium ions in general, are reported to demethylate fairly readily. Chromatographic evidence on the relative proportion of demethyl A_2 in the complex suggested that the ion was too intense to represent a pseudomolecular ion and since the FAB ionisation takes place from the solid at room temperature any significant thermal demethylation of the sulphonium ion could be discounted. Subsequent analysis of the FAB spectrum of the pure A_2 component (see Fig. 3) indicated that the ion at m/z 1400 in common with the sulphonium cation at m/z 1414 are almost certainly fragment ions from the A_2 sulphate pseudomolecular ion at m/z 1512. Despite the polarity, high mass and thermal instability of the bleomycins, spectra similar to that shown in Fig. 2 can be obtained routinely without special precautions.

Diagnostic fragment ions are apparent in the FAB spectrum of pure bleomycin A_2 (Fig. 3). The major structurally significant peaks are indicated and assignments made on the accompanying formula. It appears that most fragmentation proceeds via the m/z 1400 ion from which the terminal sugar and amino acid

Several possible mechanisms exist that might account for the heterogeneity of fragment A homologous DNA sequences reported here. DNA modification, e.g., methylation, can affect the susceptibility of DNA to cleavage by restriction endonucleases. If the methylation of the restriction enzyme sites which border the fragment A homologues were itself to be heterogeneous, several fragments of different sizes, which hybridize to fragment A, would be generated. Point mutations in some of the approximately 200 copies of the fragment A homologues would yield the same result as differences in DNA modification. Lastly, these findings may reflect different DNA fragments.

Homologous, but different, DNA fragments have previously been reported to exist due to variations in related genes. The ribosomal DNA's of Xenopus (3), Drosophila (4), mouse and man (6) are heterogeneous in size. Xenopus vitellogenin is apparently encoded by a family of related genes (5) composed of two divergent populations, each of which is composed of two more populations.

The heterogeneity of the fragment A homologues within each of the populations studied may be due to a related phenomenon. However, the heterogeneous distribution of the 5.8, 7.2 and 9.4 kb Eco R1 fragments, which are homologues to fragment A, may be due to specific selective pressures or a result of neutral drift between populations. Most particularly, the heterogeneity found within separately outbred colonies of CFE rats indicates that the variations reported here can arise due to genetic drift over relatively short periods of time. These data may also reflect the consequence of transposable genetic elements in a higher eukaryote. However, the present data, of themselves, do not constitute a rigorous proof of a eukaryotic transposon.

Cloning of the fragment A homologues and comparative sequence studies should provide a better understanding of their relationships to one another, as well as providing an assessment of just how far these sequences have drifted.

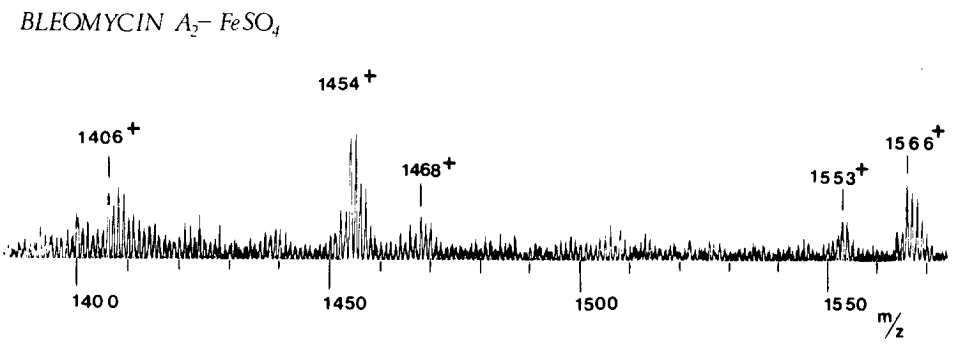

Fig. 5 Fast atom bombardment mass spectrum of bleomycin A_2. $FeSO_4$ complex

ferrous complex of bleomycin A_2 (Fig. 5) conforms with the formulation. Since the amide hydrogen of the histidine residue is lost on complexation, the salt ($A_2 - H^+ + FeSO_4$) is observed as a 'pseudomolecular' ion at m/z 1566. An interesting fragmentation pattern includes a prominent ion at m/z 472 corresponding to Fe^{2+} ion complexed to the surrounding ligands but lacking the disaccharide groups and the peptide chain. The FAB mass spectra of the Cu and other metal complexes have been obtained and are consistent with the proposed structure.

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