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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF MALIC ACID ANALOGUES OF ANGIOTENSIN II

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Summary: Fast atom bombardment mass spectrometry of underivatized L-malic acid analogues of angiotensin II yield spectra which provide confirmation of molecular weight and amino acid sequence. The characteristics of the spectra are discussed and compared with the spectra of natural Angiotensin II.

Recently we reported the synthesis and biological activities of [β-malyl^1] - and [β-malyl^1, Leu^8] - angiotensin II (AII) (1). Although these analogues were synthesized by standard methods, full characterization using classical amino acid analysis and microsequencing via Edman degradation was precluded due to the L-malic group on the amino terminus. With the development of fast atom bombardment mass spectrometry (FAB) (2,3), it is now possible to analyze peptides without prior derivatization. We now report the use of this technique to confirm the structure of the AII analogues.

METHODS

Mass spectral analyses were performed on a Kratos MS-50 mass spectrometer equipped with a Kratos FAB ion source operating at 8 kV accelerating voltage. Samples were deposited in thioglycerol solution (~10 μg/μl) onto the probe tip and irradiated by a beam of xenon neutral atoms derived by neutralizing ions which had been accelerated through 8 kV in a beam current of 0.4 mA. The resulting positive ion mass spectra were collected and analyzed using a Kratos DS-55 data system.

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RESULTS AND DISCUSSION

The amount of structural information contained in the FAB spectra of the malic acid analogues is illustrated in the spectrum of [β-malyl[1]]-AII (Figure 1). Not only is the molecular weight of the compound confirmed by a strong protonated molecular ion (MH+) at m/z 1047, but all of the major fragments can be assigned consistent with the expected structure. Loss of CO_2 and CH_2O_2 from MH+ accounts for the ions at m/z 1003 and 1001 respectively. The strong ions at m/z 110 and 136 are derived from the

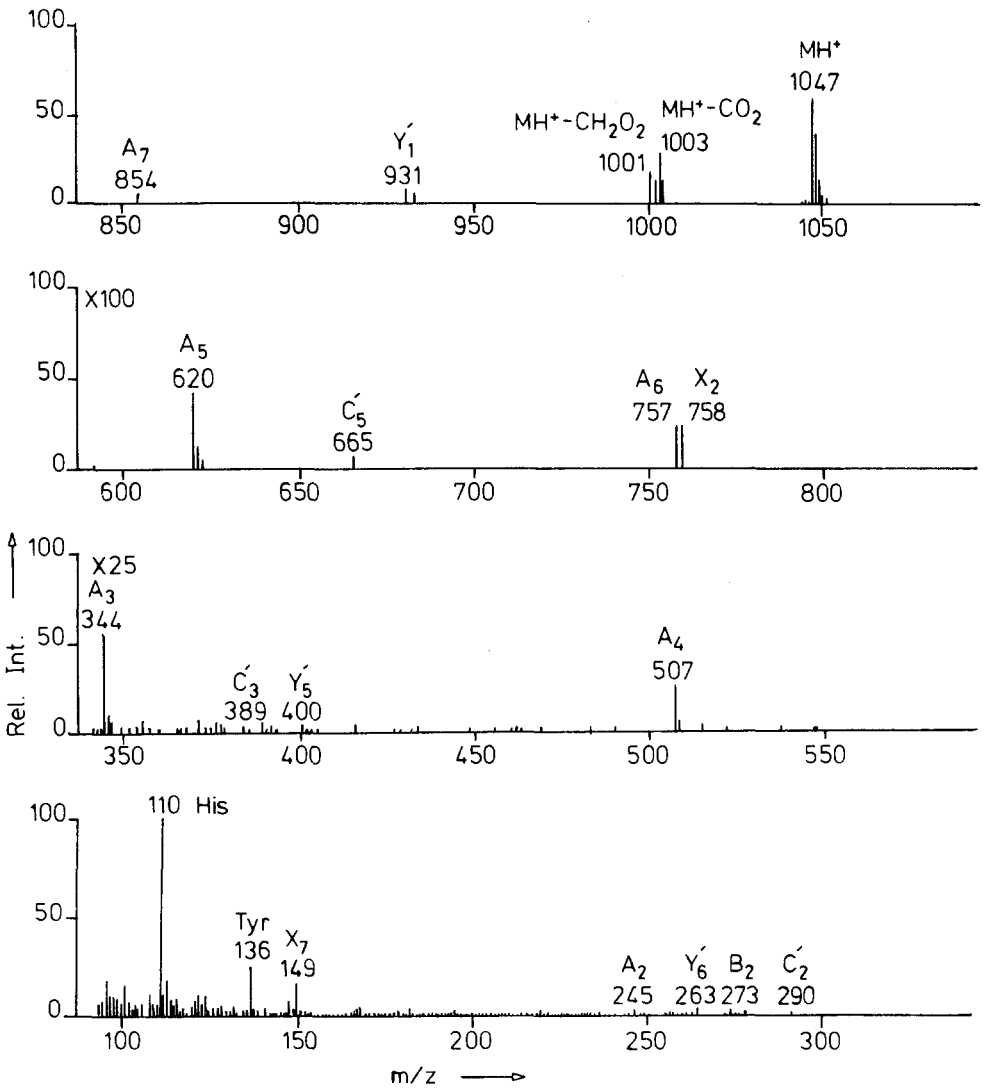

Figure 1. Positive Ion FAB Mass Spectrum of [β-Malyl[1]]-AII (MW 1046).

histidine and tyrosine amino acid residues respectively (4,5). The spectrum for [β-malyl^1-Leu^8]-AII (not shown) is similar in every respect.

Sequence information is contained in a number of fragment ion series. The fragment series for [β-malyl^1]-AII are diagrammed in Figure 2 and a comparison of fragment series for the L-malic acid analogues and AII is given in Table 1. The presence of an arginine residue near what would normally be considered the amino terminus of each peptide results in a greater proportion of N-terminal fragments. In our method of naming fragment series a prime is used to denote cleavages involving hydrogen transfer. Thus, the m/z value for a member of the Y' or C' series is the mass of fragment resulting from simple cleavage plus 2. Possible mechanisms for the formation of these fragments have been described by others (5-8).

The degree of correspondence between the spectra of the L-malic acid analogues and the spectra previously published for natural AII (5,6) is remarkable. In all the spectra, the dominate fragmentation series (series A) is that which results from cleavage of the bond between the alkyl carbon and the carbonyl carbon producing fragments which contain the arginine residue. The first member of the A series (does not contain arginine) is absent in all the spectra. The extent to which the position of the arginine residue influences the prominence of fragments is reflected in the observation that 75% of the

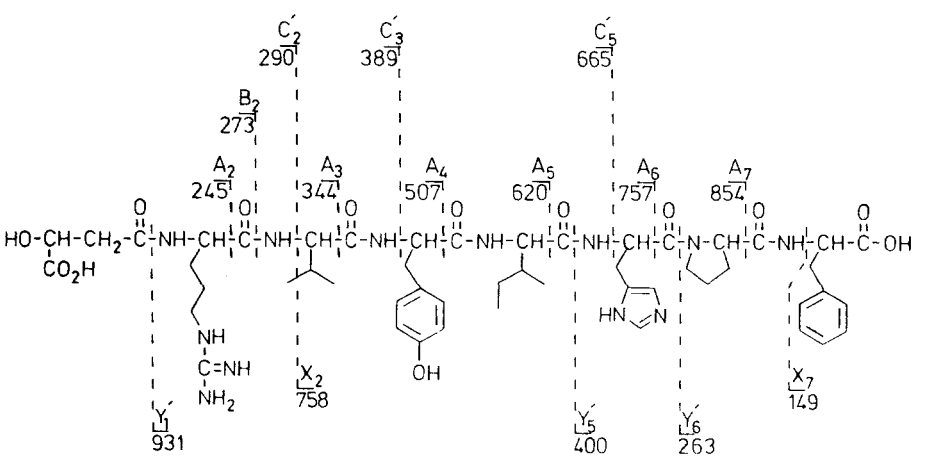

Figure 2. Sequence Fragments for [β-Malyl^1]-AII.

Table 1. Sequence Fragments in FAB Mass Spectra of Angiotensin II and L-Malic Acid Analogues.

Series	Member	[β-malyl^1]-AII m/z	[β-malyl^1-Leu^8]-AII m/z	AII (5) m/z
A	2	245	245	
	3	344	344	343
	4	507	507	506
	5	620	620	619
	6	757	757	756
	7	854	854	853
B	2	273	273	272
	6		785	784
	7		882	
C'	2	290	290	
	3	389	389	388
	4		552	551
	5	665	665	664
	7		899	898
X	2	758	724	
	7	149	115	
Y'	1	931	897	931
	3			676
	4		468	513
	5	400	366	400
	6	263	229	263

observed fragments contain that amino acid. This is the result of the preference for the positive charge to reside on the guanidine functionality.

The substitution of L-malic acid for aspartic acid at the amino terminus of AII would have little effect on the charge distribution of the peptide, and therefore, very little effect on the mass spectrum. The same argument would also apply to the substitution of leucine for phenylalanine at the carboxyl terminus as is the case for [β-malyl^1-Leu^8]-AII. It is not clear whether the differences in intensities or completeness of minor fragment series are

related to structural changes or are due to experimental error. There is a tendency for weaker ions to become obscured by the relatively high background which is characteristic of FAB spectra.

Although in this case peptides with great differences in biological activity have very similar FAB spectra, modifications involving changes in easily ionizable moieties would be expected to result in significant spectral changes. The possibility exists that a better understanding of the similarities and differences in the FAB spectra of peptides and their analogues would provide additional clues to the mechanism of their biological activities. In any event, given the simplicity of the technique and the wealth of information that it provides, FAB mass spectrometric analysis should be routinely applied to any synthetic peptide.

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