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FAST ATOM BOMBARDMENT MASS SPECTROMETRY (FABMS) ANALYSIS OF AN N- TERMINAL - BLOCKED PEPTIDE

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Abstract

FABMS analysis of T-1b peptide before and after one cycle of Edman degradation indicated an unblocked N-terminal Thr residue for this tryptic peptide. In contrast , our data showed a molecular protonated ion, MH^+ for T-1a peptide at 655 mass units (mu) which is 42 mu higher than the MH^+ ion of T-1b peptide. In addition, T-1a peptide was not amenable to one cycle of manual Edman degradation. These observations suggest that the N-terminal residue of T-1a peptide is blocked by a chemical group having a mass of 42 mu. This observation is consistent with the presence of an N-terminal acetyl group. This prediction was further investigated by complete characterization of the gas phased fragment ions, originated from the individual peptides, using link-scanning approach. The fragment ions again suggest an acetyl group attached to an N-terminal threonine. In addition, the fragment ions provide evidence that the two peptides (T-1a and T-1b) have identical structures except for the N-acetyl group.

Introduction

The versatility of mass spectrometry as a sensitive technique for structure determination of a large variety of nonvolatile biological samples has been illustrated by many investigators [1-10]. A particularly useful approach for studying the components of a biological mixture without separating them is to use a double focusing mass spectrometer to obtain different mass spectra which assist the structure determinations of each component. Molecular ions which can undergo either unimolecular or collision induced decompositions in the field free regions between the source and the first sector or between the two sectors of a double focusing instrument of a forward or reverse geometry will give daughter ions which can be investigated using linked scan mass Spectrometry [11-13]. The most commonly used linked scans with instrument of either geometry are referred to as B/E and B^2/E scans-In B/E mode of linked scan, one can select the fragment (daughter) ions derived from a given parent molecular ion [14,15]. Reversably, the parent molecular ion of a given daughter ion can be established using B^2/E mode of linked scan [16]. This paper describes the application of B/E linked scan to determine the chemical nature of the blocking group of the N-terminal tryptic fragment of the largest CNBr-treated N-terminal peptide of the Hemocyanin. This protein, which is obtained from Horseshoe crab, Limulus polyphemus, consists of 48 different subunits.

Keywords: Peptides, Protein Structure, Mass Spectrometry

The subunits can be separated into five fractions, I-V, by Chromatography on DEAE-sephadex [17]. This paper deals only with subunit(s) collected from fraction II [18].

Materials and Methods

Dithioerythritol (DTE) and Dithiothreitol (DTT) were purchased from Sigma Chemical Company. Phenylisothiocyanate (PITC) was obtained from Pierce Chemical Company. Organic reagents were reagent grade and were used without further purification. Glass distilled water was used for sample preparations.

FABMS Analysis. The purified peptides of T-1a and T-1b in maximum amounts of 0.6 and 1.3 m moles have been obtained from Horseshoe crab Hemocyanine as described previously [18] and provided to our lab by Dr. A. F. Riggs. The peptides were separately dissolved in 30 ul of 50% formic acid. A 5 ul aliquot of each peptide was mixed with 1-2 ul of a 3 to 1 mixture of dithiothreitol and dithioerythritol (DTT/DTE), respectively. One ul of this solution was loaded on a stainless steel probe tip and subjected to FABMS analysis. Positive ion FAB mass spectra were recorded using a Kratos MS-50 double focusing mass spectrometer (Kratos Scientific Instruments, Manchester, UK) at full accelerating voltage of 8 KV. All spectra have been recorded on an Osillographic recorder. Cesium iodide has been applied for manual calibration of the mass spectra. The error in molecular weight determination is less than ± 0.5 mu.

Manual Edman degradation-Ten ul of the peptide solution in 50% formic acid was vacuum dried, the dried residue was dissolved in 10 ul of 20% phenylisothiocyanate (PITC) in pyridine under N_2 gas. The mixture was kept at 55°C for 15 min followed by vacuum drying. The dried residue was then dissolved in 10 ul concentrated HCl for decoupling process. The acidic mixture was kept at 55°C for 10 min followed by drying under vacuum. FABMS analysis of the peptide after one cycle of manual Edman degradation was achieved as mentioned before [8,9].

Results and Discussion

FABMS analysis of peptide T-1b-The FAB mass spectrum of T-1b contained at least five peaks located at m/z 1263, 1247, 695, 613, and 568 mass units (mu). However, the major component of the peptide mixture appeared to be the peptide with a molecular protonated ion, MH^+ at m/z 613 (Figure 1a). All of the above

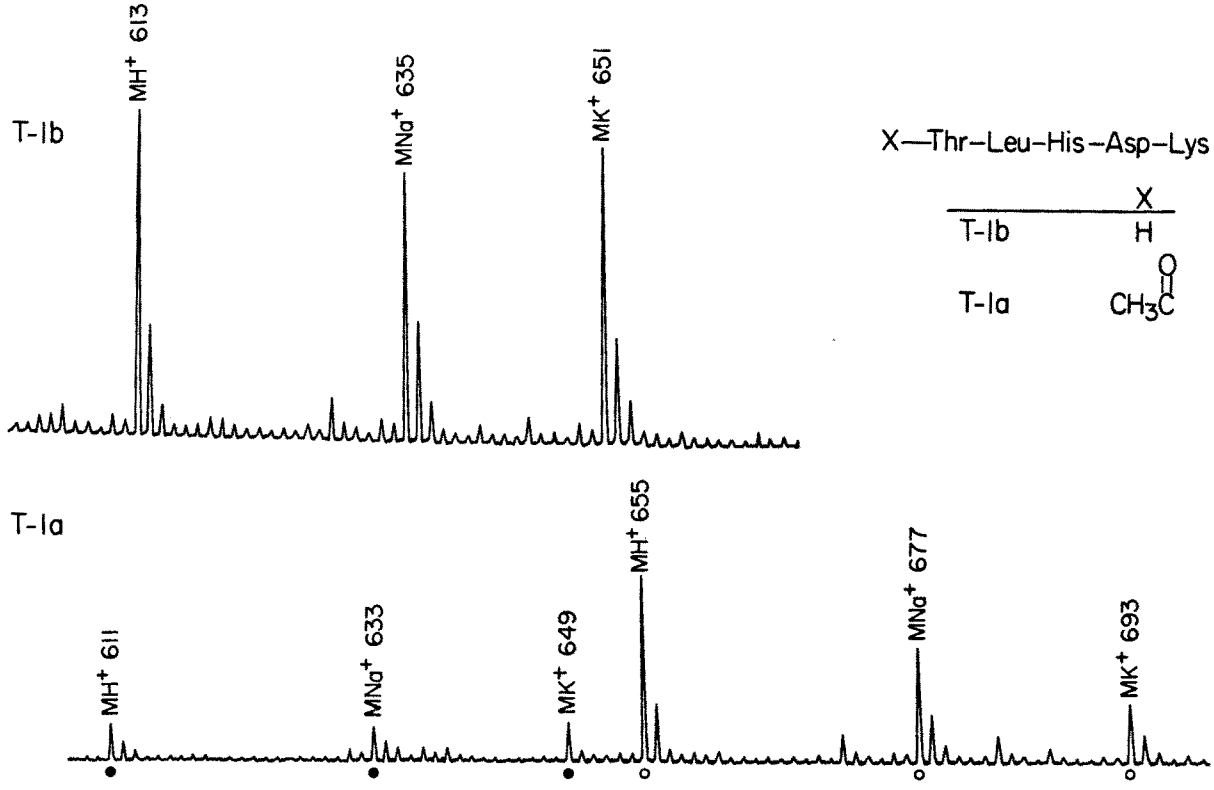

Fig.1. The partial FAB mass spectra of molecular ion region of peptides T-1b and T-1a.

mentioned peaks have been accompanied with sodium and potassium cationated ions, MNa^+ and MK^+, respectively. This criteria will differentiate the aforementioned isotopic peaks from peaks corresponding to gas phase fragmentations which are not accompanied by MNa^+ and MK^+ peaks. The assignment of the peak at m/z 613 to peptide T-1b was further confirmed by subjecting the sample to one cycle of manual Edman degradation using PITC reagent. This approach, which removes one N-terminal amino acid residue from the peptide, per cycle, is the most common and easiest approach for confirming the assignment of mass spectral peaks to peptides.

The FAB mass spectrum of peptide T-1b after one cycle of manual Edman degradation showed a peak at m/z 512 which corresponds to peptide T-1b without the N-terminal threonine residue. The FAB mass spectrum of T-1a did not have an isotopic peak at m/z 512.

FABMS analysis of peptide T-1a. The positive FAB mass spectrum of peptide T-1a showed at least nine peaks, all accompanied with Na and K cationated ions, at m/z 1367, 1347, 1068, 738, 683, 655, 649, 611, and 459 mu. The major peptide of the mixture appeared to have MH^+ ion at m/z 655 based on the absolute intensity of the peak in the FAB mass spectrum. The partial FAB mass spectrum of the molecular protonated ion region of T-1a is shown in Figure 1b.

FABMS analysis of T-1a after one cycle of Edman degradation indicated a new peak at m/z 790 in addition to a peak at m/z 655. The former peak may correspond to peptide T-1a after covalent modification of the Lysine residue (ε-NH_2) by Edman reagent (PITC). This modification will shift the MH^+ ion to higher masses by 135 mu. Consequently, it may be concluded that the N-terminal threonine residue of peptide T-1a is blocked.

Chemical nature of the N-terminal blocking group- Since both peptides T-1a and T-1b have identical amino acid analysis the mass difference between the corresponding MH^+ ion will provide some insight into the chemical nature of the blocking group. In addition , comparison of the gas phase fragmentation pattern of both peptides using a link scanning method, will assist and confirm further structure elucidation as explained in the following:

A mass difference of 42 between the MH^+ ions of T-1b and T-1a may correspond to an acetyl blocking group. The gas phase fragmentation pattern of both peptides was investigated using a link scanning approach to evaluate this prediction. In this scanning method, the mass spectrometer is set upon one particular peak followed by simultaneous scanning of both electrostatic (E) and magnetic sector (B) analysers in a manner to maintain the B/E ratio constant. The link scanned FAB mass spectra of a compound usually contains one MH^+ ion (parent molecular ion) plus its corresponding gas phase fragmentations (daughter ions). The link scanned FAB mass spectrum has the advantage of being devoid of any peak related to impurities or peaks related to the material used for FABMS analysis. However, this powerful technique suffers from poor sensitivity. This feature will limit the application of link scanning method for structural analysis of larger peptides.

The link scanned FAB mass spectra of T-1b and T-1a contained the peaks shown in Table I and II, respectively. The assignments of the observed peaks to specific sections of each peptide are shown in Figure 2. As it is shown in Table I and II, both peptides share

Table 1

Gas Phase Fragment ions of T-1b confirmed by Link Scanning technique

Molecular ion	Gas Phase fragment ions	
MH^+	N-terminal	C-terminal
	233	385
613	325	400
	353	513
	468	
	485	

Table II

Gas Phase fragment ions of T-1a confirmed by link scanning technique

Molecular ion	Gas phase fragment ions	
MH^+	N-terminal	C-terminal
	367	385
655	395	400
	510	513
	527	

fragment ions at m/z 513,400, and 385 mu. These peaks correspond to the C-terminal gas phase fragment ions, as shown in Figure 2. The remaining peaks in Table I and II appeared to correspond to the N-terminal gas phase fragment ions. As is evident from Figure 2, there is a mass difference of 42 Units between four pairs of the N-terminal gas phase fragment ions (Table III). This mass difference of 42 units unambiguously corresponds to an acetyl group at the N-terminal threonine residue of T-1a. These data are in complete agreement with the mass difference of 42 units between MH^+ ions of T-1b and T-1a. In addition, link scanned data confirm the data obtained from the Edman degradation approach and also supports the data that the two peptide do not differ from one another at any site other than the amino terminal acetyl group.

Table III

Mass difference between some of the corresponding N-terminal gas phase fragment ions of T-1a and T-1b

T-1b	T-1a	mass difference
325	367	42
353	395	42
468	510	42
485	527	42

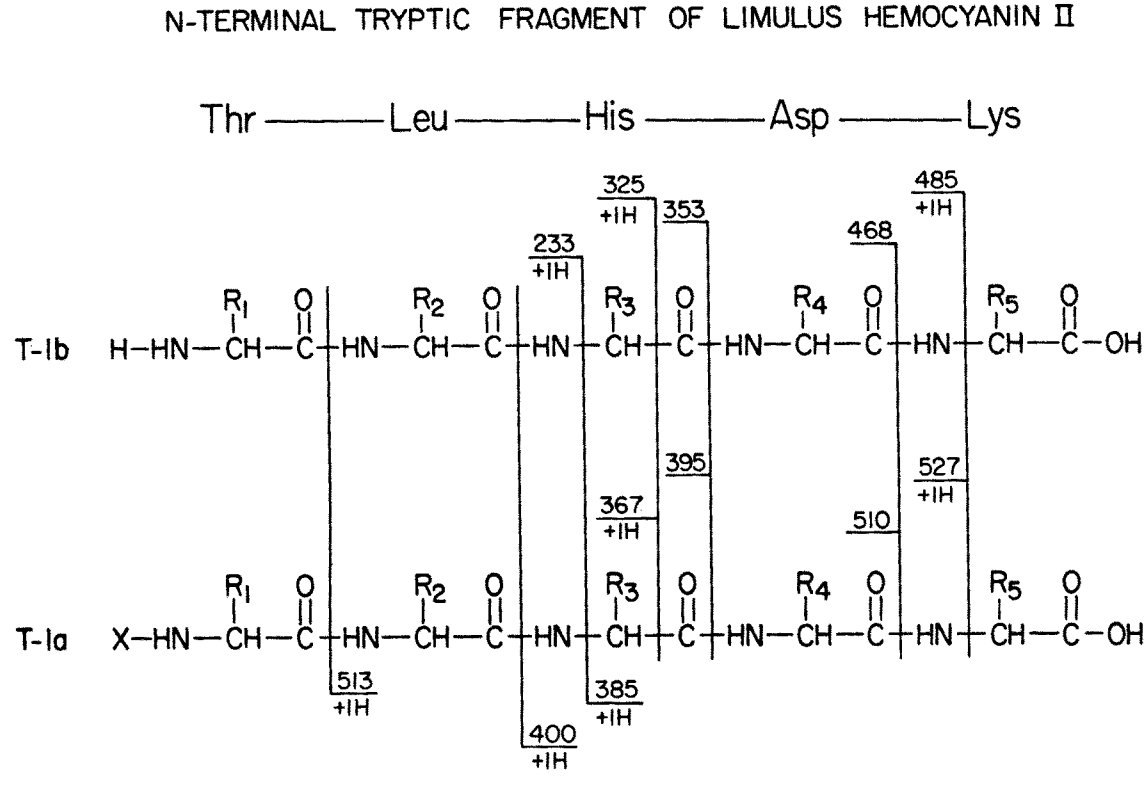

Fig.2. Gas phase dominant sequence ions observed in FAB mass spectrometry analysis of T-1a and T-1b peptides.

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