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THE OBSERVATION OF QUASI-MOLECULAR IONS FROM A PROTEIN NEUROTOXIN (MW 7821) USING ^{127}I-PLASMA DESORPTION MASS SPECTROSCOPY

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Summary. Fast heavy ions, i.e. 90 MeV ^{127}I from the Uppsala tandem accelerator have been used to desorb and ionize molecules from a cobra venom neurotoxin. The protein is built up by 71 amino acid residues in a single polypeptide chain, tightly cross-linked by 5 disulfide bridges. The molecular weight as confirmed by protein sequence analysis is 7821. The ions were mass analyzed by the time-of-flight technique. This is to our knowledge the largest protein for which it has been possible to detect quasi-molecular ions by a mass spectrometric technique.

During the last few years a number of new methods have been introduced in the field of mass spectroscopy on large thermally labile molecules. A certain class of methods use particle bombardment of solid or liquid samples to induce desorption and ionization of sample molecules. In 1974 Macfarlane and coworkers (1) reported on the use of fission fragments from a ^{252}Cf-source for such a purpose. Since then a number of important biomolecules have been studied by the Texas group with that method (2-4). The most impressing result so far is the detection of a fully protected oligonucleotide of mass 6275 amu and the corresponding dimer (4). Benninghoven (5) and others have used low energy

ABBREVIATIONS

TDC = time-to-digital-converter
SIMS = secondary ion mass spectrometry
FAB = fast atom bombardment
PDMS = plasma desorption mass spectrometry
TOF = time-of-flight
MS = mass spectrometer
P/S = particle per second
amu = atomic mass units

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(keV) ions in SIMS type studies and been able to study many compounds, hard to investigate with other mass spectroscopic techniques (6-8). A further development is the use of neutral low energy (keV) particles, i.e. the FAB-technique (9), which uses liquid samples. Recently Dell and Morris reported the detection of quasi-molecular ions of insulin with the FAB-technique (10). The first observation of quasi-molecular ions from insulin was published by ourselves in a collaboration with C.J. McNeal and R. Macfarlane (11). In that experiment a beam of 90 MeV $^{127}I^{20+}$ ions from the Uppsala tandem accelerator was used as primary ions. Fast heavy ions like fission fragments mainly interact with the electrons in a medium, in contrast to low energy ions and neutrals like in SIMS and FAB, which induce collisional cascades directly with the atoms in a medium. Therefore one might expect rather different features for these two classes of desorption tools. Here we report the result with a Thai cobra venom neurotoxin using a 90 MeV $^{127}I^{20+}$ accelerator beam.

Material

The principle neurotoxin of Thai cobra (Naja naja siamensis) venom was a kind gift from Dr. E. Karlsson. It was purified as previously described (12) by gel filtration on sephadex G-50 and ion exchange chromatography on Bio-Rex 70 in volatile ammonium acetate buffers. The protein acts in the same way as curare by blocking the acetyl choline receptors of skeletal muscle nerve connections. Envenomation is followed by muscle paralysis, arrest of respiration and thereby death by oxygen deficit. The siamensis neurotoxin has been completely sequenced (13) and found to contain 71 amino acid residues in a single polypeptide chain. The protein is tightly cross-linked by five disulfide bridges. It has been possible to crystallize the protein and the complete tertiary structure has been determined (14). It is presently being used as a tool for the isolation of the acetyl choline receptor and also in clinical use for diagnosis of the neuromuscular disease myasthenia gravis (15).

Method

Samples were dissolved in trifluoroacetic acid. The solution was electro-sprayed (16) on an aluminum foil (500 $\mu g/cm^2$). The amount of sample was about 20 µg, spread over an area of 80 mm^2. The film was mounted in the ion source of a TOF-MS. The principle of this spectrometer is described in detail in Ref. 17. The sample foil was maintained at 20 kV positive or negative potential. The primary fast ions hit the sample surface at 45^o angle and the quasi-molecular ions which are desorbed and ionized are accelerated through a 90% transmission grid on earth potential. The fast ions are detected in a solid state detector which gives the start signal for every time-of-flight event. The quasi-molecular ions are then allowed to drift in a field-free region (35 cm distance) and are finally detected in a stop-detector consisting of two channel plate electron multipliers (Mullard G-25-25) coupled in tandem. Every fast ion starts a TDC, a time-measuring electronic unit with a multiple stop option which is very important to use as every fast ion desorbes several ions. The time scan of the TDC corresponds in this experiment to a mass region of 0-20 000 amu. The outputs of the TDC are accumulated in a TOF-spectrum on a graphic terminal of a computer. The intensity of the ^{127}I-ions was about 2000 p/s and the spectra took about 1 hour each to collect. The TOF spectrum was mass calibrated with a CsI sample, watching the cluster ions $(CsI)_nCs^+$ and $(CsI)_nI^-$. Also the H^+ and Na^+ peaks in positive spectra were used to check the mass calibration. The TOF spectra for ion-induced desorption are in the high mass region characterized by a smooth exponentially decaying background. This background has been subtracted in the spectra shown in Figs. 1 and 2. The peaks left after this background subtraction are very broad. The contribution of the isotopic effect to the half-width of the peaks has been calculated to about 5 amu. It is worth noting that for high molecular weight compounds the isotopic mass-distribution approaches a Gauss-distribution around the isotopically averaged mass. In the present study the peak shapes are most likely due to meta-

stable ion decay in the field-free (flight path) region. This effect causes a symmetric distribution centered around the quasi-molecular ion mass. The centroid of this distribution can be determined to an accuracy considerably better than one channel (Figs. 1 and 2). The mass errors given are dominated by contributions from the uncertainties in the centroid determination.

Results and Discussion

The region shown in Figs. 1 and 2 is m/z=2000-15000. A number of peaks were found for m/z < 2000 but are not shown here. The rest of the mass spectra are dominated by three (four in the positive ion spectrum) peaks. The isotopically averaged mass of the neurotoxin is 7821 amu as deduced from the sequence analysis. The main peaks in the positive (Fig. 1) and negative (Fig. 2) ion spectra correspond to masses of 7861±14 and 7851±14 amu respectively. Earlier studies (18) with PDMS on small peptides show that protonation and sodium attachment are the main mechanisms for positive quasi-molecular ion formation and deprotonation the main mode of negative ion formation. Therefore the present data strongly suggest that we observe quasi-molecular ions of both polarities of the neurotoxin. The errors in the mass determination are still too large to allow for definite assignments but the data may indicate that in the positive ion case we have

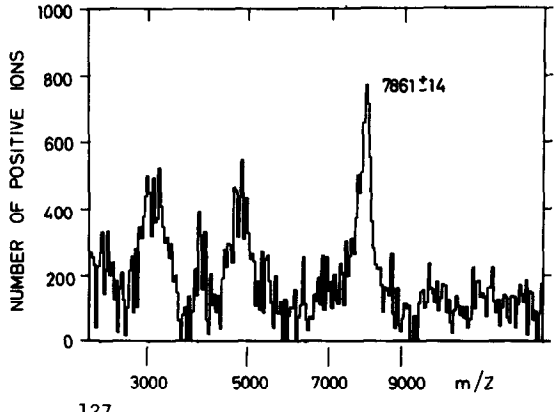

Fig. 1. Positive ^{127}I-plasma desorption spectrum of siamensis neurotoxin (MW = 7821) recorded with a primary beam of 90 MeV $^{127}I^{20+}$.

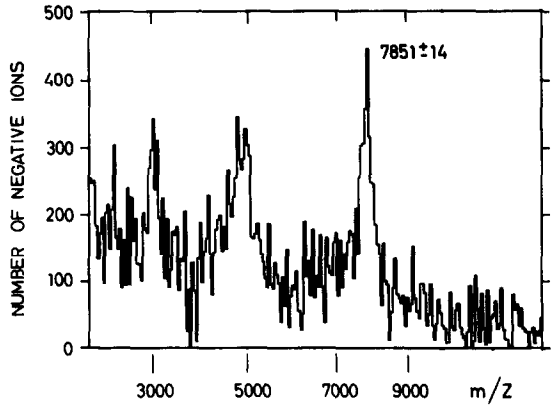

Fig. 2. The negative spectrum of the same toxin under the same conditions as in Fig. 1.

sodium attachment and that the negative ion is formed by addition of a small molecule. The two broad structures in both spectra around m/z 4700 and 3100 seem to correspond to a particular fragmentation of the polypeptide chain. On the basis of available structural information (19) we suggest a split of the molecule corresponding to the two dominating double-loops of the molecule. This would be in the region of the Cys-Ala-Ala-Thr-Cys structure in the middle of the molecule (residues 41-45) in Fig. 3. Detailed analysis indicates discrete cleavages at especially the Ala-Ala, Ala-Thr and Thr-Cys

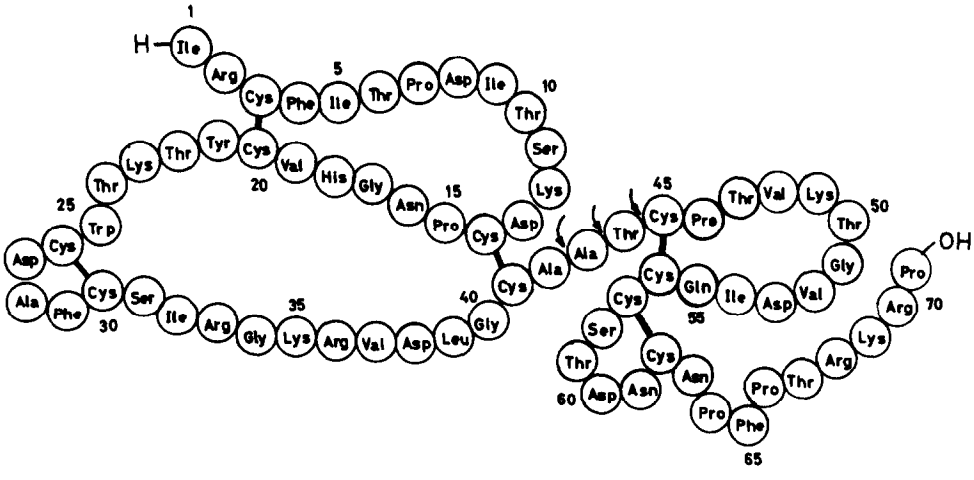

Fig. 3. Complete primary structure of Thai cobra princple neurotoxin (siamensis neurotoxin) with the disulfide pairing. Arrows indicate possible cleavage site. Courtesy Dr. E. Karlsson.

bonds. Very soon we hope to decrease the errors in mass determinations in the actual m/z region to ±1 amu. Thus the possibility of obtaining gas-phase ions of high molecular weight compounds is exciting and gives promise to routine molecular weight determinations of protein using plasma desorption mass spectroscopy.

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