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FAST ATOM BOMBARDMENT: EVIDENCE OF DISPROPORTIONATION AND RECOMBINATION OF A SYNTHETIC PORPHYRIN IN THE MATRIX†

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We have studied the mass spectra of deuteroporphyrin 6(7)methylester, 7(6)-(histidine methyl ester) obtained by fast atom bombardment (FAB) and observed the *in situ* formation of the homologous diamide. Fragmentation pathways are *defined* and a mechanism is suggested.

Fast Atom Bombardment (FAB) mass spectrometry has been shown to be of significant value in peptide (1,2) and carbohydrate sequencing (3,4), and also in numerous other applications where Electron Impact (EI) ionization MS does not give good ion intensities (5) of compounds that are thermally labile or have low volatility. The FAB technique provides a sensitive, analytical procedure for structural analysis, giving, in many cases, a pseudomolecular ion and sufficient fragmentation with which to make reasonable assignments for compound identification. It has been reported that FAB spectra may contain artifact signals arising from bombardment-induced damage to molecules by the fast atom beam, as well as from matrix molecules (6) and metal adducts (7).

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Recently, while investigating the FAB mass spectra of some synthetic porphyrins, we found in the spectrum of deuteroporphyrin 6(7)methylester, 7(6)-(histidine methylester), MW 675 [DMH], using a thioglycerol matrix, related ion signals in the M/Z region above the molecular ion not consistent with known and published artifacts. The interpretation of this observation in the FAB spectra as an ionic recombination in the matrix to form a species of a homologous series, we wish now to report.

Materials and Methods

Synthesis of subject compounds was accomplished by methods previously reported (8).

Instrumentation

FAB spectra were recorded on a VG Analytical ZAB-2H double focussing mass spectrometer (Altrincham, U.K.) fitted with an Ion-Tech saddle-field gun (Teddington, U.K.) using xenon. Samples were loaded onto the probe by dissolution or suspension in a few µl of thioglycerol. Absorption spectra were obtained on a Carey 219 spectrophotometer. IR spectra were obtained on a Perkin-Elmer 681 spectrophotometer. NMR spectra were recorded on Varian FT-80A and XL-200 FT-NMR spectrophotometers. Direct chemical ionization (DCI) spectra were obtained on a Ribermag Gas Chromatography/Mass Spectrometer System (Mass range 0-1500 a.m.u.), Model R10-10-C, RDS Nermag, Inc., Houston, TX. Methane at a source pressure of approximately 0.1 torr was used as the reagent gas. The samples were dissolved in dichloromethane, an aliquot containing 0.5- 1 µg of sample was deposited on the DCI probe, and the solvent was evaporated under a stream of dry N_2. Following insertion of the DCI probe into the source region, the current in the DCI filament was linearly programmed from 20-400 MA at 7 MA/sec in order to volatilize the sample.

Results and Discussion

Fig. 1 shows the FAB positive ion spectrum of the subject compound DMH, with a comparison of ion signals recorded by FAB and DCI-MS covering the range

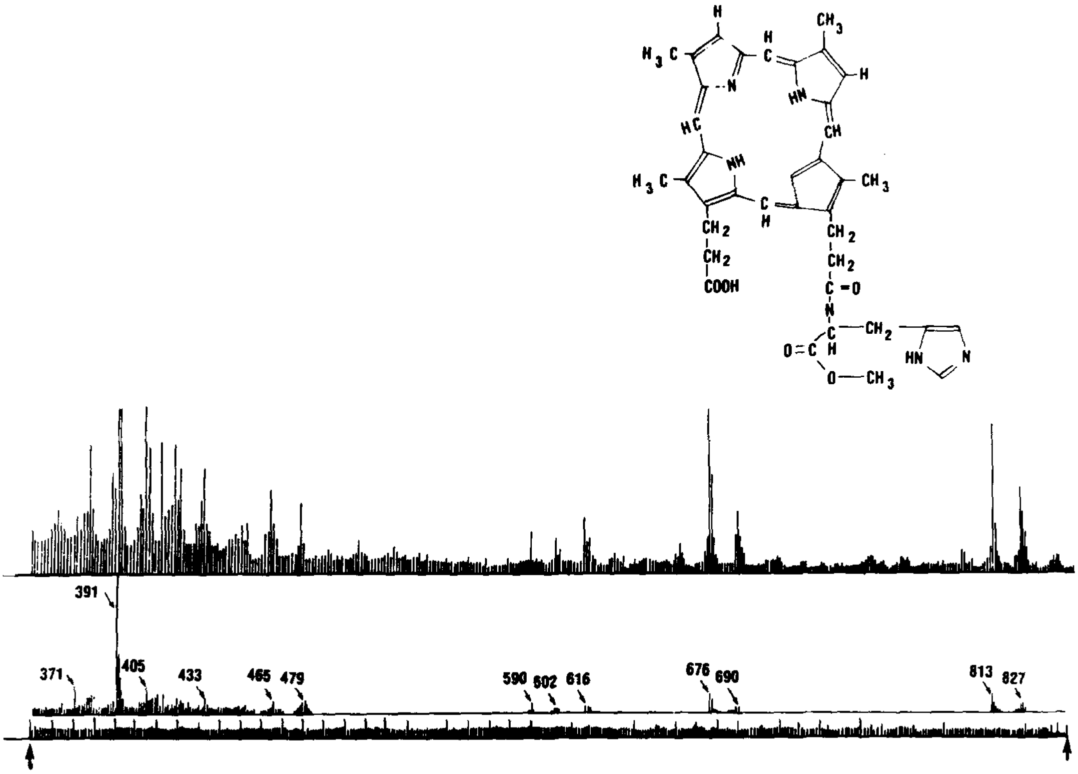


FIG. 1 FAB Positive Ion Spectrum of DMH

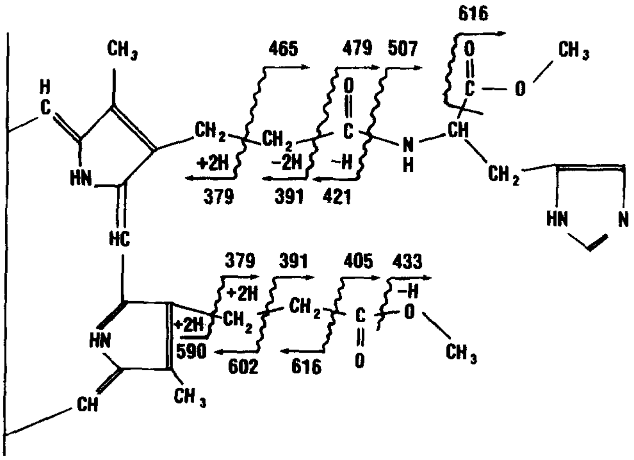
of M/Z 364 to 850 shown in Table I. Analyses within the limits of our detection by UV, IR, 'HNMR, ^{13}CNMR, thin layer chromatography, DCI-MS, and EI-MS, detected the presence of only the monoamide (9). Spectra of similar diamides and monoamides by EI-MS have been obtained by Castro (10), who has reported their preparation and characterization, finding that the diamides are more volatile than the monoamides. In the sample used for this study, neither the DCI-MS or EI-MS spectra indicated any trace of diamide. However, analysis by FAB-MS indicates that the compound is either the diamide or is contaminated with significant amounts of the diamide. The fragmentation patterns of FAB-MS spectra usually are similar to patterns obtained by CI technique with fission at C-X hetero-atom bonds; therefore the ions arising from the monoamides listed in Table I may be interpreted to occur as shown in Scheme 1. We postulate an *in situ* mechanism for the formation of the M/Z 813 ion as similarly reported for the N-acetylation reaction with amino terminal fragments in a glycerol matrix upon the addition of an acetylation reagent

Table 1. Ions Obtained Using FAB and DCI Mass Spectrometry

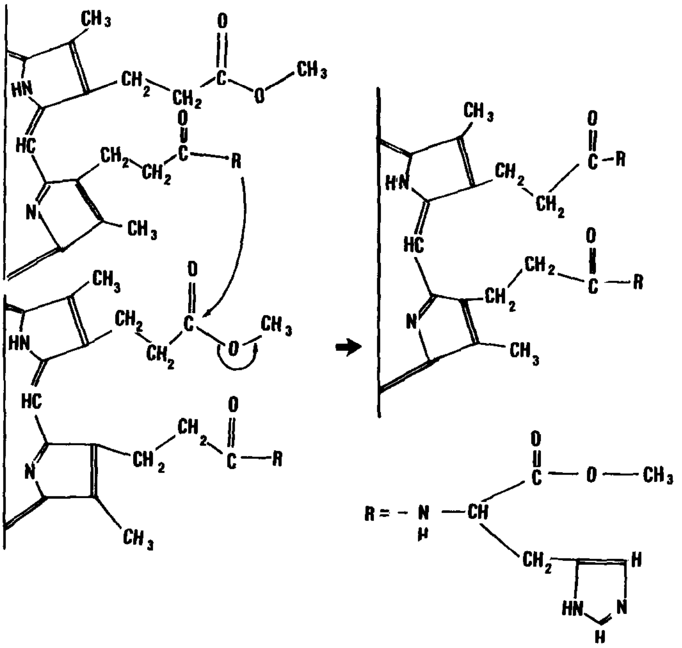
FAB	DCI (Methane)
827	704 (M+29)+
813	690 (M+15)+
690	676*
676*	612
616	597
602	552
590	539
507	524
479	510
465	492
433	466
421	451
413	434
405	407
391	391
379	377

*MH+ protonated molecule

(11). The reaction described here occurs with ions formed in the matrix as a result of FAB shown in Scheme 2 to yield the diamide.



Scheme 2. Transposition of N-Terminal Fragment



Scheme 1. FABMS Fragmentation Pattern of DMH Monoamide

The fragmentation of the parent compounds according to the patterns in Scheme 2 involves an intermolecular transposition of the N-terminal fragment. Ions appearing at M/Z 690 and 827 may be interpreted as being the result of a methylation similar to that which occurs in the matrix. As is apparent in Table I, a similar series is obtained in the DCI spectrum and also in other homologous series of porphyrins analyzed by DCI-MS. The in situ methylation of carboxyl functions has been reported to occur in glycerol matrices when MEOH/HCl is added (12).

Since the only artifact attributable to the thioglycerol matrix appears to be an ion at M/Z 107, it is of speculative interest at this time as to whether the product is a result of acidity and polarity factors inherent in the use of thioglycerol as a FAB sample matrix, the result of electronic factors in the compound itself, viz., its ability to aggregate in polar media at concentrations above 0.04 M, or a combination of both.

Summary

In addition to dimerization and polymerization of samples as previously suggested, it appears that during FAB-MS, reactions in the sample matrix can

occur to yield new compounds that are recombinations of molecular fragments. This type of reaction may be especially critical to the integrity of peptide sequencing using FAB, since the reactions cited in this report involve the formation and rupture of amides or peptide bonds.

References

1. Bradley, C. V., Williams, D. H., and Hanley, M. R. (1982) Biochem. Biophys. Res. Comm. 104, 1223-1230.
2. Williams, D. H., Bradley, C. V., Santikarn, S., and Bajesen, G. (1982) Biochem. J. 201, 105.
3. Reinhold, V. N., and Carr, S. A. (1982) Abstr. 30th Ann. Conf. Mass Spectrom. Allied Topics, Honolulu, Hawaii.
4. Farsberg, L. S., Dell, A., Walton, D. J., and Ballou, C. E. (1982) J. Biol. Chem. 257, 3555.
5. Rinehart, K. L. (1982) Science, 218, 254-260.
6. Rinehart, K. L. (1982) Science, 218, 255.
7. Campana, J. E., Ross, M. M., Rose, S. L., Wyatt, J. R., and Colton, R. J. Proc. of the Int. Workshop on Ion Form from Organic Solids (IFOS II) Munster, Germany, Sept. 1982.
8. Momenteau, M., Raugee, M., and Loock, B. (1976) Eur. J. Biochem. 71, 63.
9. Momenteau, M., Raugee, M., and Loock, B. (1976) Eur. J. Biochem. 71, 66.
10. Castro, C. E. (1974) Bioinorg. Chem. 4, 45-65.
11. Morris, H. R., Panico, M., Barber, M., Bordoli, R. S., Sedjwick, R. D., and Tyler, A. (1981) Biochem. Biophys. Res. Comm. 101, 623-631.
12. Martin, S. A., Costello, C. E., and Biemann, K. (1982) Anal. Chem. 54, 2362-2368.