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Reaction of Pentafluoropropionic Anhydride Vapor on Polypeptide as Revealed by Mass Spectrometry. A Carboxypeptidase Mimetic Degradation

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Pentafluoropropionic anhydride vapor at -18 °C degraded a dodecapeptide, AlaArgGlyIleLysGlyIleArgGlyPheSerGly to the peptides which are successively lacking from the C-terminus. The mass spectrum of the reaction mixture for 10 minutes indicated each molecular ions from the intact peptide up to the N-terminal dipeptide clearly, together with two types of successively degraded molecular ions, minus 18 and minus 45 M/Z respectively.

Protein amino acid sequencing is mainly performed by a stepwise degradation from the amino-terminus, a process commonly termed Edman degradation.[1] The method has been automated and widely accepted. As a means of reducing contamination from the reagents, the vapor method was introduced for the above degradation,[2] acid hydrolysis of protein[3] and carboxy(C)-terminal hydrazinolysis.[4] The present letter communicates that the reaction of pentafluoropropionic anhydride (PFPAA) vapor on polypeptides induces C-terminal successive degradation. This reaction provided sequence information much clearly than that commonly seen with carboxypeptidase degradation.

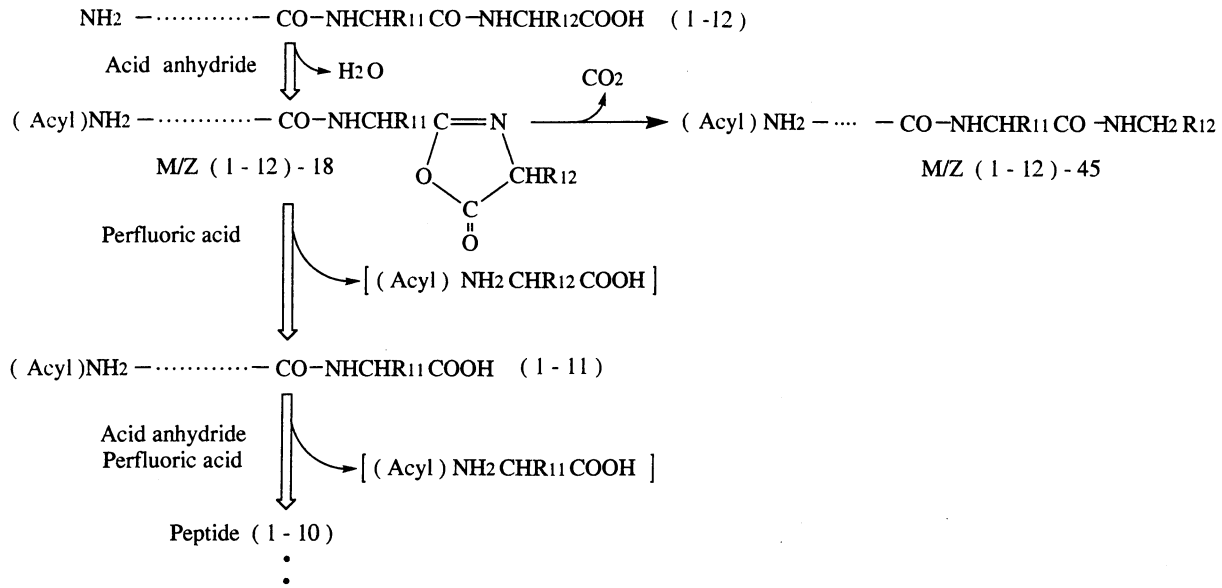
A dodecapeptide, AlaArgGlyIleLysGlyIleArgGlyPheSerGly (Peptide Institute Inc. Minoh), 3 nmol, was dried in a small test tube (6 mm x 40 mm). The tube was then placed in a large test tube (13 mm x 100 mm) which contained 100 µl (micro liter = $10^{-6}dm^3$) of 10% PFPAA in acetonitrile[5] (Nacalai Tesque Co. Kyoto, Japan). The large tube was flame-sealed under vaccum at -30 °C. The tube was maintained at reaction temperature of -18 °C for various time periods. After the reaction, the small tube was taken out and dried in vacuo. Two µl of dimethylformamide (Wako Chem. Osaka) was added to the small tube and 1 µl of the product solution was mixed with an equal volume of glycerol (Wako Chem.) as a matrix. One µl of the mixture was applied to a fast atom bombordment (possitive ion) mass

spectrometer (JEOL HX110 Japan), employing an acceleration voltage of 10 kV and xenon as an ionizing gas.

C-terminal successive degradation was observed to proceed even at -18 °C. And after only 10 min reaction time, degradation molecular ions (M/Z) were clearly detected (Fig.1b). Further successive C-terminal degradation was observed with longer reaction time. The results were quite different from the reaction of acetic anhydride vapor which afforded only oxazolone derivative at the C-terminus.

Another peptide having a C-terminal carboxyl-α-amide residue was exposed to PFPAA vapor. Under the conditions described above, the degradation by PFPAA was not observed, indicating a free α-carboxyl group is essential for the degradation.

The successively degraded molecular ions were observed to be accompanied by two types of successively degraded molecular ions, minus 18 and minus 45 M/Z. The minus 18 molecular ions may be the corresponding oxazolones at their C-termini, as indicated by their disappearence on water stream treatment of the products. The minus 45 molecular ions may due to decarboxylation. These observations suggest the following reaction scheme for this novel C-terminal successive degradation of polypeptides.



The continuation of the reaction for 30 min showed production of the other molecular ions having additional 146 M/Z (Fig.1c) indicating the pentafluoropropionylation of the main successively degraded molecular ions and their molecular ions together with the original dodecapeptide ions. Further continuation of the reaction time (up to 120 min) did not show

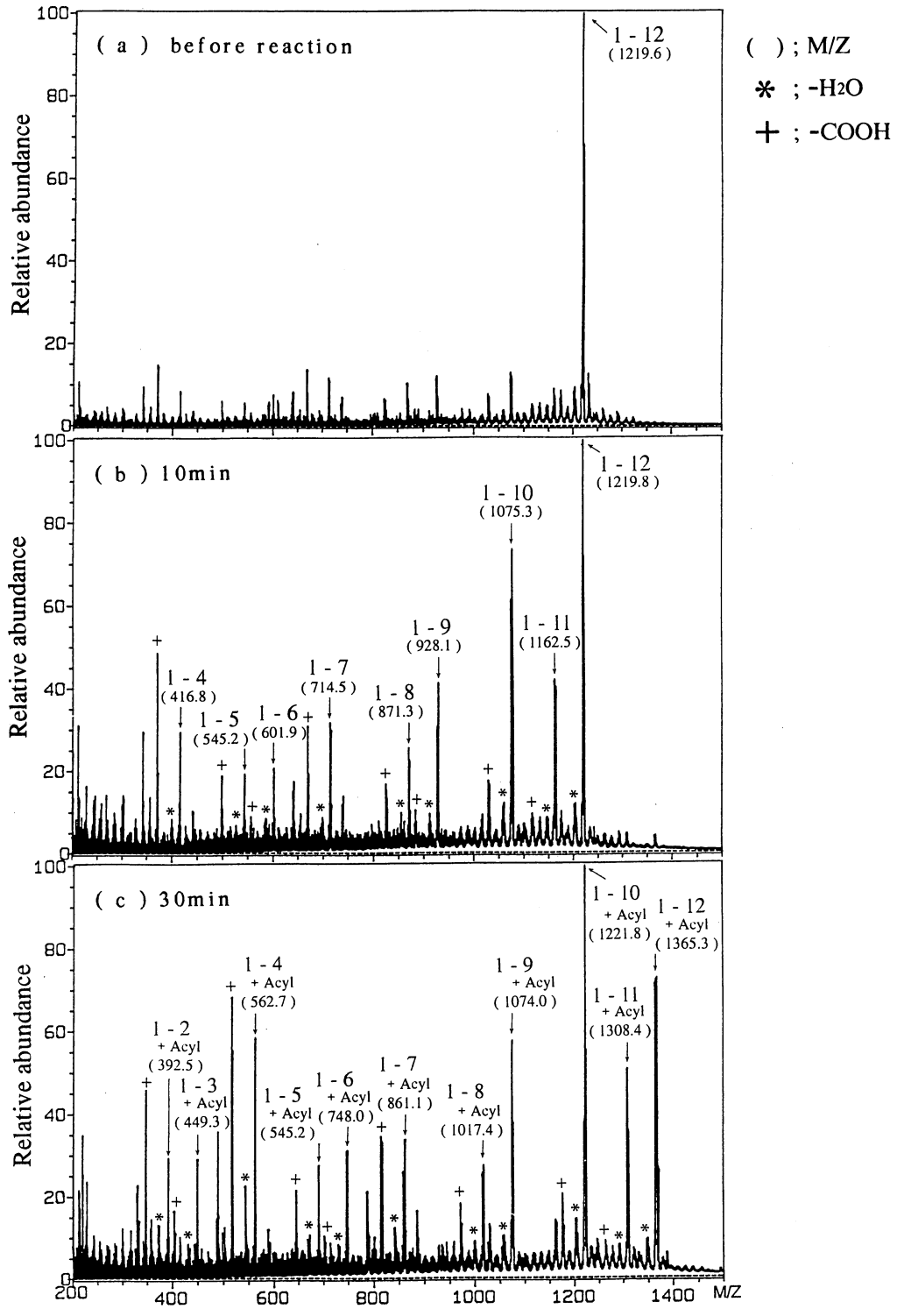


Fig. 1. FAB-mass spectra of successive degradation with PFPAA vapor on a dodecapeptide, Ala-Arg-Gly-Ile-Lys-Gly-Ile-Arg-Gly-Phe-Ser-Gly.

further successive degradation in the presence of excess amounts of the anhydride.

In summary, PFPAA vapor successively degrades polypeptides from the C-terminus. The reaction is clear and reproducible on a micro amount of peptide. The degradation of the dodecapeptide was identified almost completely through to the last dipeptide with a FAB mass spectrometry.

Complementing the conventional Edman amino-terminal sequencing, several C-terminal sequencing methods[6] have been proposed but unfortunately without practical success. C-terminal successive degradation with high concentration of perfluoroacid aqueous solutions[7] and the present work may provide a general C-terminal sequencing method of protein. Elucidation of the detailed mechanism of successive degradation is expected to be clarified.

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