# SPECIFIC CLEAVAGE OF C-METHIONYL PEPTIDE BONDS IN HYDROGEN FLUORIDE[1]

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It has recently been demonstrated (Sakakibara, Shin, and Hess, 1962; Lenard, 1964) that serine- and threonine-containing peptides undergo an N to O acyl shift in hydrogen fluoride, and that this reaction can serve as the basis for specific cleavage at N-seryl and N-threonyl peptide bonds. We now report a specific cleavage of C-methionyl peptide bonds which occurs on treatment of methionine-containing peptides with hydrogen fluoride. A different specific cleavage of C-methionyl peptide bonds has previously been reported from another laboratory (Gross and Witkop, 1961, 1962).

## MATERIALS

L-Methionyl Glycine was purchased from K & K Laboratories. Sulfone of L-Methionyl Glycine was prepared by oxidation of L-methionyl glycine with performic acid at 0° (Hirs, 1956). α-Melanocyte-Stimulating Hormone (α-MSH) was prepared according to a previously published procedure (Schally and Guillemin, 1960; Schally et. al., 1960).

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## METHODS

Reaction in Hydrogen Fluoride was carried out as previously described (Sakakibara, Shin, and Hess, 1962) at 30°. At least 0.5 ml of hydrogen fluoride and 4-10 mg of material were used in each experiment. Any N to O shift occurring at the serine residues in the α-MSH was reversed, after removal of the hydrogen fluoride, in basic aqueous solution (Lenard, 1964).

Determination of Cleavage of L-Methionyl Glycine was made by Van Slyke amino nitrogen analysis (Van Slyke, 1929; modification of Kendrick and Hanke, 1940). After removal of hydrogen fluoride, the reaction product was dissolved in a measured volume of distilled water and aliquots were taken for the analysis.

Paper Chromatography was used to confirm methionine as a product of the L-methionyl glycine reaction. Two solvent systems were used: (1) n-butanol: acetic acid: water (4:1:1); and (2) ethanol: water: 1N ammonia (90:5:5). For further confirmation, aliquots of the reaction mixture solutions were also applied to the paper over a dish of hydrogen peroxide in order to convert methionine to its sulfoxide (Thompson and Morris, 1959), which has a much lower Rf value in both solvent systems.

Paper Electrophoresis in pyridine-actate buffer at pH 4.5 (Galmiche, 1962) was used to separate components of the reaction mixtures. Products from the dipeptide were separated at 70 v/cm (one hour), and products from α-MSH at 17.5 v/cm (3-4 hours).

Gel filtration on Sephadex was used to separate components of the α-MSH reaction product. Sephadex G-25 was equilibrated with 50% acetic acid (Crestfield, Moore, and Stein, 1963) and poured into a 150 x 0.9 cm column. The column was kept at 25° during the separation. A flow rate of 12 ml per hour was maintained, and 1 ml fractions were collected. Aliquots were analyzed with the Folin phenol reagent (Lowry et. al., 1951).

## RESULTS

As shown in Table I, treatment of the dipeptide L-methionyl glycine with hydrogen fluoride results in the release of amino nitrogen. After 36 hours in hydrogen fluoride, 100% cleavage is obtained, as judged by amino nitrogen release. By contrast, the sulfone of L-methionyl glycine is stable to treatment with hydrogen fluoride (Table I).

The products from L-methionyl glycine were identified as methionine and glycine by: (1) Paper electrophoresis, which separated methionine, glycine, and the starting dipeptide. No dipeptide was detected in the L-methionyl glycine product after 36 hours of treatment, while after similar treatment the sulfone sample showed only the single spot corresponding to the dipeptide. (2) Paper chromatography in two different solvent systems, before and after oxidation of the material (see METHODS). (3) Analysis on an automatic amino acid analyzer, which showed the presence of equimolar amounts of methionine and glycine, accounting for over 95% of the starting material.

TABLE I

Reaction of L-Methionyl Glycine and its Sulfone in Hydrogen Fluoride, 30°

| Compound | Time in HF (Hours) | Moles Amino Group / Mole Dipeptide |
|---|---|---|
| L-Methionyl Glycine | 0 | 0.97 |
| | 12 | 1.64 |
| | 36 | 1.98 |
| Sulfone of L-Methionyl Glycine | 0 | 0.86 |
| | 34 | 0.89 |

The amino acid sequence of α-MSH is as follows (Harris and Lerner, 1957):

Ac-Ser-Tyr-Ser-MET-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val-$NH_2$.
1 2 3 4 5 6 7 8 9 10 11 12 13

Cleavage at the C-methionyl bond yields two peptides, 1-4 and 5-13. After 48 hrs. treatment

of α-MSH with hydrogen fluoride, these two fragments were separated by paper electrophoresis and by Sephadex chromatography (Figure I). Amino acid analysis of fractions is compared, in Table II, with theoretical values for these two peptide fragments. As shown in the table, an amino acid analysis corresponding to peptide 5-13 was obtained for the fraction separated by electrophoresis as Spot B (Figure I). This peptide was also obtained in Peak I (Figure I) from the Sephadex column, in about 45% yield (based on the starting material); the unchanged material, peptide 1-13, was present in the same fraction in about 30% yield. Peptide 1-4 was obtained in the Peak III fraction (Figure I) from the Sephadex column in about 35% yield. Peak II contained very little material, despite its apparent size, and could not be characterized.

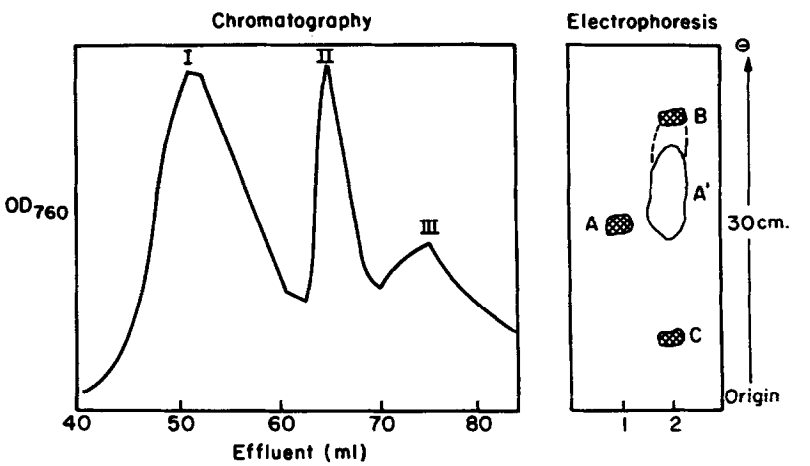


Figure 1

Separation of fragments after treatment of α-MSH in Hydrogen Fluoride for 48 hrs., 30°

The applicability of this cleavage to other proteins of known sequence is under study.

TABLE II

Amino Acid Composition of Fractions Isolated From α-Melanocyte-Stimulating Hormone After Treatment with Hydrogen Fluoride

| Residue | Theoretical 1-4 | Found (a) | Theoretical 5-13 | Found (b) | Found (c) |
|---|---|---|---|---|---|
| Arginine | 0 | T | 1 | .86 | .90 |
| Glutamic Acid | 0 | .29 | 1 | .80 | .91 |
| Glycine | 0 | .09 | 1 | 1.24 | .98 |
| Histidine | 0 | .29 | 1 | .82 | .94 |
| Lysine | 0 | T | 1 | 1.15 | .90 |
| Methionine | 1 | 1.10 | 0 | 0 | .41 |
| Phenylalanine | 0 | 0 | 1 | .83 | .96 |
| Proline | 0 | T | 1 | 1.09 | 1.28 |
| Serine | 2 | 1.91[e] | 0 | 0 | .72[e] |
| Tyrosine | 1 | 1[d] | 0 | 0 | .22 |
| Valine | 0 | T | 1[d] | 1[d] | 1[d] |

a - Fraction III, Figure I
b - Fraction B, Figure I
c - Fraction I, Figure I
d - Taken as basis for calculation
e - Uncorrected
T - Trace

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