THE N,O PEPTIDYL SHIFT IN ANHYDROUS HYDROGEN FLUORIDE

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The reversible, pH dependent, N,O acyl migration first observed by Bergmann (1923) has been the basis of several attempts to develop a chemical method for the specific cleavage of protein chains adjacent to seryl and threonyl residues. Although in strong acids the N,O acyl shift has so far been investigated only with two serine containing model peptides (Moore et al, 1954; Hoermann et al, 1956), a great number of attempts have been made to induce acyl migration in proteins by means of strong acids (Cohen and Witkop, 1961). In this regard, concentrated sulfuric acid appeared most promising (Elliot, 1953), even though a great number of complications were encountered; the acyl migration occurred in low yields with unspecific hydrolysis of peptide bonds; the formation of sulfate esters and the destruction of tryptophyl, arginyl, tyrosyl, and methionyl residues has been reported (Cohen and Witkop, 1961).

Recent reports by Katz (1954a) suggested that anhydrous hydrogen fluoride is vastly superior to sulfuric acid as a solvent for proteins. In the instances investigated, the biological activity of proteins was not altered irreversibly by anhydrous HF (Katz, 1954b). This solvent, unlike sulfuric acid, is easily removed from proteins dissolved in it. The fact that anhydrous HF is a strong acid (The Hammet acidity function (H_0) is approximately -10), (Hyman

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et al, 1957), suggested to us that the N,O acyl migration would proceed in good yields in this solvent. This possibility has now been explored with the use of synthetic serine and threonine containing peptides. For comparison purposes, some experiments were conducted using concentrated sulfuric acid (Mallinckrodt Reagent Grade) as the solvent.

The peptides or amino acid derivatives which were prepared for these studies are: glycyl-DL-serine, O-glycyl-DL-serine, glycyl-L-threonine, and O-glycyl-L-threonine which has been synthesized here for the first time. In experiments using anhydrous hydrogen fluoride, about 0.1 g. of dried peptide or amino acid derivative was placed in Kel-F reaction vessels which were attached to a monel vacuum line. Hydrogen fluoride was dried over cobaltous fluoride and about 5 ml. were distilled into the reaction vessels which were held at liquid nitrogen temperature. The reaction was then allowed to proceed at room temperature. In experiments in concentrated sulfuric acid about 0.1 g. of peptide was dissolved in 2 ml. of concentrated sulfuric acid at -20° and the reaction was then allowed to proceed at room temperature. All experiments were followed by Van Slyke amino nitrogen determinations, paper electrophoresis, and by crystallization and identification by classical means of the products obtained in hydrogen fluoride.

The Van Slyke amino nitrogen values were standardized with glycine, serine, threonine, and the synthetic peptides. Excellent agreement with the theoretical values were obtained with the exception of the values for the N-glycyl peptides which were higher than expected, in agreement with earlier reports (Kendrick and Hanke, 1940). Glycyl-DL-serine gave 115% of the theoretical value, glycyl-L-threonine gave 117%. The amino nitrogen values were also obtained after treating the O-peptides with bicarbonate to obtain the N-acyl derivatives. About 100 μM. of the O-peptide were treated with 50 ml. of 0.05 N sodium bicarbonate for 24 hours at room temperature. If the treatment with the HF had caused hydrolysis of the peptide bond instead of an N,O shift, then the migration in alkali could not take place and the amino nitrogen value

would be very high. This reasoning was tested by subjecting the synthetic O-peptides to the bicarbonate treatment. The actual amino nitrogen values of the products obtained from bicarbonate treatment of the synthetic O-peptides were about 5% less than that expected for complete reversal.

The Van Slyke amino nitrogen values obtained in the experiments are listed in Table I.

TABLE I

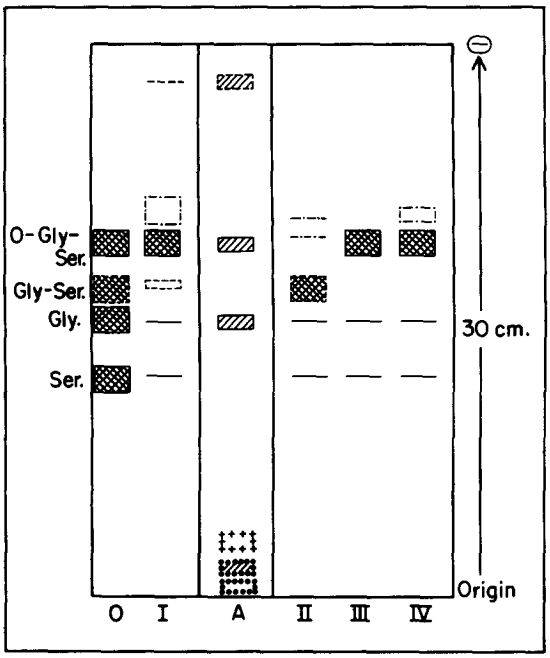
N,O ACYL MIGRATION IN HYDROGEN FLUORIDE

Reaction Mixture	Days of Reaction	Micromoles Amino Nitrogen	
		Reaction Mixture	Reaction Mixture After Bicarbonate Treatment
glycyl-DL-serine 617 μM. in 5 ml.HF (710 μM. amino nitrogen)	15	1210 (96%)[a]	680 (100%)[b]
glycyl-L-threonine 568 μM. in 5 ml.HF (665 μM. amino nitrogen	12	1110 (96%)[a]	630 (100%)[b]
DL-alanyl-DL-serine 568 μM. in 5 ml.HF (560 μM. amino nitrogen)	12	1040 (86%)[a]	570 (101%)[b]

[a](μM. amino nitrogen observed - μM. amino nitrogen of N-acyl compound) x 100 / (μM. amino nitrogen of synthetic O-acyl compound - μM. amino nitrogen of N-acyl compound).
[b]The amino nitrogen value obtained after treating the synthetic O-acyl compound with bicarbonate was taken as 100%.

As judged by this method the peptides are converted in about 90% yield to the corresponding O-peptides in anhydrous hydrogen fluoride. The data obtained after bicarbonate treatment indicate that the increase in amino nitrogen obtained in HF is due to a N,O shift and not due to hydrolysis of the peptide. This conclusion is further substantiated by the paper electrophoresis ex-

periments. Similar electrophoretograms were obtained with all peptides treated with HF. The data obtained with glycyl-DL-serine are documented in Figure 1. It can be seen (Figure 1) that in HF almost quantitative conversion of glycyl-DL-serine to O-glycyl-serine is obtained. Only traces of starting material and of side products can be detected. N,O acyl migration in HF was further substantiated by crystallization of O-glycyl-DL-serine from the HF reaction mixture. The compound was obtained as the mono-hydrochloride in 70% yield and has the same I.R. spectrum and melting point as the authentic synthetic compound.



FIGURE

N,O Acyl Migration in Glycyl-DL-Serine. Paper Electrophoresis Experiments. O - Mixture of synthetic compounds; I - 12 day hydrogen fluoride reaction mixture; II - 12 day hydrogen fluoride reaction mixture after bicarbonate treatment (See Text); III - Synthetic O-glycyl-DL-serine HCl; IV - Crystalline O-glycyl-DL-serine HCl, isolated from 15 day hydrogen fluoride reaction mixture; A - 6 day concentrated sulfuric acid (See Text) reaction mixture. Experiments run in 1 N acetic acid, 15 volts cm^{-1}, at 0° for 3 hours. Shading and size of spots denotes relative concentrations. Lines denote traces of material. Lines surrounding spots denote color: Ninhydrin purple _____; Ninhydrin yellow with a change to purple after several hours ---; Ninhydrin yellow with a change to purple after one day -•-•-; Ninhydrin yellow +++ ; Ninhydrin yellow brown • • • .

In Figure 1A are the results obtained with glycyl-DL-serine in sulfuric acid. Van Slyke amino nitrogen determinations indicate a maximum N,O acyl shift of 35%. The paper electrophoresis experiment leads to the detection of a great number of side products. Some of the side products are due to the formation of the sulfate ester of serine. Treatment of serine with sulfuric acid for two hours under the conditions used in these experiments leads to the isolation of the crystalline O-serine sulfate ester in over 60% yield (Reitz et al, 1946).

The experiments presented indicate that N,O acyl migration proceeds in anhydrous HF in high yields without unspecific cleavage of amide bonds or the formation of significant amounts of side products. Anhydrous HF is vastly superior to sulfuric acid as a solvent in the experiments performed and its use in a method for the specific cleavage of protein chains appears very promising. However, the influence which steric or inductive effects in these peptides have on the N,O acyl migration is not known. These questions must be answered with model peptides before investigating the use of HF as an agent for inducing acyl migration in proteins. Experiments along these directions are in progress.

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