amino acids by the dinitrophenylation method. In both cases, it was found that tyrosine and serine were present in a one to one molar ratio.

As these experiments indicated, about 0.8 mole of the acetyl peptide/mole was obtained from the chymotryptic digests of I-peptide and TMV-protein. The synthetic acetyl peptide was recovered in a similar yield. The loss of 20 % might be caused by an acetyl shift from N to O under the conditions of the chromatography used. In conclusion, the good agreement in recovery in all chromatographic treatments suggests that the I-peptide actually contains one mole of N-acetylseryltyrosine.

The fact that the I-peptide does not contain lysine indicates that the acetyl peptide is located in the N-terminal position of the I-peptide and the possibility of branching from the ε-amino group of lysine must be excluded. Thus it may be concluded that N-acetylseryltyrosine occupies the N-terminal position of TMV-protein and of the I-peptide located at the N-terminus of the TMV-peptide chain.

Appreciation is expressed to Dr. H. FRAENKEL-CONRAT, Dr. L. K. RAMACHANDRAN and Dr. W. M. STANLEY for their helpful suggestions and discussions throughout the investigation. This work was aided by U.S. Public Health Service Training Grant CRTY-5028.

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Received June 30th, 1959

Biochim. Biophys. Acta, 38 (1960) 145–146

The stabilization of unstable amino acids in polypeptides by desulphurization and hydrogenation using tritium

The instability of several amino acids during the cleavage of peptide chains presents one of the main problems encountered in the investigation of protein structures. Major difficulties in this field are caused by tryptophan which is destroyed by the process of hydrolysis by acid and during oxidation by performic acid of sulphur-containing amino acids. Further obstacles arise from the instability of tyrosine, cysteine and methionine.

Making use of our previous experience[1] we attempted to overcome these procedural difficulties by transforming tryptophan into the stable octahydrotryptophan derivative by hydrogenation. The application of this procedure to protein hydrolysates, however, requires desulphurization to be performed as a first step, since the hydrogenation of tryptophan is impeded by sulphur-containing amino acids. On desulphurization, cysteine is converted into alanine, and methionine into α-aminobutyric acid. Using tritium, alanine arising from cysteine may be labelled and thus distinguished from the original alanine residues of the protein molecule. The hydrogenation of tryptophan is paralleled by the reduction of tyrosine and phenylalanine.

A chromatographic procedure involving the passage of solutions of sulphur-containing amino acids or the corresponding peptides through a column of Raney

nickel[2] operated with cooling was used for desulphurization. When using 400 mg of Raney nickel (column dimensions 10 × 3 mm) quantitative desulphurization of 5 mg cysteine and 0.7 mg methionine was obtained. Obviously, the total desulphurizing capacity of the column is much higher, but the filtrate then contains both the desulphurized and the original material. Under the same conditions, glutathione was transformed quantitatively to pure γ-glutamylalanylglycine. Using this procedure the losses in aromatic peptides due to adsorption are insignificant.

The hydrogenation of aromatic amino acids depends both on pH and on the catalyst used. In the acidic range, and in the presence of palladium or platinum, tryptophan and phenylalanine are readily converted into the basic octahydrotryptophan and cyclohexylalanine, respectively. In the case of tyrosine, the formation of a mixture of hexahydrotyrosine and cyclohexylalanine in the slightly acidic or neutral range was observed, in accordance with the findings of WASER AND BRAUCHLI[4]. The ratio of these compounds cannot be influenced by a change of catalyst, *e.g.* palladium for platinum. From our observations[3], hexahydrotyrosine is unstable during acid hydrolysis, being decomposed mainly into cyclohexenylalanine. On hydrogenation in the acid (0.08 *N* to 0.1 *N* HCl) and using the platinum catalyst, tyrosine is transformed only into cyclohexylalanine, which is quite stable under the conditions of hydrolysis.

The procedures described have been carried out also using tritium. The technical process of the desulphurization reaction is very simple, since it consists in passing a solution of the substance through a column of Raney nickel, previously equilibrated on standing with ^3H_2O. In a typical experiment cysteine was converted to [^{3}H]alanine with a stable specific activity of 4 mC/mmole. The hydrogenation reactions were carried out in a closed system where gaseous tritium was developed by the decomposition of ^3H_2O by metallic lithium. The technical process is more complicated in comparison with that used for desulphurization, since an excess of isotope is required. In a typical experiment a chromatographically homogeneous sample of octahydrotryptophan possessing a stable specific activity of 60 mC/mmole was obtained from tryptophan. In all cases the isotope was removed from ionizable groups by repeated lyophilization.

The application of the procedures described to a peptic hydrolysate of chymotrypsinogen resulted in the conversion of cysteine, methionine, and the aromatic amino acids residues into [^{3}H]alanine, [^{3}H]α-amino-butyric acid, and [^{3}H]octahydrotryptophan or [^{3}H]cyclohexylalanine, respectively. All these compounds are stable during the subsequent treatment. It is in our opinion that the procedure described may be of general value in the elucidation of protein structures.

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Received June 18th, 1959