Short Communications

Studies on the N-terminal sequence of tobacco-mosaic-virus protein

In a recent paper from this laboratory, NARITA reported the separation of approximately one mole of N-acetylseryltyrosine from the chymotryptic and peptic digests of the protein of tobacco mosaic virus[1]. Even though his work suggested that the acetyl peptide was located in the N-terminal position of TMV-protein, there remained the possibility that the acetylpeptide was derived from a branch chain linked to the ε-amino group of one of the lysine residues of TMV-protein[1,2]. Definite evidence for the location of N-acetylseryltyrosine at the N-terminus of the protein is reported in this paper.

The following three products were investigated: a chymotryptic digest of TMV-protein and synthetic N-acetylseryltyrosine, both prepared according to NARITA[1]; and a chymotryptic digest of the insoluble peptide (I-peptide) obtained as a precipitate at pH 4.5–4.7 after digestion of TMV-protein with trypsin. From a study of the I-peptide[3] it was known that it consists of about 40 amino acid residues and that it does not contain a lysine residue nor any amino terminal residue which is reactive to dinitrofluorobenzene. This peptide was digested to a point where 3.7 moles of amino residues were liberated.

The three materials were chromatographed under almost identical conditions on a column (0.9 × 20 cm) of Dowex 50 X 2 in hydrogen form. The absorption of the effluent was measured at 280 mμ, and those tubes which showed absorption were again measured from 300 to 250 mμ using the Cary recording spectrophotometer. The fractions which showed the typical absorption spectrum of tyrosine were pooled. An aliquot of each fraction was made 0.1 *N* in NaOH and the absorption was measured. The absorption value obtained and the volume of each fraction gave the recovery of acetylseryltyrosine of each sample based on the molecular extinction coefficient of tyrosine. The results are shown in Table I.

Lastly, to confirm that the absorption values observed with the digests were due to acetylseryltyrosine, the two peptide fractions obtained from the chymotryptic digests of I-peptide and of TMV-protein were hydrolyzed by acid and analyzed for

TABLE I

RECOVERY OF ACETYL PEPTIDE FROM I-PEPTIDE, TMV-PROTEIN AND SYNTHETIC PEPTIDE

	Amount used (μmoles)	*Amount recovered (μmoles)**	*Recovery (%)*
I-peptide	4.95	3.93	79.4
TMV-protein	4.47	3.47	77.6
Acetylseryltyrosine	3.96	3.21	81.1

* These values are calculated using a molecular extinction coefficient of $1.97 \cdot 10^{-3}$ for tyrosine in 0.1 *N* NaOH at 284 mμ.

Abbreviation: TMV, tobacco mosaic virus.

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.

Virus Laboratory, University of California, Berkeley, Calif. (U.S.A.) A. TSUGITA

[1] K. NARITA, *Biochim. Biophys, Acta*, 28 (1958) 184.
[2] H. FRAENKEL-CONRAT AND L. K. RAMACHANDRAN, *Advances in Protein Chemistry*, in the press.
[3] L. K. RAMACHANDRAN AND A. TSUGITA, to be published.

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