THE ISOLATION OF THE C-TERMINAL PEPTIDE FROM A TRYPTIC DIGEST OF TOBACCO MOSAIC VIRUS (TMV) PROTEIN ESTABLISHING A THIRD TRYPTOPHAN RESIDUE IN TMV‡

Duane T. Gish

Virus Laboratory, University of California
Berkeley 4, California

Determination of the tryptophan content of TMV by earlier workers using a variety of methods yielded values that would correspond to about 2-3 residues of tryptophan per protein subunit. By a spectrophotometric method Fraenkel-Conrat and Singer (1957) had found a tryptophan content of the virus varying from 2.6 to 3.2%. Knight and Stanley (1941) using the glyoxylic acid procedure of Shaw and McFarlane (1938), found the tryptophan content of TMV to be 4.5%. Using a microbiological assay technique Knight (1947) had reported 2.3% and Stokes, Gunness, Dwyer and Caswell (1945) had reported 2.1%. Ramachandran (1958) used a colorimetric method and reported values varying from 2.2 to 3.1% for the tryptophan content of TMV protein which differs only about 5% in weight from the intact virus. A value of about 2.3% would indicate two tryptophan residues per protein subunit and a value of about 3.4% would indicate three tryptophan residues per subunit. Thus considerable uncertainty remained concerning the actual tryptophan content of TMV protein. This problem has now been resolved by the isolation of three tryptophan peptides from a tryptic digest of TMV protein, the third of which is the subject of this paper.

From tryptic digests of TMV protein, a peptide fraction insoluble at pH 4.7 had been isolated and designated as the I-peptide (Gish, Ramachandran and Stanley, 1958). This peptide, after purification, was

‡ Aided by a grant from the United States Public Health Service.

found to contain about 40 amino acid residues, including one mole of tryptophan (Ramachandran and Tsugita, to be published). The I-peptide was shown to be derived from the N-terminal end of TMV protein since it yielded upon chymotryptic digestion about one equivalent of N-acetylseryltyrosine (Tsugita, 1959). Narita (1958) had previously reported the isolation of N-acetylseryltyrosine from chymotryptic and peptic digests of the complete TMV protein.

The peptide material from the tryptic digest of TMV protein soluble at pH 4.7 was recovered by lyophilization. This material was stirred with a mixture of 2-butanol and 0.1 M dichloroacetic acid (DCA) and the fraction soluble in this solvent was subjected to countercurrent distribution in the same solvent system (Gish, et al, 1958). From the fraction with distribution coefficient (K) of about 0.7 was isolated, after further purification by electrophoresis, a second tryptophan-containing peptide designated K-0.7-A. This peptide was shown to have the sequence pyroglu. phe. ser. glu.^{NH_2} val. try. lys. pro. ser. pro. glu.^{NH_2} val. thr. val. arg* (Gish, 1959). By its amino acid composition and sequence this peptide is known not to contain the same tryptophan residue found in the I-peptide.

When, as previously mentioned, the peptide material from the tryptic digest of TMV protein soluble at pH 4.7 was stirred with the 2-butanol-0.1 M DCA solvent used for the countercurrent distribution, a fraction, amounting to about 10-15% of the total, remained insoluble. This DCA-insoluble fraction was subjected to electrophoresis at pH 7 or 9 and was found to contain three components: two arginine-containing peptides, one in minor amount, and a peptide which gave a negative test for arginine but which gave a positive test for tryptophan. The amino acid composition of this third tryptophan-containing peptide, as

* Abbreviations for the amino acid residues are those suggested by E. Brand and J. T. Edsall, Ann. Rev. Biochem. 16 (1947) 244, pyroglu being used to represent pyroglutamic acid (pyrrolidone carboxylic acid).

TABLE I

The Amino Acid Composition of the C-Terminal Peptide from a Tryptic Digest of TMV Protein

Amino Acid	Molar Ratio	Assumed Number of Residues
ala	1.1	1
glu	1.3	1
gly	1.7	2
leu	1.0	1
phe	1.0	1
pro	1.1	1
ser*	5.9	6
thr	2.2	2
val	1.0	1
try**	1.0	1

*Serine was identified as the N-terminal amino acid by its DNP-derivative.

**Tryptophan was determined spectrophotometrically and colorimetrically before hydrolysis.

determined by the dinitrophenylation (DNP) method of Levy (1954), was that shown in Table I. Serine was shown to be N-terminal by the same method. Tryptophan was determined before acid hydrolysis by the spectrophotometric method of Goodwin and Morton (1946) and the colorimetric method of Spies and Chambers (1949). Leucine was distinguished from isoleucine by hydrolysis of the DNP-leucine in concentrated ammonium hydroxide and chromatography of the liberated leucine in 2-butanol-3% ammonium hydroxide (3:1) (Roland and Gross, 1954). DNP-glutamic acid was distinguished from DNP-aspartic acid by the method previously described (Ramachandran and Gish, 1959).

When this peptide was treated with carboxypeptidase approximately

one mole of threonine, and only threonine, was released. Threonine, and only threonine, is split from TMV by the action of carboxypeptidase (Harris and Knight, 1952, 1955). From a chymotryptic digest of the peptide, by a combination of paper chromatography in n-butanol-acetic acid-water (4:1:5) and paper electrophoresis at pH 5.5 in a pyridine-acetic acid buffer, a peptide was isolated which, when analyzed by the DNP method, showed the following composition: N-terminal thr, 0.63, ser, 0.94, gly, 0.95, pro, 1.14, ala, 1.00, and thr, 1.12. Niu and Fraenkel-Conrat (1955 a, b) had earlier reported the C-terminal sequence of TMV protein to be thr. ser. gly. pro. ala. thr. It is apparent then, that this tryptophan-containing peptide is from the C-terminal end of TMV protein.

The crude DCA-insoluble material, which, as previously mentioned, contained three peptides, was treated with N-bromoacetamide (NBA) essentially according to the procedure of Patchornik, Lawson and Witkop (1958). This reagent had been shown by these workers to split tryptophyl peptide bonds, though the yields are sometimes rather low. The following results were obtained: N-terminal amino acids (mole ratio) before NBA cleavage (determined by the DNP method), ser, 1.00, ileu, 0.73, glu, 0.13: after NBA cleavage, ser 1.00, ileu, 0.53, glu, 0.10, thr, 0.18. Thus with the crude mixture, the reagent cleaved a tryptophan-threonine bond in about 20% yield. When the purified tryptophan-containing peptide itself was treated with this reagent, the yield of N-terminal threonine was about 10% of the N-terminal serine. This information, supplemented by the fact that a threonine N-terminal peptide was found in a chymotryptic digest of the peptide, established a tryptophyl-threonine bond in the peptide.

A partial structure for this peptide may thus be written: ser (ser_4, glu, gly, leu, phe, val) try. thr. ser. gly. pro. ala. thr. The presence of three tryptophan residues in TMV has thus been definitely established: one each in the N-terminal I-peptide, in the peptide designated K-0.7-A, and in the C-terminal peptide.

REFERENCES

Fraenkel-Conrat, H., and Singer, B. (1957). Biochim. Biophys. Acta 24, 540.

Gish, D. T., Ramachandran, L. K., and Stanley, W. M. (1958). Arch. Biochem. Biophys. 78, 433.

Gish, D. T. (1959). Biochim. Biophys. Acta. in press.

Goodwin, T. W., and Morton, R. A. (1946). Biochem. J. 40, 628.

Harris, J. I., and Knight, C. A. (1952). Nature 170, 613.

Harris, J. I., and Knight, C. A. (1955). J. Biol. Chem. 214, 215.

Knight, C. A. (1947). J. Biol. Chem. 171, 297.

Knight, C. A., and Stanley, W. M. (1941). J. Biol. Chem. 141, 39.

Levy, A. L. (1954). Nature 174, 126.

Narita, K. (1958). Biochim. Biophys. Acta 28, 184.

Niu, C. I., and Fraenkel-Conrat, H. (1955a). Biochim. Biophys. Acta 16, 597.

Niu, C. I., and Fraenkel-Conrat, H. (1955b). Arch. Biochem. Biophys. 59, 538.

Patchornik, A., Lawson, W. B., and Witkop, B. (1958). J. Amer. Chem. Soc. 80, 4747.

Ramachandran, L. K. (1958). Virology 5, 244.

Ramachandran, L. K., and Gish, D. T. (1959). J. Amer. Chem. Soc. 81, 884.

Roland, J. F., and Gross, A. M. (1954). Anal. Chem. 26, 502.

Shaw, J. L. D., and McFarlane, W. D. (1938). Canad. J. Research, Sect. B. 16, 361.

Spies, J. R., and Chambers, D. C. (1949). Anal. Chem. 21, 1249.

Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C. (1945). J. Biol. Chem. 160, 35.

Tsugita, A. (1959). Biochim. Biophys. Acta (in press).

Received August 6, 1959