LOCALISATION OF THE THREE AMINO ACID EXCHANGES IN THE COAT-PROTEIN OF TMV-06

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1. Introduction

Tobacco mosaic virus strain 06 (TMV-06) was originally isolated from the orchid *Phalaenopsis* by Kado et al. [1]. On the basis of amino acid analyses of the coat-protein these authors suggested the following amino acid exchanges: Histidine, methionine, and arginine in TMV-06 instead of glutamic acid or glutamine, isoleucine, and alanine in vulgare. It was the aim of this work to establish the position of these amino acid exchanges in the primary structure of the protein. Of particular interest was the position of the methionine residue in TMV-06. Since wild type TMV contains no methionine, single methionine mutations [2] proved a good opportunity for studying physicochemical and structural properties of large peptides which are derived by cyanogen bromide cleavage.

2. Materials and methods

2.1. *Virus and virus protein*

A sample of TMV-06 was kindly provided by Dr. R.N. Perham, Cambridge, England. From this sample more virus was grown on *Nicotiana tabacum* L. *cv. Samsun*. The virus was extracted from frozen leaves and purified by the method of Leberman [3]. The coat-protein was prepared by the acetic acid method [4].

2.2. *Carboxymethylation*

Prior to all experiments described in this paper the coat protein was carboxymethylated at the cysteine-residue [5].

2.3. *Cleavage with cyanogen bromide*

An equal weight of carboxymethyl-protein and cyanogen bromide was used and the reaction carried out in 70% formic acid for 20 hr [6].

2.4. *Digestion with proteases*

Trypsin (TPCK, 198 μ/mg) and chymotrypsin were obtained from Worthington Biochem. Corp., Freehold, N.J. They were kept frozen as 1% solutions in 1 mM HCl. Digestions were done in 0.5% NH_4HCO_3 at a concentration of carboxymethylated protein of 2–5 mg/ml for 6 hr at room temperature. A ratio of 1:100 (w/w) of protease to protein was used. The digestion was stopped by lyophilisation. Carboxypeptidase A (COADFP, Worthinton) was used according to the procedure of Ambler [7].

2.5. *Peptide maps*

Peptide maps were prepared in two or three dimensions [8, 9]. High voltage paper electrophoresis (HVPE) [10, 11] at pH 6.5 for 45 min at 3000 V was used in the first dimension, and descending chromatography in butan-1-ol-acetic acid–water–pyridine (15:3:12:10, by vol.) [12] in the second dimension. For those peptides which were neutral at pH 6.5, HVPE at pH 3.5 for 45 min at 3000 V was used in the third dimension. The peptide maps were stained with ninhydrin–cadmium acetate reagent [13]. For localising the histidine containing peptide Pauly's reagent was used [14]. When maps were to be used for preparing peptides, they were stained with 0.02% ninhydrin [15] prior to eluting the peptides from the paper with 0.02 M ammonia. In

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general peptides were prepared by dissolving the lyophilised digest in 0.01 M ammonia (approx. 10 mg digest/0.1 ml) and applying this solution as a band of 1 mg/cm on Whatman 3 MM paper. After each separation step guide strips from the sides of the peptide bands were stained with ninhydrin–cadmium acetate reagent. The corresponding unstained bands were then cut out and sewn onto new sheets of chromatography paper for further purification. When a peptide band was considered pure, it was eluted from the paper and dried over concn. H_2SO_4 in vacuo.

2.6. *Amino acid analysis*

Amino acid analysis was carried out on a Locarte amino acid analyser [16] after hydrolysis of the peptides in 6 M HCl at 108°C in vacuo for 22 hr. When the lactone of homoserine was expected to be present the dried hydrolysate was treated with 2 M NH_3 for 1 hr at 37°C, and dried again prior to amino acid analysis. By this procedure homoserine lactone is converted to homoserine [17].

2.7. *Dansylation*

The N-terminal amino acid residues of the isolated peptides were identified by means of the dansyl technique [18, 19]. The dansyl amino acids were separated by thin layer chromatography on polyamide sheets (Cheng Chin Trading Co., Taipeh, Taiwan) by use of the solvent systems of Woods and Wang [20].

2.8. *Nomenclature of the peptides*

Peptides produced by cleavage with trypsin were assigned a capital T, chymotryptic peptides a Ch. The numbers behind T or Ch, respectively, represent the position of a given peptide in the primary structure of the protein [21, 22]. Peptides derived from cyanogen bromide cleaved protein are designated as CB followed by T, according to the way of further digestion.

3. Results

3.1. *Localisation of histidine*

The chymotryptic peptide containing histidine (Ch 3–10, see table 1) was treated with carboxypeptidase A. After 10 min 0.92 μmoles phenylalanine and 0.76 μmoles of histidine were released per μmole of peptide. After 240 min in addition to Phe and His 0.15 μmoles of serine were liberated per μmole of peptide. This confirms the position of serine as no 8, histidine as no 9, and phenylalanine as no 10 in the sequence of TMV-06 protein.

3.2. *Localisation of arginine*

On a two-dimensional map the only tryptic peptide of vulgare negatively charged at pH 6.5 (T 62–68) is not present on the peptide map of 06 under the same conditions. The bands which had been neutral at pH 6.5 were subjected to HVPE at pH 3.5 yielding one band (T 114–122) in the case of vulgare protein and three bands in the case of 06 protein. These three bands were shown to contain four peptides (see table 1): 1) A mixture of T 114–122 and T 62–65; 2) T 62–68; 3) T 66–68.

This result shows that TMV-06 has an arginine residue in position 65 and that tryptic cleavage at this residue was incomplete under these conditions – probably due to the neighboring aspartic acid residues.

3.3. *Localisation of methionine*

Dansylation of CNBr cleaved TMV-06 protein showed that valine was a new N-terminal residue and therefore had been C-terminal neighbor to methionine in the intact protein. CNBr-cleaved TMV-06 protein and intact TMV-06 protein were digested with trypsin. The two-dimensional peptide map showed no difference outside the peptide band neutral at pH 6.5. After HVPE at pH 3.5 of the neutral band two new peptides were seen on the map of the CNBr treated digest and were identified as CB T 123–129 and CB T 130–134 (see table 1). The tryptic peptide CB T 123–129 contains no arginine and no lysine but one homoserine residue. Homoserine indicates the C-terminus of a CNBr-fragment and hence also the C-terminus of CB T 123–129. This means that position 129 of TMV 06 is a methionine residue and that cleavage with CNBr at this position results in two fragments, CB 1–129 and CB 130–158. Structural studies on these two fragments are in progress.

Table 1
Amino acid composition (moles/mol of peptide) and color of TMV-06 peptides after staining with ninhydrin–cadmium acetate.

	Ch 3–10	T 62–68	T 66–68	T 62–65 and T 114–122	CB T 123–129	CB T 130–134
Lys		1.0	1.0			
His	0.9					
Arg		1.0		1.8		0.9
Asx		2.0	0.8	2.4	1.95	
Thr	2.2			1.0		
Ser	1.9				1.0	
Glx						1.2
Pro	1.1	1.0		0.5		
Ala				2.0	1.0	
Val				2.0		1.0
Homoser					0.8	
Ile	1.0			1.0	1.0	1.0
Leu					1.0	1.2
Phe	0.9	1.9	1.1	0.5		
No. of residues	8	7	3	4 and 9	7	5
N-terminal residue	Ser	Phe	Asp		Ser	Val
Color	red	orange	red	orange and red	orange later red	red (very weak)

Inferred sequences (amino acid residues which differ from those in TMV-vulgare are indicated by capital letters): Ch 3–10, Ser–Ile–Thr–Thr–Pro–Ser–HIS–Phe; T 62–68, Phe–Pro–Asp–ARG–Asp–Phe–Lys; CB T 123–129, Ser–Ala–Ile–Asn–Asn–Leu–MET; CB T 130–134, Val–Glu–Leu–Ile–Arg; T 114–122, Val–Asp–Asp–Ala–Thr–Val–Ala–Ile–Arg.

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