ISOLATION OF THE COAT PROTEINS OF FOOT-AND-MOUTH DISEASE VIRUS AND ANALYSIS OF THE COMPOSITION AND N-TERMINAL ENDGROUPS

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SUMMARY: The three coat proteins of foot-and-mouth disease virus type O can be separated in milligram amounts using a SE-cellulose ionic exchanger, both from each other and viral RNA not quantitatively precipitated by acetic acid. The amino acid composition of the separated proteins has been determined and a statistical method has been used to calculate, for each, the most probable number of amino acids and molecular weight. The N-terminal amino acids of the three proteins are glycine, asparagine and threonine.

In a previous paper a useful method for the identification of the structural proteins of foot-and-mouth disease virus (FMDV) was described (1). Three coat proteins from various types of FMDV could be detected, and the similarity of their molecular weight and the charge of the proteins was shown by polyacrylamide gel electrophoresis. Small quantities of the particular proteins could be isolated by this method.

Bachrach et al. (2) separated the proteins of FMDV type A 119 using continuous flow electrophoresis and determined their C-terminal sequences with carboxypeptidase A. We have developed a separation method using SE cellulose exchanger, and we have controlled the uniformity of the separated proteins by the described gel electrophoresis in an acid buffer system (1). The amino acid compositions and the N-terminal amino acids have been determined and the most probable molecular weights calculated by a least squares method.

MATERIALS AND METHODS

FMDV type O_1 Kaufbeuren was grown in rolling bottle cultures of BHK 21 cells. After concentration by precipitation with polyethylene glycol the virus was purified by CsCl cushion centrifugation followed by a sucrose density gradient centrifugation, as described (1).

The purified virus suspension was mixed with two volumes acetic acid in the cold (3). The acid-soluble protein was oxidized by performic acid (4) and lyophilized.

SE cellulose (Serva, Heidelberg) was prepared as described in the laboratory manual of Whatman, equilibrated with 0.01 M sodium formate buffer pH 3.5 in 8 M urea and packed in a 0.9 x 15 cm column. About 20 mg virus protein dissolved in 1 ml equilibration buffer was layered onto the column. The elution was carried out using a linear gradient of 110 ml buffer 0.01 to 0.2 M sodium formate in 8 M urea. The flow rate was 14 ml/hr. The elution was detected with a modified Uvicord uv photometer (LKB) and tested by gel electrophoresis as described (1).

For amino acid estimation the three protein fractions as well as a control from collector fractions 40 - 42 (fig. 1) were dialysed against bidistilled water and evaporated to dryness. The isolated proteins were hydrolysed in 6 N NCl at 110° C for 20, 52, and 110 hr, desiccated and resuspended in 0.2 N sodium-citrate buffer pH 2.2. An Unichrom amino acid analyser (Beckman Instruments, Munich) was used with one column and 3 buffers as suggested by Kremen and Vaughn (5). Cysteic acid and methionine sulfon (Serva, Heidelberg) were added to the amino acid test mixture. Values from the longest hydrolysis times for Val, Ile, and Leu, and those extrapolated to zero time for Thr and Ser were used to calculate the respective amounts of these amino acids (6).

Dansylation was carried out following the instructions of Grey (7). 0.2 - 0.6 µg protein dissolved in 0.1 ml 1 % SDS and 0.1 ml N-ethylmorpholine was combined with 0.15 ml of a solution of 2.5 % dansylchloride in dimethylformamide. After 2 hours the dansylated proteins were precipitated and washed with acetone and vacuum dried. After hydrolysis in 6 N HCl at 110° C for 18 hr, the hydrolysate was dried, redissolved in 0.2 ml water and twice extracted with 0.2 ml ethylacetate. Both phases were dried separately, and dissolved in 0.01 ml of 50 % pyridine. Chromatography on polyamide sheets 7.5 x 7.5 cm (Schleicher & Schüll) was developed in the first dimension with water - 90 % formic acid (200 : 3) and in the second dimension with benzene - acetic acid (9 : 1) and again in the same direction with ethylacetate-methanol-acetic acid (20 : 1 : 1).

RESULTS

Separation of virus proteins.

Fig. 1 A shows the uv absorption diagram of the elution

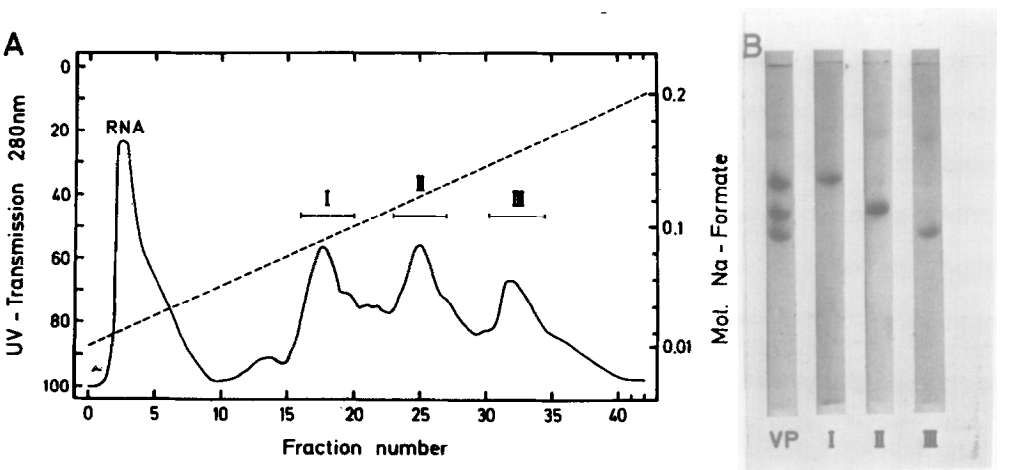

Fig. 1: Separation of FMDV protein on a SE cellulose column. A: Elution diagram of the optical transmission at 280 nm; ---- Molarity of Na formate buffer pH 3.5 right ordinate; RNA: unadsorbed RNA peak. B: Polyacrylamide gel electrophoresis of the oxidized virus protein (VP) and the protein fractions I, II, and III of diagram A. 10 % gel. Buffer: 1 M propionic acid 8 M urea.

from the SE column. The fractions I, II, and III were collected and tested by gel electrophoresis showing that the desired separation could be achieved (fig. 1 B). The fractions between the peaks were repeatedly tested, but in each case only the preceding protein was found, recovery amounted to between 50 - 70 % for each protein. The increase in volume of the original sample to 6-8 ml per protein fraction still gave good results. In all runs the first peak detected by uv absorption contained no protein. This fraction consists of nucleic acid not quantitatively eliminated by the acetic acid precipitation. If this step was omitted, the nucleic acid peak overlapped the protein fraction I. Virus protein 4, fast migrating in SDS gel electrophoresis and present in small quantities in the starting material has never been found in the eluate.

The determination of the N-terminal amino acids of protein I, II, and III is shown in fig. 2. Comparison with a test mixture of dansylated amino acids revealed the following N-terminal amino acids: fraction I, glycine; fraction II, aspartic acid; fraction III, threonine. These three amino acids were detected in about the same quantities in the starting material and only these three. Tyrosine can be dansylated at the side chain and DNS-O-Tyrosine was not washed out quantitatively by water. No other amino acids could be detected in the water phase of ethylacetate extraction.

Amino acid composition.

The amino acid composition of the separated proteins I, II and III are listed in table 1. Cysteic acid and methionine sulfon are designated as Cys and Met respectively.

To estimate the most probable number of amino acids per protein a factor f was calculated, such that the function

$$F(f) = \sum_{n=1}^{m} \frac{(A_n - N_{An,f})^2}{N_{An,f}}$$

would be a minimum. (A_n: Mol percent of the n^{th} amino acid; $N_{An,f}$: nearest integral number of $A_n \cdot f$; m: total number of amino acids taken into account). The nominator of the term is the square of the deviation and the denominator results in a decreasing weight of increasing numbers of amino acids. By means of a program for the desk computer IME 80 S the function F(f) was calculated and the minima estimated. N_{An} of the lowest minimum represents the most probable number of the amino acid A_n in the monomeric protein. By multiplication with the molecular weight of each amino acid and summation, the most probable molecular weight of the protein can be estimated. (A more detailed presentation is in preparation.) The numbers of amino acids and the molecular weights are: Protein I, 288 and 30170

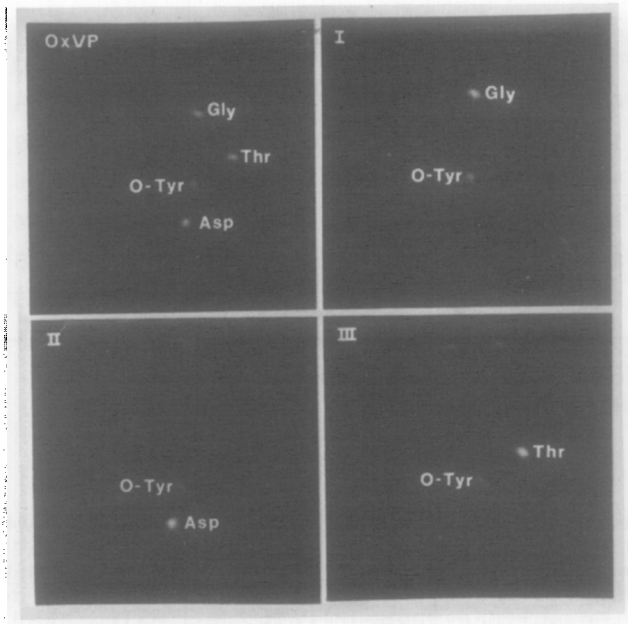

Fig. 2: Dansylated N-terminal amino acid of the oxidized virus protein (OxVP) and protein fractions I, II, and III. DNS-O-Tyr (O-Tyr) is present in all fractions but detects no N-terminal tyrosine.

Table 1

Amino acid composition of the isolated proteins

Amino acid	Protein fraction I Mol percent [1]	I MPN [2] of amino acids	II Mol percent [1]	II MPN [2] of amino acids	III Mol percent [1]	III MPN [2] of amino acids
Cys [3]	1.71	5	1.22	3	1.25	4
Asp [4]	10.09	29	9.83	24	9.35	28
Met [3]	2.06	6	1.57	4	1.42	4
Thr	8.40	24	11.37	28	10.11	30
Ser	7.76	22	5.32	13	4.06	12
Glu [4]	7.24	21	10.12	25	9.11	27
Pro	6.92	20	5.59	14	7.11	21
Gly	8.09	23	7.40	18	5.61	17
Ala	12.07	35	6.39	16	9.45	28
Val	6.39	18	10.79	27	8.16	24
Ile	2.75	8	2.91	7	4.00	12
Leu	7.65	22	7.73	19	9.45	28
Tyr	4.14	12	3.18	8	3.69	11
Phe	5.79	17	4.73	12	2.66	8
His	2.12	6	3.98	10	3.74	11
Lys	3.68	11	3.92	10	4.35	13
Arg	3.12	9	3.96	10	6.45	19
MPN of amino acids in the protein		288		248		287
Mol weight		30,170		26,767		32,218

The order of the amino acids is that of the analysis in one column with 3 buffers. 1) The Mol percents are calculated without tryptophan. 2) MPN: Most probable number calculated as described in results. 3) Cys and Met are detected as cysteic acid and methionine sulfone respectively, but calculated as cystine and methionine. 4) Asn and Gln are detected as acids. 5) The molecular weights are calculated without tryptophan.

Daltons; protein II, 248 and 26767 Daltons; protein III, 287 and 32218 Daltons respectively. Tryptophan cannot be detected after oxidation. Bachrach and Polatnik (8) found a content of 1.6 % in typ O_9 corresponding to an average molecular weight of 558 - 744 Daltons per protein.

DISCUSSION

The experiments demonstrate the possibility of separating the coat proteins of FMDV in milligram amounts using SE cellulose exchanger. It is a prerequisite of the technique that the sulphur bonds of cystine are broken. Oxidation with performic acid has been found to be a suitable preparation. Laport (9) used DEAE Sephadex at pH 8.6 and 10.5 to isolate one protein and the RNA. Under these conditions the other two proteins elute together in one peak.

The molecular weight, calculated on the basis of the most probable number of amino acids agrees about with the values estimated with SDS gel electrophoresis but the order in relation to previous findings (1) has changed. Swaney *et al.* (10) have shown, that molecular weights estimated by SDS gel electrophoresis depend on the quality of SDS. This fact may explain the discrepancy.

The estimation of N-terminal amino acids gives clear results. Not only the unseparated virus protein but also the isolated fractions show the same spots. It was necessary to dissolve the dansylchloride in dimethylformamide because acetone precipitates the proteins and prevents the reaction.

In a heat stable mutant of type O virus Laport (9) has found the N-terminal amino acids isoleucine, threonine, and leucine, and Matheka and Dietzschold (11) in type A_2 Spain the

amino acids arginine, asparagine and glycine. If these findings could be confirmed, it might be suggested that differences between N-terminal amino acids provide the chemical background for the serological differences of types and subtypes.

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