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Amino Acids of Turnip Yellow Mosaic Virus.*† (19536)

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A study has been undertaken in our laboratory of the adsorptive properties of the recently described crystalline turnip yellow mosaic virus(1) and of the nucleic acid derived therefrom. A complete analysis of the nucleic acid moiety of the virus has been published(2). The present communication contains the analysis of the protein prepared from the virus.

Experimental. The protein was liberated from the virus by treatment with 35% ethanol at room temperature at a neutral pH(1). The precipitated protein was washed free of nucleic acid. Suitable quantities of the protein were hydrolyzed and analyzed for amino acids by microbiological and paper chromatographic procedures which have been described in detail in a previous communication(3).

Results. Chromatographic findings. A typical 2-dimensional chromatogram prepared from a volume of acid hydrolysate corresponding to 1 mg of the original protein is shown in Fig. 1. Eighteen ninhydrin-reactive substances were detected. In addition to the usually occurring amino acids small amounts of constituents which have been *tentatively* identified as glucosamine (spot 2), α-amino-n-butyric acid (spot 6), and γ-aminobutyric acid (spot 11) were also observed. It is not certain whether the latter 3 substances are present as impurities or are actually constituents of the virus protein. Tryptophan was not detected because of destruction during acid hydrolysis, and the phenylalanine spot was obscured by the large spot produced by the leucines. The most noteworthy finding on the chromatograms were the large quantities of proline, serine, and threonine. The proportion of threonine was greater than that found in any protein previously examined in our

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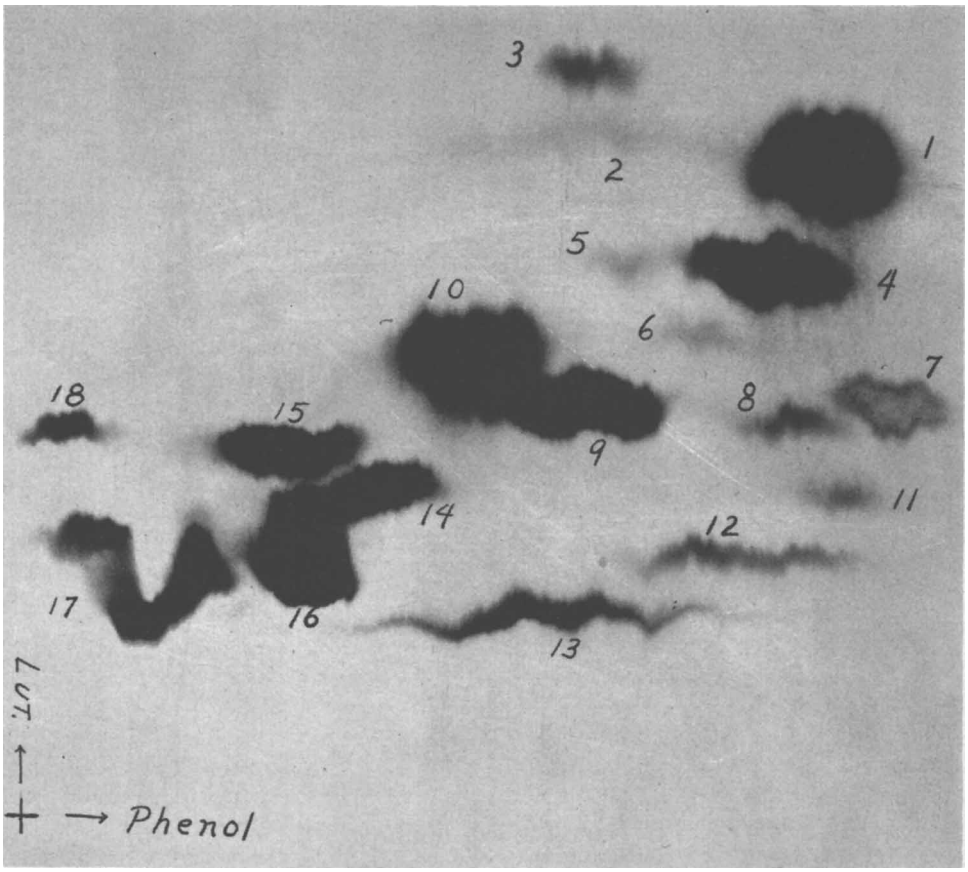

FIG. 1. Chromatogram of peroxide-treated hydrolysate of turnip yellow mosaic virus protein. Leucine and isoleucine, 1; glucosamine (?), 2; tyrosine, 3; valine, 4; methionine (methionine sulfone), 5; α-amino-n-butyric acid (?), 6; proline, 7; histidine, 8; alanine, 9; threonine, 10; γ-aminobutyric acid, 11; arginine, 12; lysine, 13; glycine, 14; serine, 15; glutamic acid, 16; aspartic acid, 17; cystine (cysteic acid), 18.

laboratory. The proline spot (spot 7) did not photograph with the same intensity as did the spots given by the other amino acids, since proline gives a yellow color while the other amino acids give colors ranging from blue to purple. No hydroxyproline was detected.

Microbiological determinations. From the results shown in Table I it is seen that almost all of the protein was accounted for by the analyses. There is a considerable excess of free basic groups over free carboxyl groups, making potentially available a number of loci which are capable of forming salt linkages with the phosphate groups of the nucleic acid. The presence of a large number of residues of serine and threonine suggests that the hydrogen bonding of the aliphatic hydroxyl groups may be important both in maintaining the internal structure of the protein and also in binding the protein to the nucleic acid.

Discussion. The finding of a large quantity of the aliphatic hydroxy amino acids in the protein moiety of the turnip yellow mosaic virus is of considerable interest since other proteins with interesting biological activities, human γ-globulin, pepsin, chymotrypsinogen, ribonuclease (see Tristram's compilation) (4), and Bence-Jones protein (3) have been found to have particularly high levels of these amino acids. In addition, it was found that the tobacco mosaic virus proteins (5) also contained relatively large amounts of serine and threonine. However, it is not possible at the present time to make any specific suggestions about

TABLE I. Amino Acid Composition of Protein of Turnip Yellow Mosaic Virus. All values are expressed on a moisture-free basis. Nitrogen content, 16 g/100 g protein.

	Amino acid			
	g/100 g protein	g residue /100 g. protein	g N/100 g protein	Moles residue/10^5 g protein
Glycine	2.90	2.20	.54	38.6
Alanine	5.70	4.54	.91	64
Valine	8.45	7.14	1.01	72.1
Leucine	10.30	8.89	1.10	78.6
Isoleucine	10.80	9.31	1.15	82.4
Phenylalanine	3.80	3.39	.32	23
Proline	10.70	9.04	1.30	93
Tryptophan	1.14	1.04	.16	5.6
Cystine	2.50	2.31	.29	10.4
Methionine	2.85	2.50	.27	19.1
Glutamic acid	9.60	8.41	.92	65.2
Aspartic acid	5.10	4.41	.54	38.3
Histidine	2.10	1.86	.57	13.5
Lysine	10.70	9.40	2.05	73.2
Arginine	2.50	2.24	.80	14.4
Serine	8.10	6.71	1.08	77.1
Threonine	15.10	12.80	1.77	126.8
Tyrosine	2.45	2.20	.19	13.5
Amide NH_3	1.25	1.25	1.03	73.5
Total	116	99.6	16	982

Free basic groups, 101; free acid groups, 30; aliphatic hydroxy groups, 204.

the role that these amino acids might play in the biological activity of the proteins in which they are found in large amounts.

Summary. 1. The amino acids of the protein of crystalline turnip yellow mosaic virus were studied by paper chromatographic and microbiological technics. 2. Virtually all the protein was accounted for by microbiological assay for 18 amino acids performed on hydrolysates of the protein. 3. The protein was characterized by the presence of large amounts of serine, threonine, and proline. Hydroxyproline was not detected.

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Preparation of a Stable Form of Inulin for Tissue Analysis.* (19537)

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Direct analysis of tissue for inulin is essential for a correct evaluation of the volume distribution of this substance. Ross and Mokotoff(1) have described a method of analysis for muscle tissue using dilute alkali and heat to destroy the high inulinoid blank of this tissue. They were unable to obtain adequate recoveries of inulin added to muscle tissue by this method, unless the inulin had been previously treated with heat and dilute NaOH and reassayed before adding it to the muscle. Therefore, the analysis of the tissue of animals injected with the usually available inulin would not determine the total inulin present if this method were used. In studies involving the simultaneous determination of the total body inulin space and tissue distribution, it became necessary either to develop a method for tissue analysis which would not destroy the inulin, but, at the same time, would destroy the large inulinoid blank of muscle; or to develop a stable form of inulin for injection into these animals.

Table I indicates the results of treating inulin and muscle with various forms of digestion. From this, it is apparent that some inulin is destroyed by any procedure involving acid, alkali or heating. Inulin probably consists of at least 3 fractions—a heat or dilute acid labile one, a hot alkali labile one, and a hot alkali stable one. This is also suggested by the variation in molecular weight deter-

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