[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Basic Amino Acids in Strains of Tobacco Mosaic Virus

BY C. A. KNIGHT

It was shown in a recent communication from this Laboratory that the Holmes ribgrass strain of tobacco mosaic virus differs strikingly from ordinary tobacco mosaic virus in aromatic amino acid content.[1] It was also shown that cucumber viruses 3 and 4, which are related to tobacco mosaic virus, possess the same amount of tyrosine as the latter but contain distinctly different amounts of tryptophane and phenylalanine. These results demonstrated clearly for the first time the nature of some of the chemical differences between strains of tobacco mosaic virus. However, no distinctive difference was detected between the aromatic amino acid composition of ordinary tobacco mosaic virus and that of the yellow aucuba, green aucuba, J14D1, or masked strains of the virus. These strains are serologically, and in certain other respects, much more closely related to the common tobacco mosaic virus than are the other strains mentioned above. It seemed desirable, therefore, to extend the original investigation to include other amino acids, in order to determine whether there are demonstrable chemical differences between tobacco mosaic virus and some of its more closely related strains. The present report deals with analyses of the 8 previously described strains of tobacco mosaic virus[1] for arginine and histidine.

Experimental

Preparations of the viruses were made by purely physical methods involving filtration and differential centrifugation of the juices from infected Turkish tobacco plants, or from cucumber plants in the cases of cucumber viruses 3 and 4. Highly purified solutions of each virus in distilled water were frozen, dried *in vacuo*, and then further dried to constant weight at 110° in a drying oven. The white fluffy material thus obtained was used for amino acid analyses.

Arginine.—Arginine was determined by a colorimetric procedure based on the Sakaguchi reaction and by a gravimetric method involving precipitation of arginine as the flavianate.[2,3]

For the colorimetric test, 15 to 20 mg. of virus was hydrolyzed in 1 ml. of concentrated hydrochloric acid in a sealed tube in an oven at 110° for twenty hours. The hydrolysate thus obtained was transferred quantitatively to a 100 ml. volumetric flask, the solution was made to volume with distilled water, and 1 ml. aliquots were taken for analysis. The Sakaguchi reaction was carried out essentially as described by Thomas and co-workers,[2] with the exception that readings were made with a Klett-Summerson photoelectric colorimeter using Green Filter 54. The method used for the gravimetric determination of arginine involved a preliminary isolation of the basic amino acids by the electrolytic procedure of Albanese.[3]

The arginine content of tobacco mosaic virus was found by colorimetric and gravimetric methods to be 9.2 ± 0.1%. As may be observed in Table I, the arginine values obtained by the two different methods agreed remarkably well for all of the strains. Four of the 8 strains were found to contain about 9.2% arginine. On the other hand, cucumber viruses 3 and 4 were found to contain significantly less arginine than ordinary tobacco mosaic virus, while the green and yellow aucuba viruses were found to contain significantly more arginine than the type strain.

TABLE I

ARGININE AND HISTIDINE IN STRAINS OF TOBACCO MOSAIC VIRUS

Virus	Arginine: No. of preparations	Arginine: Sakaguchi method, %[a]	Arginine: No. of preparations	Arginine: Flavianate method, %[a]	Histidine, %
Tobacco mosaic	7	9.2	8	9.2	None
Yellow aucuba	7	10.0	2	10.0	None
Green aucuba	7	10.0	1	10.0	None
Holmes' ribgrass	4	9.1	1	9.2	0.55
Holmes' masked	2	9.2	1	9.0	None
J14D1	2	9.2	1	9.2	None
Cucumber virus 4	5	8.7	2	8.7	None
Cucumber virus 3	1	8.7	1	8.8	None

[a] The results of individual analyses for arginine showed a maximum deviation from the averages listed of ±0.2% for the Sakaguchi method and ±0.1% for the flavianate method.

Histidine.—Ross was unable to detect histidine in ordinary tobacco mosaic virus upon examination of whole hydrolysates of the virus or portions of hydrolysates obtained by chemical fractionation, although an amount of histidine equivalent to less than 0.1% of the virus could be recovered when added to various fractions.[4] Hence, it was concluded that histidine is not present in the tobacco mosaic virus molecule. The present experiments confirm this conclusion.

The Albanese technique for the estimation of histidine[3] was found to be quite unreliable for the determination of very small amounts of histidine in mixtures which contained large amounts of arginine. On the other hand, the conditions required in the Jorpes modification of the Pauly reaction[5] seemed admirably suited to such analyses and

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

(2) L. E. Thomas, J. K. Ingalls, and J. M. Luck, *ibid.*, **129**, 263 (1939).

(3) A. A. Albanese, *ibid.*, **134**, 467 (1940).

(4) A. F. Ross, *ibid.*, **138**, 741 (1941).

(5) E. Jorpes, *Biochem. J.*, **26**, 1507 (1932).

particularly adaptable to the determination of small amounts of histidine in the histidine–lysine fraction obtained from tobacco mosaic virus by the electrolytic method. In the present investigation, the Jorpes procedure was slightly modified by substituting for the Zeiss photometer a Klett–Summerson photoelectric colorimeter with Green Filter 54. Results of tests with a standard solution of histidine showed that a true proportionality was obtained between the colorimeter reading and the amount of histidine present in the range 0.005 to 0.05 mg. per ml.

Application of the Pauly test to the histidine–lysine fractions of the strains indicated the absence of histidine from all except the ribgrass strain (Table I). Analyses on 2 different preparations of the ribgrass virus gave histidine values of 0.39 and 0.43%.

It has been suggested that the presence of carbohydrate may result in the destruction of histidine during the acid hydrolysis of proteins.[6] In order to evaluate fairly the apparent absence of histidine from all except one of the strains, the destructive effect of the virus carbohydrate was tested in two ways. In the first, small amounts of histidine were added to tobacco mosaic virus during hydrolysis and the recovery of histidine was noted, and, in the second, nucleic acid was removed from tobacco mosaic and the ribgrass viruses before hydrolysis and the effect on the histidine analyses was observed. In two trials in which small amounts of histidine were added to tobacco mosaic virus during hydrolysis, recoveries of 36 and 64% were made. In the other approach, tobacco mosaic and the ribgrass viruses were freed from nucleic acid by treatment with alkali. Negative phosphorus and carbohydrate tests on 25- to 30-mg. portions of the proteins indicated that essentially all of the nucleic acid had been removed. Five hundred and ten mg. of nucleic acid-free tobacco mosaic virus protein and 470 mg. of the corresponding preparation of ribgrass virus protein were then hydrolyzed as usual and the basic amino acids were isolated by the electrolytic procedure. No histidine could be demonstrated in the histidine–lysine fraction of the tobacco mosaic virus protein by the Pauly reaction, but 0.55% of histidine (calculated on the basis of the intact virus) was detected by the same method in a similar fraction of the ribgrass virus protein.

The histidine–lysine fraction of the nucleic acid-free ribgrass virus protein was also analyzed for histidine by the nitranilic acid gravimetric procedure as described by Block.[6] After allowance was made for the 6% nucleic acid in the ribgrass virus, a histidine value of 0.57% was obtained, in good agreement with the 0.55% found by the colorimetric procedure, and both figures are almost one-third greater than the highest value obtained on whole virus.

In general, the results of the above experiments appear to confirm earlier observations regarding the destruction of histidine in the presence of carbohydrate during acid hydrolysis of proteins. In particular, the data emphasize the absence of histidine from tobacco mosaic virus and its presence in the ribgrass virus. This is the first demonstration of the presence in a strain of tobacco mosaic virus of a constituent which is apparently entirely lacking in the common strain.

(6) R. J. Block, *J. Biol. Chem.*, **133**, 67 (1940).

Lysine.—If the amino nitrogen of the intact virus[7] is attributed to epsilon amino groups of lysine, tobacco mosaic virus contains about 1.35% of lysine. However, the experiments of Ross[8,9] have led him to conclude that tobacco mosaic virus contains very little, if any, lysine. In the present investigation, analyses of the electrolytically obtained lysine fractions of eight strains of tobacco mosaic virus indicated the presence of amino nitrogen which was unattributable to known constituents of the virus and which might therefore represent lysine. The amounts of this nitrogen were too small to permit the confirmatory isolation of lysine derivatives, but attempts to isolate such derivatives starting with considerably larger samples of virus are now in progress and will be described in detail in a later communication.

Discussion

Analysis of eight strains of tobacco mosaic virus indicated that each strain possesses a characteristic and constant amount of arginine. Thus, in seven preparations of tobacco mosaic virus obtained from different groups of diseased tobacco plants over a period of two to four years, the arginine content was found to be 9.2 ± 0.1%. On the other hand, seven preparations of green aucuba virus and seven of yellow aucuba virus obtained over a similar period of time were found to contain 10.0 ± 0.1% of arginine. The difference between the compositions of the aucuba viruses and ordinary tobacco mosaic virus is particularly striking in view of the very close relationship of these strains. For example, it is almost impossible to distinguish between the symptoms produced by ordinary tobacco mosaic virus and the green aucuba strain in diseased Turkish tobacco plants. It is generally necessary to inoculate to *Nicotiana sylvestris* Spegaz. and Comes to differentiate between the two strains. The green aucuba strain produces local lesions on *N. sylvestris*, whereas the ordinary strain does not. It is now possible, on the basis of arginine content, to distinguish definitely between these strains with considerably less than the amount of virus obtained from a single diseased plant. The significantly lower amounts of arginine found in cucumber viruses 3 and 4 than in ordinary tobacco mosaic virus are somewhat less surprising because the relation of the cucumber strains to the latter is less close.

In general, the data from the basic amino acid analyses of the virus strains support and extend the observations resulting from the aromatic

(7) G. L. Miller and W. M. Stanley, *ibid.*, **141**, 905 (1941).

(8) A. F. Ross and W. M. Stanley, *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, **128**, p. lxxxiv (1939).

(9) A. F. Ross, *J. Biol. Chem.*, **143**, 685 (1942).

amino acid analyses.[1,10] It is quite clear that the protein components of strains of tobacco mosaic virus differ in their amino acid compositions. Whether or not these differences are responsible for the different biological properties of the various strains remains to be shown. In any case, they must be considered when sufficient data have been gathered to permit an intelligent correlation of biological properties with chemical structure. The nucleic acid components of the viruses cannot, of course, be entirely neglected. There is no unequivocal evidence at present that the nucleic acids of various strains of tobacco mosaic virus differ either quantitatively or qualitatively. On the other hand, the results of phosphorus analyses indicate that 8 of the strains contain essentially the same amount of nucleic acid, and colorimetric tests show that all of these 8 strains contain ribose nucleic acid.[1] Nevertheless, it will not be certain that the nucleic acids of strains of tobacco mosaic virus are identical until thorough analytical studies have been completed.

The discovery of the presence of histidine in the ribgrass virus is especially interesting, for it represents the first case in which one strain of tobacco mosaic virus has been found to contain a constituent apparently lacking in another. The ribgrass virus has not yet been obtained directly by mutation of ordinary tobacco mosaic virus, as have certain yellow strains[11,12] and the Holmes masked strain.[13] Thus, the ribgrass strain may represent a stable product resulting from many variations rather than from one. However, if the ribgrass strain developed originally from ordinary tobacco mosaic virus, it is necessary to assume that at one stage some histidine was added to a virus molecule which previously contained none. Unless it can be established experimentally that direct changes of this type can occur in completely formed virus molecules, it seems reasonable to assume that the introduction of a new amino acid occurred as a result of a departure from the usual pattern of the synthetic process of virus multiplication. On this basis the phenomena of virus multiplication and virus variation become directly related, the latter representing simply a modification of the former.

The author is indebted to Dr. W. M. Stanley for his interest and encouragement during the course of this investigation.

(10) W. M. Stanley and C. A. Knight, *Cold Spring Harbor Symposia on Quant. Biol.*, **9**, 255 (1941).

(11) J. H. Jensen, *Phytopathology*, **23**, 964 (1933).

(12) J. H. Jensen, *ibid.*, **26**, 266 (1936).

(13) F. O. Holmes, *ibid.*, **24**, 845 (1934).

Summary

Eight strains of tobacco mosaic virus were analyzed for arginine and histidine. Analyses by two methods of seven different preparations of tobacco mosaic virus indicated that this virus contains 9.2 ± 0.1% of arginine. A similar amount of arginine was found in the Holmes masked, Holmes ribgrass, and J14D1 strains. On the other hand, the green and yellow aucuba strains were found to contain 10.0% of arginine and the cucumber viruses 3 and 4, 8.7% of arginine. No histidine could be detected in seven of eight strains, but about 0.55% of histidine was found in the ribgrass strain. Indirect analyses indicated that the eight strains contain a small amount of lysine, but this finding has not yet been verified by isolation methods.

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