AMINO ACID COMPOSITION OF HIGHLY PURIFIED VIRAL PARTICLES OF INFLUENZA A AND B*

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The mutation of viruses to form new strains (1, 2) is a phenomenon which has been recognized for many years in the form of its various biological manifestations, but only recently has it become possible to attempt a correlation of this knowledge with the fundamental chemistry of the viruses themselves. Thus, amino acid analyses made on strains of tobacco mosaic virus have revealed differences in the composition of the virus proteins which presumably can explain their different biological properties and which also conceivably illustrate the nature of the chemical changes which accompany the mutation of a virus to form a new strain (3–7). In view of these findings, it was naturally of interest to determine whether or not similar chemical differences exist between strains or types of an animal virus. Highly purified preparations of influenza viruses (8–12) were available for this purpose and the PR8 strain of influenza A and the Lee strain of influenza B were chosen for comparison. These viruses produce clinically indistinguishable diseases (13) and appear to be very similar in gross chemical properties (9). However, they are serologically and immunologically distinct (13) and seem to differ slightly in size (12, 14). In this communication there are presented the results of an attempt to discover, at least in part, a chemical basis for the similarities and differences between these two types of influenza viruses. The approach employed has centered upon the protein components of the viruses and microbiological assays for amino acids have been made on hydrolysates of the highly purified viral particles of PR8 and of Lee influenza viruses obtained from the allantoic fluids of infected chick embryos.

Methods and Findings

Preparation of Virus for Assay.—Highly purified preparations of the PR8 and Lee strains of influenza virus were obtained from allantoic fluids of infected chick embryos by a combination of the methods of differential centrifugation and adsorption on and elution from chicken red cells (8-12). Such preparations were found to consist of particles which were highly active biologically and which were uniform in size, in electrochemical behavior, and in serological reactions (11). The purified viruses were freed of salt and dried as recently described (15). Hydrolysates of the viruses were obtained by heating 50 mg. samples in 2 ml. portions of 2.7 N hydrochloric acid in sealed tubes in an autoclave at 15 pounds pressure for 10 to 12 hours.

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The hydrolysates were neutralized and filtered and the combined filtrate and washings for each sample was brought to a volume of 250 ml. For the tryptophane assays, separate samples of 11 to 15 mg. were hydrolyzed in 1 ml. portions of 20 per cent sodium hydroxide in sealed tubes in an autoclave at 15 pounds pressure for 15 hours. The hydrolysates were neutralized, filtered, and brought to a volume of 100 ml. For the sake of comparison, an hydrolysate of the sedimentable component of normal allantoic fluid (16) was also prepared and simultaneous assays were made on this and the virus hydrolysates.

TABLE I

Amino Acid Content of Highly Purified PR8 and Lee Influenza Virus Particles and of the Sedimentable Particles of Normal Allantoic Fluid

Amino acid	PR8 influenza virus	Lee influenza virus	Normal allantoic particles	MD*
	per cent	*per cent*	*per cent*	*per cent*
Alanine	2.5	2.6		0.1
Arginine	5.0	4.0	3.9	0.2
Aspartic acid	7.4	7.3	6.2	0.1
Glutamic acid	7.7	6.2	6.1	0.2
Glycine	2.5	2.9	1.8	0.1
Histidine	1.4	1.5	0.8	0.03
Isoleucine	5.2	5.4	4.1	0.1
Leucine	5.3	5.5	4.3	0.1
Lysine	3.6	4.7	2.5	0.1
Methionine	2.3	2.1	1.1	0.1
Phenylalanine	3.7	3.4	3.6	0.2
Proline	2.6	2.7	2.8	0.2
Serine	2.2	2.2	2.1	0.1
Threonine	3.7	4.0	3.8	0.1
Tryptophane	1.1	0.7	0.7	0.02
Tyrosine	3.1	2.1	2.2	0.05
Valine	3.4	3.2	3.2	0.1

* Mean deviation of the values of single determinations from the averages given in the table.

Microbiological Assays.—The methods used were largely those of Stokes and co-workers (17–19), to whom the author is also indebted for original cultures of the bacteria employed. Alanine, glutamic acid, proline, and glycine were not determined by Stokes and collaborators. However, they were determined in the present investigation by microbiological assays which satisfied fairly well the usual criteria of reliability in this type of analysis (17). Thus, *Streptococcus faecalis* was employed to assay for alanine, arginine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophane, and valine. *Lactobacillus delbrückii* LD5 was used to determine phenylalanine, serine, and tyrosine; and *Leuconostoc mesenteroides* P-60 (20) was employed in assays for proline, aspartic acid, glycine, and in some instances, for lysine. The basal medium of Stokes (18) was used in all cases.

Five preparations of PR8, four preparations of Lee virus, and a combination sample representing several preparations of normal allantoic particles were analyzed. The averages of the results obtained in these analyses are given in Table I. From the mean deviations, also presented in Table I, one can obtain an estimate of the reproducibility of each analysis and

hence a judgment of which of the observed differences are probably significant. On this basis, the values shown in Table I indicate significant differences between the PR8 and Lee influenza particles in their contents of arginine, glutamic acid, lysine, tryptophane, and tyrosine. There may also be significant differences in the glycine and histidine values for the two strains, although the present data do not clearly indicate this.

DISCUSSION

In evaluating the differences observed in analyses made on the whole particles of influenza viruses, account must be taken of their chemical complexity. The highly purified particles of PR8 and Lee viruses contain protein, polysaccharide, lipid, and nucleic acid components. The sums of the percentages of these last three components are essentially the same for the two strains and the percentages of nitrogen contained in the intact virus particles, which can be determined more precisely, coincide (9, 21). From these facts, one can conclude that both strains probably contain identical quantities of protein and hence that the differences observed in amino acid assays of the whole particles are real and do not merely reflect variations in quantity of the non-protein constituents. This conclusion is strengthened by the nature of the analytical results. Five differences were observed and one of these was in the opposite direction from the other four. No significant differences were observed with respect to the value for ten of the seventeen amino acids determined. Therefore, the assumption that PR8 and Lee virus particles contain equal quantities of protein appears to fit the facts better than the alternative hypothesis. Furthermore, it should be noted that even if this assumption should prove false, the major premise of the report, namely that the protein components of the two strains of virus are markedly different in composition, still holds.

The analysis of highly purified preparations of influenza viruses has revealed, as in the case of similar analyses made on plant viruses, a noteworthy uniformity in the composition of successive preparations. The compositions of the PR8 and Lee influenza viruses were so characteristic of the strains used in these studies that they could undoubtedly have been used to identify them, as has been done with certain strains of tobacco mosaic virus (5). While the differences found in the present study are pronounced both in number and kind, it is interesting to note that the two strains appear to contain identical quantities of at least ten different amino acids. It seems that this should provide a chemical basis for the biological fact that these are influenza viruses, although more data would be required to establish this point firmly. In this connection, it can be seen from the results shown in Table I that the composition of the sedimentable particles of normal allantoic fluid closely resembles that of Lee virus in eight or nine cases and that of PR8 virus in four or five respects. However, a close and perhaps fundamental relationship has been established among these materials by immunochemical studies (11).

The present findings resemble those obtained in studies made on strains of a

plant virus, tobacco mosaic virus, both in character and in extent. In both instances the protein components have been found to differ. The results of the plant virus analyses demonstrated the presence of only a few differences between closely related and many deviations among presumably distantly related strains (3–7). The well known immunological distinction between the viruses of influenza A and B (13, 22–24) strongly suggests that they are not closely related, and it was found in the present analyses that the protein components of PR8 and of Lee viral particles differed in five or more rather than in one or two respects.

On the basis of current knowledge one can only speculate regarding the relationship of the present findings to the different biological and physicochemical properties of PR8 and Lee viruses. However, in the absence of data to the contrary, it seems reasonable to assume that a substantial portion of the serological activity and of the biological specificities of these viruses can be attributed to the protein components, which constitute approximately two-thirds of the weight of the viral particles. For example, the differences found herein might well account at least in part for the lack of immunological relationship between the two strains (13, 22–24), for their different pH stability ranges (25), their different red cell agglutinating capacities (11), and for the widely divergent heat stabilities of their agglutinating capacities (26). However, it is apparent that further studies of the sort reported herein will be required before it will be possible to draw more specific conclusions regarding the relationship of viral composition to viral properties.

SUMMARY

Microbiological assays for amino acids were made on hydrolysates of four to five highly purified preparations each of influenza A virus (PR8 strain) and influenza B virus (Lee strain). The results of the assays indicated that these strains of influenza virus contain approximately the same amounts of alanine, aspartic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, and valine. However, significant differences were found in the values for arginine, glutamic acid, lysine, tryptophane, and tyrosine. It is believed that these differences may provide, at least in part, a chemical explanation for some of the differing properties of the PR8 and Lee strains of influenza viruses.

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