THE ISOELECTRIC POINTS OF SOME STRAINS OF TOBACCO MOSAIC VIRUS

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The detailed amino acid analysis of several strains of tobacco mosaic virus carried out by Knight (1) has revealed interesting chemical similarities and differences among these strains, although they are morphologically very similar (2). In the present work the isoelectric points of the strains which Knight has analyzed were determined by a method developed by the author (3). The virus strains show maximum coagulation at the isoelectric point and this may be observed by light scattering. Since these virus strains are closely related substances, the present work also provides a comparative study of the amphoteric properties of a series of related proteins.

Methods and Results

Preparation of Virus Strains—The virus strains used in this work are identical with those studied by Knight (1). The viruses were purified by the differential centrifugation method of Stanley (4) and were resuspended twice in distilled water before use.

Turbidity-pH Studies—To 3 cc. of aqueous solutions of the ordinary strain of tobacco mosaic virus (4.0 mg. of virus per cc.) were added 3 cc. of 0.04 M solutions of Sørensen's citrate-HCl buffers of various pH values. The pH of the final solution was measured with a glass electrode in a Beckman pH meter. Fig. 1 shows the relative turbidity, measured in a Klett-Summerson colorimeter with a blue filter, as a function of pH. For comparison, the relative turbidity of the PR8 strain of influenza virus as a function of pH is also shown. The latter virus was purified by the method described by Knight (5). For the ordinary strain of tobacco mosaic virus, the maximum turbidity occurs at pH 3.5 ± 0.1 and, for the influenza virus, the maximum occurs at pH 5.0 ± 0.1. An experiment similar to that performed for the ordinary strain of tobacco mosaic virus was run with the Holmes rib-grass strain and the maximum was found to occur at pH 4.0 ± 0.1.

The isoelectric points of these viruses at the same buffer concentration

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were also determined by microelectrophoresis with the Kunitz-Northrop apparatus. In all these cases the virus, when adsorbed on collodion particles, gave isoelectric points in agreement with those found by the turbidimetric method.

The turbidimetric method described above is not sufficiently sensitive for distinguishing between the isoelectric points of some of the strains of tobacco mosaic virus. Furthermore, the method gives isoelectric points which are strongly dependent on the nature and concentration of the salts

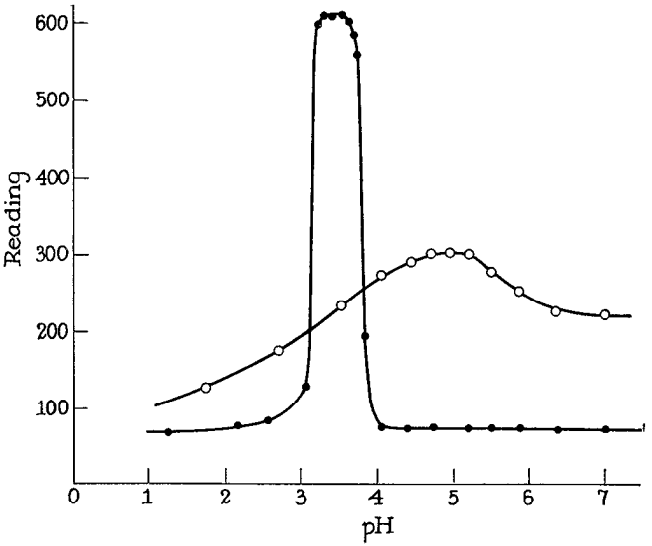

FIG. 1. The relative turbidities as a function of pH (buffered) of solutions of the ordinary strain of tobacco mosaic virus (●, virus concentration 2.0 mg. per cc.) and of the PR8 strain of influenza virus (○, virus concentration 0.005 mg. per cc.). Turbidity read in Klett-Summerson colorimeter.

present. A superior method, which, in addition, requires a much smaller sample, is the following. To a salt-free solution of the purified virus is added dilute HCl, and the pH at which the turbidity shows the greatest rate of change with increment of acid, *i.e.* the point of discontinuity in the curve, is taken as the isoelectric point. Fig. 2 shows a typical turbidity curve. To 5 cc. of a 0.3 per cent solution of purified virus in distilled water were added small increments of 0.0015 M HCl, and turbidity and pH measurements were made after the addition of acid. The turbidity readings rise rapidly just before the isoelectric point and then flatten out just after it. The point of the break in the curve corresponds to the isoelectric point, since the precipitate at this point when adsorbed on collodion failed to move in the Kunitz-Northrop electrophoresis apparatus. If,

however, a trace of acid or alkali was added to the solution, the particles moved rapidly. It is not feasible to measure the isoelectric points of proteins in salt-free solutions by electrophoresis methods since, because

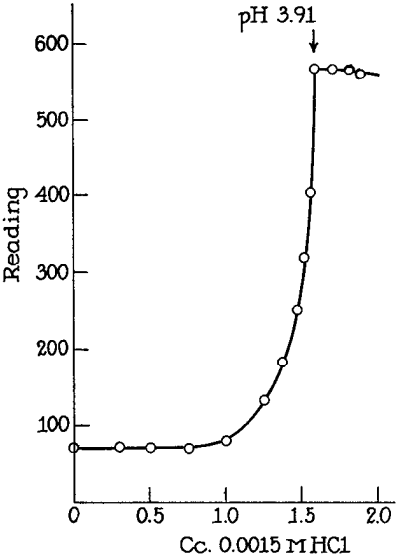

FIG. 2. The relative turbidity of the ordinary strain of tobacco mosaic virus as a function of volume of 0.0015 M HCl added to 5.0 cc. of a salt-free solution of the virus at 3.0 mg. per cc. of virus. Turbidity read in Klett-Summerson colorimeter.

TABLE I

Isoelectric Points in Water of Some Strains of Tobacco Mosaic Virus

	pH
Ordinary tobacco mosaic virus	3.91
Holmes' masked strain	3.91
J14D1 strain	4.22
Holmes' rib-grass strain	4.49
Green aucuba strain	4.50
Yellow " "	4.64
Cucumber virus 4	4.90

of the low electric conductivity and, therefore, the high voltage gradient, the particles move too rapidly and the point of zero mobility is easily overstepped.

Table I gives the isoelectric points in water of seven strains of tobacco mosaic virus determined by the turbidimetric method. The values are easily reproducible and the accuracy is limited only by the accuracy of the standard buffers used to calibrate the pH meter. It was found that

mixtures of purified virus strains gave turbidity curves which resemble those constructed by superposing the curves for the individual strains. The PR8 strain of influenza virus gave an isoelectric point in water of pH 5.88.

The isoelectric points of the viruses as determined by the turbidity method are about 0.5 pH unit higher in water than in the presence of buffers. Neutral salts also lower the isoelectric point; for example, it was found that the isoelectric point of the ordinary strain is pH 3.6 in the presence of 0.5 M NaCl. The salt renders the turbidity curve less distinct; the virus at the isoelectric point is more soluble in salt solutions than in salt-free water.

DISCUSSION

The strains of tobacco mosaic virus have minimum solubility at their isoelectric points, and, if no salt is present, the viruses are insoluble at their isoelectric points. This property may be used to purify the virus and, in fact, was one of the methods employed by Stanley (6) when he first purified the ordinary strain. Since all of the strains examined have distinct isoelectric points, it is possible to separate two or more strains, if their isoelectric points differ, by differential precipitation. In this way, purified virus from a plant suffering from yellow aucuba, but contaminated with the ordinary strain, may be separated into two virus components. It was found, however, that the two strains could not be separated completely by differential precipitation, and the method is therefore useful only for concentrating the strains. For a complete separation of virus strains the isolation of single lesions as employed by Kunkel (7) is necessary.

The increase in turbidity of solutions of tobacco mosaic virus at the pH of minimum solubility is due to the increase in light scattering by the large aggregates of the rod-shaped particles packed side to side (8). Some viruses, for example tomato bushy stunt virus, do not coagulate at their isoelectric points. Apparently, in these cases, the hydration of the particles is so great that the particles cannot aggregate, although they possess a zero net charge at the isoelectric point.

A comparison of the observed isoelectric points of the virus strains with the results of Knight (1) of the amino acid analysis of the strains shows that the isoelectric points follow in the same sequence as do the ratios of the number of basic groups (lysine, histidine, and arginine) to the aspartic acid content of the strains. This relation is valid if it is assumed that the free charged groups are independent and that the glutamic acid residues are not available (*e.g.*, are present in the form of glutamine). It should be further noted that the ratios are close in the case of the green and yellow

aucubas and in the case of the ordinary strain and the masked strain, in agreement with similar isoelectric points observed for these two sets of strains.

There is a rough correlation between the isoelectric points of the strains and their movement in their host plant. Thus, the various strains may be written in the order of decreasing speed of movement in *Nicotiana tabacum* L. var. Turkish as follows: ordinary strain, masked strain, ribgrass strain, green aucuba, and yellow aucuba; *i.e.*, in the order of increasing isoelectric points. The cucumber 4 strain does not infect this host. The expressed sap of the host had an average pH value of 5.70 (the acidity is greater the older the plant and the lower the temperature at which it is grown). It might be expected, since the virus aggregates more in solution the closer it is to its isoelectric point, that the higher the isoelectric point of the strain in a given host, the more aggregation will occur and, hence, the less rapid will be the movement of the virus in its host. Until we know the pH of the medium in which the virus moves, however, nothing further can be said.

SUMMARY

The isoelectric points of seven strains of tobacco mosaic virus in water were determined by a turbidimetric method.

There is a correlation between the isoelectric points observed and the amino acid content of the strains. A further rough correlation exists between the isoelectric points and the rate of movement of the strains in their host.

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