THE PHYSICAL CHEMISTRY OF TOBACCO MOSAIC VIRUS PROTEIN[1]

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INTRODUCTION

Tobacco mosaic, a disease which manifests itself by causing a mottling and a distortion of the leaves of tobacco plants, was first recognized over eighty years ago. In 1892 Iwanowski (19) observed that the juice of plants diseased with tobacco mosaic remained infectious after passing through a Chamberland filter, a device capable of holding back all of the living organisms then known. After repeating and confirming Iwanowski's experiments, Beijerinck (5), in 1898, realized that the infectious agent differed from ordinary bacteria and described it as being a "*contagium vivum fluidum.*" Tobacco mosaic was thereby established as the first disease recognized as being caused by an agent now known as a virus.

In 1935, by a process consisting of fractionation by chemical means, a crystalline protein was isolated from the juice of Turkish tobacco plants infected with tobacco mosaic virus (40). This protein has been shown to be in chemical combination with a nucleic acid closely resembling yeast nucleic acid and is, therefore, a nucleoprotein (2, 27). It has definite and specific chemical composition and physical characteristics. It gives the usual protein color reactions, is precipitated by the usual protein precipitating agents, has characteristic heat and pH stability ranges, and is denatured under certain definite conditions. Very dilute solutions give a specific precipitin reaction with antiserum to the protein. The protein is insoluble at its isoelectric point and in 20 per cent ammonium sulfate solutions. It crystallizes in definite needle-shaped crystals in the paracrystalline or mesomorphic state, visible only with the microscope.

All of the evidence available at present indicates that this crystallizable nucleoprotein is the active disease-causing agent. The virus protein

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isolated from many different batches of diseased Turkish tobacco plants and from other plant species infected with the virus possesses the same chemical, physical, biological, and immunological properties. This protein is capable of infecting other plants, will multiply in them, and can be isolated from such diseased plants. There is no reason to believe that the protein is not pure, for its chemical, physical, biological, and immunological properties remain unchanged following fractionation of the protein by various procedures. The ultracentrifugally isolated preparations of the material are completely homogeneous with respect to sedimentation constant and electrochemical behavior (14, 53). It was found impossible to demonstrate the presence of an impurity in such preparations even by the sensitive precipitin and anaphylactic tests. It has never been found possible to separate the virus activity from the protein by any one of several procedures. The ultraviolet light absorption spectrum of the protein agrees essentially with the destruction spectrum of virus activity (2, 10, 26). The pH stability range of the protein was found to coincide with that of virus activity. Partial or complete denaturation of a preparation by each of several procedures was found to result in a corresponding loss of virus activity. Finally, it is possible not only to inactivate and reactivate the virus protein, but also to demonstrate that the inactivation and reactivation are accompanied by simultaneous changes in the structure of the protein molecule (36). For complete and detailed descriptions of the properties of the tobacco mosaic virus protein, the reader is referred to other reviews (41, 42, 43, 44, 45). In this paper a summary of many of the physical and physicochemical properties of the protein is presented, and an attempt is made to unify the interpretation of these properties.

THE SHAPE AND SIZE OF THE TOBACCO MOSAIC VIRUS PROTEIN PARTICLES

Optical studies

Takahashi and Rawlins (48) first showed that the juice from tobacco plants diseased with tobacco mosaic exhibited stream double refraction, and it has since been demonstrated that solutions of the purified tobacco mosaic virus protein show the phenomenon to a marked degree (2, 3, 25, 49) (figure 1). This property may be the result of one or more of the following three factors: (*1*) the photoelastic effect, (*2*) the orientation of plate-like bodies in the flowing stream, and (*3*) the orientation of rod-like particles. The possibility that stream double refraction may result from the first (25) or second of these factors (48) has been eliminated; hence it may be concluded that the double refraction of flow shown by the tobacco mosaic virus protein is due to the orientation of rod-shaped particles in the flowing stream.

Wiener (52) has shown on theoretical grounds that it is possible to obtain

double refraction by orienting perfectly isotropic rod-shaped particles parallel to each other in a liquid medium, if the rods have a different refractive index from that of the medium. This is called morphic double refraction. If the rod-shaped particles have intrinsic double refraction, the double refraction of the system of oriented rods in a liquid medium is the sum of the morphic and intrinsic double refractions. By dispersing the rods in a medium having the same refractive index as the rods, the morphic double refraction can be completely eliminated, and any remaining double refraction is the intrinsic double refraction of the rods. It has been shown by the method indicated that the tobacco mosaic virus protein particles

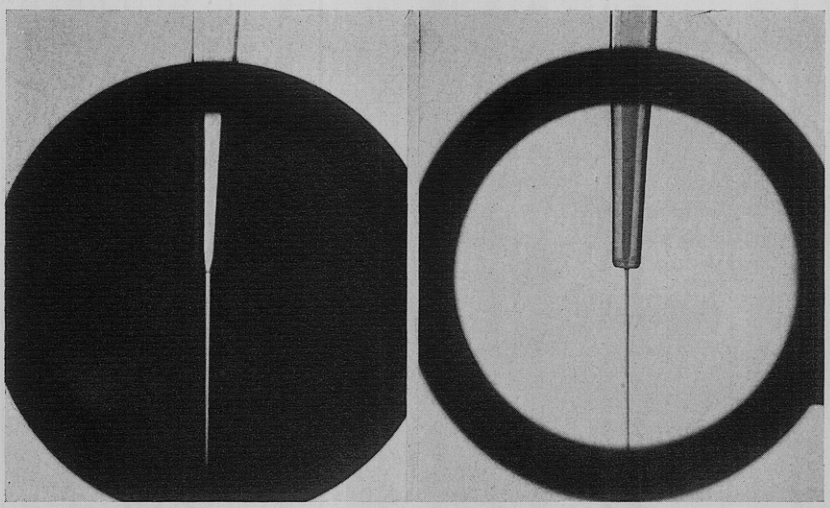

FIG. 1. Left: doubly refracting stream of tobacco mosaic virus protein solution photographed between crossed Polaroid plates arranged so that the vibration direction of each plate makes an angle of 45° with the direction of flow. Right: same system photographed between parallel Polaroid plates. (From Lauffer and Stanley (25)).

possess very little if any intrinsic double refraction and that the double refraction shown by solutions of the oriented molecules must be largely morphic double refraction (figure 2) (22). This result is entirely consistent with the observation of Bawden *et al.* (3) that dried films of the protein show very much less double refraction than wet films.

Solutions of the protein also show electrical birefringence (3). The birefringence is positive with respect to the direction of the electrical field, showing that the particles orient themselves parallel to the direction of the field (24).

Bawden and Pirie (2) first noticed that, upon standing, some solutions

of virus protein separate into two layers, of which the lower more concentrated one is liquid crystalline (figure 3). The jelly-like pellets obtained by ultracentrifuging solutions of the protein have also been shown to be

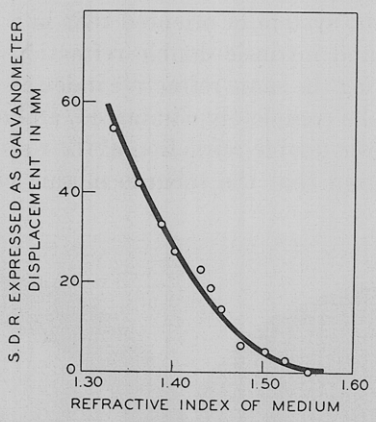

FIG. 2. The dependence of stream double refraction of tobacco mosaic virus protein upon the refractive index of the solvent. (From Lauffer (22)).

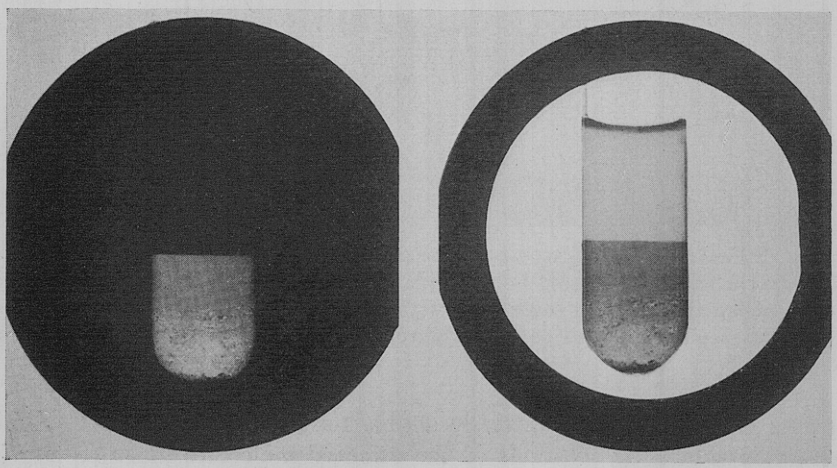

FIG. 3. Left: sample of tobacco mosaic virus protein solution which has separated into two layers. The photograph, taken with the aid of crossed Polaroid plates, shows that the bottom layer material is spontaneously doubly refracting or liquid crystalline. Right: same system photographed between parallel Polaroid plates. (From Lauffer and Stanley (25)).

typically liquid crystalline (22). This property of liquid crystallinity is generally associated with materials having rod-shaped molecules, and may therefore be regarded as constituting additional evidence of the rod-

like character of the tobacco mosaic virus protein particles. The layering behavior illustrated in figure 3 is entirely analogous to that described previously by Zocher and Jacobsohn (57) for vanadium pentoxide sols.

As one should expect of a substance having rod-shaped particles, solutions of the tobacco mosaic virus protein show the Ganz depolarization effect (22). The depolarization of the scattered light observed in the case of this protein is of the type regarded as being due to asymmetry or anisotropy of particles of the disperse phase, rather than to particle size being beyond a critical limit. All of these pieces of evidence, then, in addition to the fact that the virus protein crystallizes in needle-shaped mesomorphic crystals, leave little doubt but that the molecules of the tobacco mosaic virus protein are rod-shaped bodies.

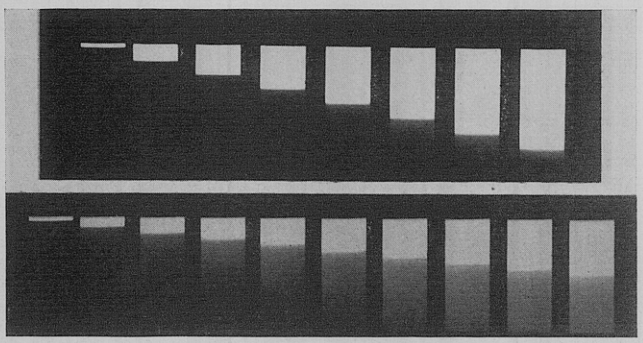

FIG. 4. Upper: sedimentation photograph of ultracentrifugally isolated tobacco mosaic virus protein showing single sharp boundary indicative of homogeneity. Lower: sedimentation photograph of an ultracentrifugally isolated sample which had stood in contact with electrolytes, showing two sharp boundaries indicating the presence of two molecular species. (Courtesy of Wyckoff.)

Ultracentrifugation studies

The first sedimentation studies on the tobacco mosaic virus were made on infectious juice by Bechhold and Schlesinger (4) before the virus was isolated as a purified protein. They followed the sedimentation by means of measurement of virus activity and obtained data from which a sedimentation constant may be calculated that is in good agreement with that found later for the purified protein. Sedimentation studies on the purified tobacco mosaic virus protein have been made by Eriksson-Quensel and Svedberg (14) and by Wyckoff (53, 54, 56). It was found that the protein isolated and purified by differential centrifugation gives a single sharp boundary in the ultracentrifuge (53) (figure 4), with a sedimentation constant, $S_{20°}$, of 174×10^{-13}.

It is not possible to interpret directly this sedimentation constant in

terms of molecular weight, because it is known that the molecules of the tobacco mosaic virus protein are not symmetrical. In order to calculate the molecular weight from these studies, it is necessary to know the dissymmetry factor of the protein. This is usually obtained from sedimentation equilibrium measurements (46), but, because of technical difficulties, it was not found possible to obtain satisfactory results by this method (14).

Viscosity studies

It is possible to obtain an idea of the dissymmetry of rod-shaped particles from studies of the viscosity of solutions or suspensions of these particles. Viscosity studies on this protein have been reported by Stanley (43), by Frampton and Neurath (15), and by Lauffer (21, 23). In figure 5 the data of Frampton and Neurath and of Lauffer are presented graphically. It is seen that viscosity is a linear function of concentration for very dilute

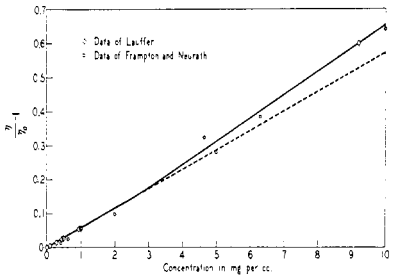

FIG. 5. The relationship between specific viscosity and concentration of tobacco mosaic virus protein. (Drawn from data of Frampton and Neurath (15) and of Lauffer (21, 23)).

solutions of the virus protein, but that this linearity does not hold for solutions as concentrated as 1 per cent. The results of the viscosity studies on the virus protein in urea solutions recently reported by Frampton and Saum (15a) have no direct relationship to the present problem, for they refer to degradation products which have been found not to possess virus activity (24).

Kuhn (20) has derived the following equation expressing the viscosity of a solution or suspension of rod-shaped particles as a function of the relative volume and the relative dimensions of the particles of the disperse phase:

$$\frac{\eta}{\eta_0} = 1 + 2.5G + \frac{G}{16}\left(\frac{b}{a}\right)^2 \qquad (1)$$

This equation was derived for a rod-like model composed of rigidly joined spheres. Guth (16) has derived the same expression for a model consisting

of an elongated ellipsoid of revolution. G is the volume of the dispersed material per cubic centimeter of solution, η/η_0 is the relative viscosity of solution, and b/a is the ratio of length to diameter of the rods of the disperse phase. An alternate equation has been derived by Eisenschitz (12) for elongated ellipsoids of revolution. When $b \gg a$, this equation takes the form,

$$\frac{\eta}{\eta_0} = 1 + G \frac{\left(\frac{b}{a}\right)^2}{15\left(\ln 2\frac{b}{a} - \frac{3}{2}\right)} \tag{2}$$

The Eisenschitz equation, in its expanded form, reduces to the Einstein viscosity equation for $(b/a) = 1$, just as does the Kuhn–Guth equation. Using equation 1, assuming little or no hydration of the protein, and taking 0.73 as the specific volume of the protein (2, 43), the value of b/a calculated from the limiting slope of the viscosity–concentration curve is 35. Using equation 2 and the viscosity data presented in figure 5, one obtains a value of 63 for b/a.

An equation describing the relationship between the ratio of minor to major axes of an ellipsoid of revolution and the dissymmetry factor of Svedberg, (D_0/D), has been presented by Herzog, Illig, and Kudar (18) and Perrin (34). It may be used in the following form when $b \gg a$:

$$(D/D_0) = \frac{\left(\frac{a}{b}\right)^{2/3}}{\sqrt{1 - \left(\frac{a}{b}\right)^2}} \ln \frac{1 + \sqrt{1 - \left(\frac{a}{b}\right)^2}}{\frac{a}{b}} \tag{3}$$

The diffusion constant of particles of any shape, D, is related to the sedimentation constant, S, according to the following equation:

$$D = \frac{RTS}{M(1 - Vd)} \tag{4}$$

where R is the gas constant, T is the absolute temperature, M is the molecular weight, V is the partial specific volume of the dispersed particles, and d is the density of the solvent (46). The diffusion constant of spherical particles, D_0, is given according to the Einstein equation:

$$D_0 = \frac{RT}{6\pi\eta_0 N\left(\frac{3MV}{4\pi N}\right)^{1/3}} \tag{5}$$

where N is Avogadro's constant and M is the molecular weight. Since D_0/D may be evaluated by equation 3, the molecular weight can be calcu-

lated from sedimentation data by combining equations 4 and 5. A value of 42.6×10^6 is obtained for the molecular weight of the tobacco mosaic virus protein by this method when the viscosity data are interpreted according to equation 1. This would correspond to rod-shaped particles 12.3 mμ in diameter and 430 mμ in length. If the value b/a obtained from the viscosity data employing equation 2 is used in conjunction with equations 3, 4, and 5, a value of 63.2×10^6 is obtained for M, corresponding to particles 11.5 mμ in diameter and 725 mμ in length.

Diffusion

Another means of evaluating the molecular weight of material having asymmetrical molecules is by combining diffusion and sedimentation measurements in the manner indicated by equation 4 (46). Diffusion studies on purified solutions of tobacco mosaic virus have been made by Waugh and Vinson (51) and by Hills and Vinson (18a). It is probable that the diffusion constants reported by the latter workers are too high because of failure to suppress the accelerating effect of smaller oppositely charged ions. Their virus solutions, which contained small amounts of electrolytes, were allowed to diffuse through a porous membrane into distilled water. Neurath and his associates (15, 32, 33) have measured the diffusion coefficient of chemically isolated virus protein, using the refractory method of Lamm. They found a value of about 3×10^{-8} for $D_{25°}$. From equation 4 it follows that this value corresponds to a particle with a molecular weight of 59.6×10^6. By the use of equations 3 and 5, it can be shown that such particles should have a ratio of length to width of 58, corresponding to dimensions of 675 mμ in length and 11.6 mμ in diameter (32, 33).

It is readily apparent that the molecular weight may be estimated from viscosity and diffusion data by the use of equations 1 or 2, 3, and 5 (32, 33, 35). If the data are treated in the indicated manner using equation 1, a value for the molecular weight of 116.5×10^6 is obtained, corresponding to particles 600 mμ long and 17.2 mμ in diameter. However, if equation 2 is used instead of equation 1, the calculated molecular weight is found to be 53.4×10^6, corresponding to particles 690 mμ long and 10.9 mμ in diameter.

The rotational diffusion coefficient

Boeder (9) has derived an equation in which the average orientation of rod-shaped particles in a flowing liquid is expressed as a function of the ratio of the velocity gradient tending to align the particles to the rotational diffusion constant. The latter is a measure of the tendency of the particles to become randomly oriented. Kuhn (20) has expressed the relationship

between the rotational diffusion constant, θ, and the length of a rod-shaped particle, b, by the equation:

$$\theta = \frac{8kT}{\pi\eta b^3} \tag{6}$$

where k is the Boltzmann constant. From quantitative measurements of double refraction of flow and the average orientation of the particles, Mehl (31), following the method of Boeder, estimated the rotational diffusion constant for chemically isolated tobacco mosaic virus protein to be 25 at 2°C. By substituting this value into equation 6, he obtained the value of about 610 mμ for the length of the particle, a value which is of the same order of magnitude as those obtained from the other data considered.

Filtration of the tobacco mosaic virus protein

The whole body of knowledge of viruses began with the observation that the infectious agent of tobacco mosaic would pass through a Chamberland filter (5, 19). In 1916 Allard (1) reported that, although the virus of tobacco mosaic would easily pass a Berkefeld filter, it was held back by a Livingstone atmometer porous cup. Later Duggar and Karrer (11) reported that the virus had about the same filterability as colloidal hemoglobin, and suggested a value of 30 mμ for the diameter of the particles of the infectious agent. MacClement and Smith (30) found that two strains of tobacco mosaic would pass collodion filters of pore diameter of 51 mμ, while a third strain, aucuba mosaic, would pass through pores 112 mμ in diameter but would be held by those 100 mμ in diameter. On the basis of Elford's studies (13), these results correspond to particle diameters of 17 to 25 mμ and 37 to 56 mμ, respectively. Thornberry (50) found that eight strains of tobacco mosaic virus, including the aucuba mosaic virus, as well as several other plant viruses, when diluted with a solvent containing nutrient broth, filtered through pores as small as 45 mμ, indicating that they all have diameters of about 15 to 22 mμ. All of the studies thus far reported were on plant juice containing the virus. Thornberry also studied tobacco mosaic virus protein purified by the modified lead acetate method of Stanley and found that this material would go through pores 33.8 mμ in diameter. In contrast with this observation, Bawden and Pirie (2) and Smith and MacClement (39) have reported that purified tobacco mosaic virus protein will not pass through a collodion membrane with a pore size as great as 450 mμ. They regard this as evidence of an aggregation of the protein due to purification. However, results obtained with protein isolated by differential centrifugation in the Princeton laboratory of The Rockefeller Institute for Medical Research indicate that this purified protein will pass freely through membranes with a pore size of 450 mμ, al-

though a diminished rate of passage in some membranes of smaller pore size has been noticed (29, 45). Smith and MacClement reported that in an electrical field the tobacco mosaic virus will pass through a membrane having an average pore diameter of 13 mμ (39).

If it is assumed that rod-shaped particles pass through the pores of the filter lengthwise, and that the ratio of pore diameter to particle diameter obtained by Elford (13) for symmetrical particles holds for the diameter of the rods, the results of the filtration of the virus protein would indicate a diameter of about 15 mμ,—a value in good agreement with that found by other means.

X-ray studies

X-ray diffraction patterns of the tobacco mosaic virus protein have been studied in the laboratories of Bernal (3, 6, 7) and of Wyckoff (55). Bernal and Fankuchen reported studies on the virus protein dissolved in water, on the liquid crystalline "bottom layer" material (see figure 3), on films of the protein obtained by evaporation and designated as wet and dry gels, respectively, and on oriented crystals of the protein. Wyckoff and Corey studied the diffraction patterns of crystals and of pellets obtained with the ultracentrifuge. The findings from the two laboratories agree on the intramolecular structure of the material. Bernal and Fankuchen regard the unit, which, because of its physical properties, is regarded as a molecule, as having an *internal* crystalline structure of great regularity, analogous to the structure of crystalline proteins. The lines that indicate this internal structure are found in the virus in all of the states studied, including solutions, and therefore must define the molecule rather than the crystal.

A lateral spacing of 15.2 mμ is found in the "dry gel" studied by Bernal and Fankuchen. The wet gel gives corresponding spacings varying around 21 mμ depending upon the composition, the liquid crystalline bottom layer shows analogous spacings of between 30 and 47 mμ, again depending on the concentration of the protein, and the ordinary solutions show no corresponding spacings. The process of drying a solution of the virus protein, then, consists of the removal of water until the solution reaches the concentration where the particles line up parallel to each other, giving a liquid crystal with rather wide intermolecular spacings. Further evaporation results in the removal of water from between these particles, gradually decreasing the intermolecular spacings until the dry gel state is reached, when most of the water has been removed. The intermolecular distance then becomes 15.2 mμ. Since this spacing is also obtained in the dried crystals, it probably represents the closest possible spacing of the molecules, and therefore provides an estimate of the molecular diameter.

Whereas Bernal and Fankuchen found this lateral spacing of 15.2 mμ in

the oriented crystals, they were unable to find evidence of intermolecular regularity of less than 120 mμ in directions other than at right angles to the length of the crystal. They accordingly suggested that the crystals are made up of rod-shaped molecules arranged parallel in hexagonal symmetry with respect to cross section, the intermolecular distances being 15.2 mμ, and without intermolecular regularity in the direction of the long axis. In figure 6 this picture is presented diagrammatically.

It should be emphasized that the x-ray diffraction data indicate a particle 15.2 mμ in diameter and probably more than 120 mμ in length. These figures for the dimensions of the particle are in good agreement with those arrived at from viscosity and sedimentation studies.

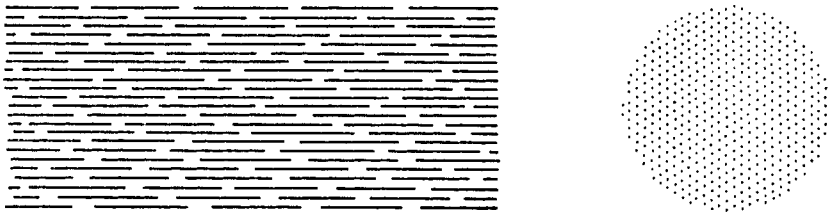

FIG. 6. The arrangement of the rod-shaped molecules of tobacco mosaic virus protein in the needle-shaped crystals. Left: longitudinal diagram showing parallel orientation of particles and no intermolecular regularity in lengthwise direction. Right: cross-sectional diagram showing hexagonal intermolecular symmetry. (Drawn from Bernal and Fankuchen (7)).

Adsorption on solids

Langmuir and Schaefer (20a) adsorbed tobacco mosaic virus protein on an A-layer of stearic acid conditioned with aluminum chloride and found by means of interference colors that the film was about 30 mμ thick. They found further that a surface conditioned by a monolayer of egg albumin takes up an adsorbed film of the virus protein having a thickness of 12.5 mμ. This latter they suggested, may be the thickness of the molecules lying flat on the surface, and indeed this value is in agreement with those obtained by the other methods just discussed.

General considerations

In summarizing the knowledge of the size of the tobacco mosaic virus protein molecule, it becomes evident that several experimentally determined constants for the protein, as well as adsorption, ultrafiltration, and x-ray diffraction data, are available. The sedimentation constant at 20°C., $S_{20°}$, is 174 $\times$ 10^{-13}, the specific viscosity per gram per milliliter is 57.9, the diffusion constant at 25°C., $D_{25°}$, is 3 $\times$ 10^{-8}, the rotational

diffusion constant at 2°C., $\theta_{2°}$, is about 25, and the specific volume, V, is 0.73. From various combinations of the above constants, using equations 1 to 6, the size, shape, and weight of the molecule may be evaluated. A summary of the results of various evaluations is found in table 1. The failure of the various sets of values calculated from sedimentation, diffusion, and viscosity data to coincide more closely is probably due to the simultaneous effect of three sources of error. In the first place, the results obtained from the viscosity data, especially when treated by the method of Kuhn, probably underestimate the asymmetry somewhat. This is true because both equations 1 and 2 were derived for the case in which there is no preferred orientation of particles, a condition which is not fulfilled by the tobacco mosaic virus protein solutions when flowing through the capillary of the viscometer. In the second place, the diffusion measurements have

TABLE 1

Summary of estimates of the shape and size of the tobacco mosaic virus protein particles

METHOD	LENGTH / WIDTH	LENGTH	WIDTH	MOLECULAR WEIGHT × 10^{-6}
		milli-microns	*milli-microns*	
Viscosity and sedimentation (Kuhn)	35	430	12.3	42.6
Viscosity and sedimentation (Eisenschitz)	63	725	11.5	63.2
Viscosity and diffusion (Kuhn)	35	600	17.2	116.5
Viscosity and diffusion (Eisenschitz)	63	690	10.9	53.4
Diffusion and sedimentation	58	675	11.6	59.6
Stream double refraction		610		
Ultrafiltration			15	
X-ray		>120	15.2	
Adsorption			12.5	

been made on solutions sufficiently concentrated so that the interparticle distance is of the order of magnitude of the probable length of the particles (32, 33). Hence it is entirely possible that the diffusion constant measured is somewhat too small, owing to interparticle attraction. Finally, the virus protein on which the diffusion studies were carried out was probably heterodisperse, since it had been prepared by chemical means. If the molecules are arbitrarily assumed to have the dimensions calculated from a combination of the viscosity data, using the Kuhn treatment, and the sedimentation data, it follows directly from equation 4 that the diffusion constant should be 4.2×10^{-8} at 25°C. It can readily be shown, by applying equations 3, 4, and 5, that the diffusion constant of a component with particles consisting of two molecules attached end to end would be 2.4×10^{-8} at 25°C. The intermediate value of 3×10^{-8}, as observed

experimentally, could obviously be that of a mixture of single and paired molecules.

In a general way, however, all of the data relative to the size and shape of the virus protein molecule are in reasonably good agreement. Of the various constants measured, the greatest precision has been obtained in the case of the viscosity and sedimentation values, and these are the only constants which have been measured on ultracentrifugally isolated material. For these reasons, bearing in mind that the molecular weight evaluated from viscosity and sedimentation data is probably somewhat too small, the value of 42.6×10^6 obtained by this means will be used, although tentatively, in further considerations. Frampton and Saum (15a) have objected to the use of viscosity and diffusion data in conjunction with sedimentation data for the calculation of the molecular weight of tobacco mosaic virus protein, because solutions of this material deviate somewhat from the behavior demanded by Poiseuille's and Fick's laws. Whereas it is evident from the preceding discussion that this objection is sound in principle, it seems probable, from a consideration of all the data pertaining to the subject, that under the more favorable circumstances these deviations are not of sufficient importance to impair the usefulness of the methods.

The first preparations of the virus protein obtained by chemical means were not homogeneous with respect to sedimentation velocity (14). Furthermore, ultracentrifugally isolated material, which is homogeneous, may develop a second more rapidly sedimenting component when allowed to stand in contact with electrolytes (53) (figure 4). This phenomenon may be explained by assuming that the molecules of the original compound associate end to end to form a second component with particles of twice the weight of the original molecule. If two rod-shaped particles, each from a supply having a sedimentation constant, $S_{20°}$, of 174×10^{-13}, a molecular weight of 42.6×10^6, and a ratio of particle length to diameter of 35, come together and associate end to end, they will contribute to a second supply having a molecular weight of 85.2×10^6 and a ratio of particle length to diameter of 70. Using equations 3, 4, and 5, it can be shown that such a supply would have a sedimentation constant, $S_{20°}$, of 202×10^{-13}. This value is in very close agreement with the actual sedimentation constants of the second components measured by Wyckoff (53), as may be seen in table 2. In view of this agreement, it has been suggested that the double boundary is due to a second component composed of particles arising from the end to end association of two original molecules (23).

It has already been pointed out that Bawden and Pirie (2) and Smith and MacClement (39), because of the lower filterability which they ob-

served for the purified virus protein, regard it as being an aggregated form of the virus as it occurs in the natural state and in untreated plant juice. This seems a reasonable interpretation of their data. However, the rather meager sedimentation data at present available indicate that this interpretation is not valid for the homogeneous ultracentrifugally isolated material. The sedimentation constant of the virus protein in a sample of untreated plant juice was found (56) to be the same as that of the protein later isolated from the same juice. If it is to be supposed that an aggregation took place in the purification process, the virus particles in the juice must have been at most half as large as the particles in purified preparations. The type of aggregation which would affect the sedimentation constant least is an end to end association of the hypothetical sub-units.

TABLE 2

Sedimentation constants of molecular species in tobacco mosaic virus proteins prepared at various intervals after plant inoculation

(Data of Wyckoff (53))

TIME AFTER INOCULATION	SEDIMENTATION CONSTANTS ($S \times 10^{13}$)	
	Ultracentrifuged samples	Chemically extracted samples
weeks		
1	174.3	177.1
2	180.0	179.3, 203.5
3	177.6	170.6, 198.7
4	177.1	172.1, 201.9
5	173.1, 198.8	172.7, 201.1
6	172.9, 199.7	170.9, 195.4
7	175.2, 198.9	175.2, 196.6
8	175.3, 197.9	178.3, 203.2
13	174.0, 205.3	

By the same method as was just used, it was shown that sub-units which could associate to give whole molecules of molecular weight 42.6×10^6, ratio of length to diameter of 35, and $S_{20°}$ of 174×10^{-13} would have a molecular weight of 21.3×10^6, a ratio of length to diameter of 17.5, and a sedimentation constant of 145×10^{-13} (23). Such a value differs sufficiently from 174×10^{-13} so that it could easily be differentiated. Any other set of assumptions would lead to an even lower sedimentation constant for the virus in juice. Since no more slowly moving boundary was found in the sample of juice studied, it must be concluded that the virus molecules in this sample of plant juice were of the same weight and size as those in purified samples prepared by careful differential centrifugation.

FURTHER PHYSICAL-CHEMICAL STUDIES

Absorption spectrum

The ultraviolet light absorption spectrum of crystalline tobacco mosaic virus protein has been studied by Lavin and Stanley (26) and by Bawden and Pirie (2). It shows a spectrum similar to that of other proteins, excepting that it has a maximum at 2650 Å. instead of at 2800 Å. This is an important observation, inasmuch as the inactivation of the virus by irradiation is at a maximum for wave length approximating 2650 Å. (10).

Surface spreading

Using the Langmuir trough, Seastone (37, 38) studied the surface spreading of tobacco mosaic virus protein. He found that the protein would not spread on dilute solutions of electrolytes (0.02 *M*) except in the

TABLE 3

The spreading of tobacco mosaic virus protein and of egg albumin on the surfaces of ammonium sulfate solutions

(Data of Seastone (38))

CONCENTRATION OF AMMONIUM SULFATE (PER CENT SATURATION)	FILM AREA AS M^2 PER MILLIGRAM OF PROTEIN	
	Tobacco mosaic	Egg albumin
	square meters	*square meters*
0	0.000	0.1
12.5	0.008	0.8
25.0	0.024	1.0
50.0	0.020	1.0
75.0	0.025	1.0
90.0	0.022	1.0

region of pH 1, where it spread to a small extent. His results show, however, that the protein will spread on concentrated ammonium sulfate solutions to a maximum extent of from 0.020 to 0.025 square meter per milligram (38). This is in contrast to the square meter per milligram occupied by films of such proteins as egg albumin and pepsin made in a similar manner (17). Evidently the tobacco mosaic virus protein does not unfold into a monolayer of a thickness corresponding to that of one amino acid, as do the proteins of lower molecular weight. It should be noted (table 3) that the spreading of the tobacco mosaic virus protein on solutions of ammonium sulfate is a function of salt concentration only in the lower ranges, and that, in the cases of the more concentrated salt solutions, the area of the film obtained is essentially a constant; this behavior parallels that of egg albumin. This suggests a definite stable arrange-

ment of the tobacco mosaic virus protein molecules at the air–solution interface.

Electrophoretic behavior

The first electrophoretic studies on tobacco mosaic virus were made by Takahashi and Rawlins (47) in experiments on extracts of plants diseased with tobacco mosaic. They followed the migration of the virus by means of infectivity measurements and reported the material as being isoelectric below pH 4. Best (8), from precipitation studies, has reported the isoelectric point to be at pH 3.4. Electrophoretic measurements of chemically isolated tobacco mosaic virus protein dissolved in acetate buffers of ionic strength of about 0.02 were made by Eriksson-Quensel and Svedberg, using the Tiselius apparatus (14). The mobility–pH curve was found to have a slope, $d\mu/dpH$, of 1.23 μ per second per volt per centimeter per pH unit, and the protein was found to be isoelectric at pH 3.49. In spite of the fact that the protein sample studied by these workers was not homogeneous with respect to sedimentation velocity, it was found to be electrophoretically homogeneous. Using the microcataphoresis apparatus of Northrop and Kunitz and adjusting the pH with hydrochloric acid and sodium hydroxide, Loring and Stanley (28) found that the isoelectric point of suspensions of crystals of the tobacco mosaic virus protein prepared by adding a small amount of ammonium sulfate to the solutions of the protein varied from pH 3.2 to 3.35. When acetate buffer was added the values were raised from 0.1 to 0.3 of a pH unit, depending upon the concentration of salts present.

Changes due to hydrogen-ion effects

In addition to the electrophoretic velocity which has already been discussed, there are other properties of the protein which vary with changes in the hydrogen-ion concentration. Sedimentation studies have been carried out by Eriksson-Quensel and Svedberg (14) and by Wyckoff (54) at various hydrogen-ion concentrations covering a wide range of the pH scale. It was found that, with the exception of the isoelectric zone, the sedimentation rate of the protein is essentially constant between pH 1.8 and 9.0, and that at hydrogen-ion concentrations greater or lower than those within this range the protein is rapidly broken down into components with lower sedimentation rates. It is interesting and important to note that this zone of approximately constant sedimentation rate coincides almost exactly with the pH zone of stability of the protein as measured by biological activity (figure 7).

Studies on the change of viscosity induced by altering the hydrogen-ion concentration have been made by Stanley (43) and by Lauffer (23). The

results of such a study are presented in figure 8. Double refraction of flow as measured by the method of Lauffer and Stanley (25) shows a behavior in general, though not rigorous, agreement with this viscosity behavior (figure 9) (23). These several behaviors suggest that, as one approaches the isoelectric point from either side, one encounters aggregation of the rod-shaped particles, at first end to end aggregation predominating and

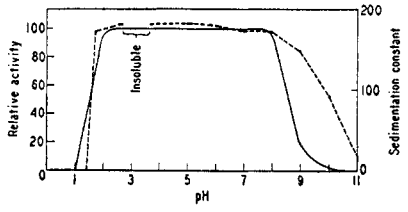

FIG. 7

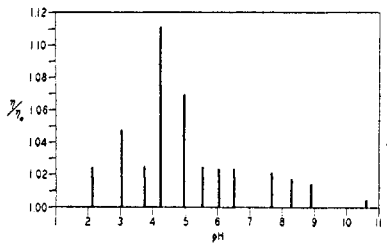

FIG. 8

FIG. 7. pH stability range of tobacco mosaic virus protein as measured by virus activity (solid line) and by sedimentation constant (dotted line). (Drawn from data of Best and Samuel, Stanley, Eriksson-Quensel and Svedberg, and Wyckoff, by Stanley (44)).

FIG. 8. The relative viscosity of solutions of tobacco mosaic virus protein (0.5 mg. per cubic centimeter) in buffers of ionic strength 0.02 at various pH values. (Drawn from data of Lauffer (23)).

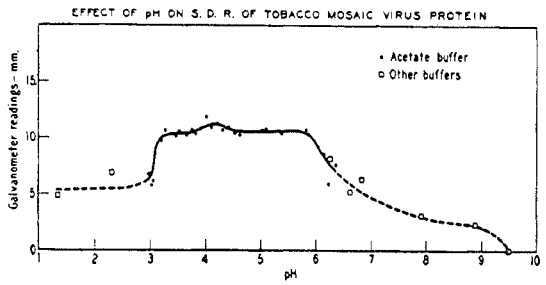

FIG. 9. Effect of pH changes on the stream double refraction of tobacco mosaic virus protein. (From Lauffer (23)).

later side to side aggregation predominating. This suggestion is in agreement with the observation that at the isoelectric point the protein exists in the form of needle-shaped crystals containing rod-shaped molecules packed side by side and end to end (7). Furthermore, the data of Mehl (31) show that the rotational diffusion constant evaluated from measurements of the orientation of tobacco mosaic virus particles in a mechanical field falls from 25 at pH 6.8 to 0.75 at pH 4.5. This is a direct indication

of a very considerable increase in length of the particle, substantiating the conclusion drawn from the viscosity and double refraction data.

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