CCVIII. PHYSICAL PROPERTIES OF BUSHY STUNT VIRUS PROTEIN

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It has not hitherto been possible to determine accurately the molecular size or weight of any of the virus proteins. X-ray diffraction patterns show in the case of tobacco mosaic virus protein that the cross sectional area of the rod-shaped molecules is 20,100 sq. Å. [Bawden *et al.* 1936] and assuming the rod to be cylindrical this corresponds to a diameter of approximately $16\text{m}\mu$. The same technique fails to give any indication of the length of the molecule and it is not at all certain that the molecular units have all the same length. Sedimentation, osmosis and diffusion measurements are also unsatisfactory when applied to the anisotropic plant viruses owing to the combination of large molecular size with gross asymmetry which they possess.

Some attempts have recently been made to apply to solutions of the anisotropic virus proteins equations relating the viscosity of a suspension of rods to the dimensions of the rods themselves. Frampton & Neurath [1938] and Lauffer [1938] deduced from viscosity measurements that the length of the tobacco mosaic molecule is 35–40 times the width. For a width of $16\text{m}\mu$ the length is therefore 560–640 $\text{m}\mu$ and the volume of the molecule can be calculated. If the density is 1.37 [Bawden & Pirie, 1937], the molecular weight by this procedure is $94\text{--}107 \times 10^6$, or approximately 100 millions. An alternative method of obtaining the molecular weight is to calculate the dissymmetry constant from the ratio of molecular length to width and to substitute this in the sedimentation equation. This method gives a weight of 42 millions [Lauffer, 1938]. Assuming, on the other hand, that the dissymmetry constant is 1.3, a value shared by a number of non-spherical proteins, a molecular weight of only 17 millions is obtained [Svedberg & Eriksson-Quensel, 1936]. The value of 1.3 is the lowest which could reasonably be proposed for a substance with the anisotropic properties of tobacco mosaic protein. Thus it seems that the molecular weight of the tobacco mosaic protein is more than 17 millions—probably considerably more—but less than 100 millions.

Recently a fully crystalline virus protein has been isolated [Bawden & Pirie, 1938, 1], the molecule of which appears to be spherical. This protein is obtained from the tomato plant infected with Bushy Stunt disease and is capable of transmitting the infection. It shows no anisotropy of flow and crystallizes in forms belonging to the cubic system. These properties suggest that the molecule is symmetrical and therefore adapted to exact measurements of size and weight by familiar physical methods. Our main object in the work described here has been to determine the size and weight of the virus protein molecule, but the opportunity has also been taken of investigating certain other properties, notably the electrophoretic behaviour.

The specimens of Bushy Stunt virus protein used in this work were prepared for us by Bawden & Pirie and a full description of the preparation is to

be found in their paper [1938, 2]. One specimen had been electro dialysed until the ash value, measured as sulphate, was 4%. The solutions were colourless and water-clear by transmitted light.

Partial specific volume. This was determined pyknometrically at 25° and the values 0.743 and 0.734 obtained. The mean of 0.739 is approximately the same as Bawden & Pirie's value for tobacco mosaic virus protein and falls within the density range of the proteins as a class.

Sedimentation constant. Table I contains data from a series of sedimentation velocity determinations. These were carried out in the Svedberg equilibrium centrifuge at 21° and at a speed of 18,120 r.p.m. corresponding to a force of 20,000 times gravity in the middle of the fluid column. The ultraviolet light absorption technique was used for optical observation and the source of light was a high pressure mercury vapour lamp. A band in the ultraviolet was selected by means of chlorine and bromine gas filters. Exposure times of 15–20 sec. were used with Ilford Ordinary plates.

Fig. 1. A series of sedimentation pictures of the Bushy Stunt virus protein, 0.27% in 0.02 *M* acetate buffer. Centrifugal force 20,000 *g*. 9 min. between each exposure.

Table I. *Sedimentation velocity of Bushy Stunt virus protein*

Protein concentration	Buffer composition	pH	$S_{W, 20} \times 10^{13}$
0.11	0.01 <i>M</i> CH ₃ COONa; 0.01 <i>M</i> CH ₃ COOH	4.70	147
0.20	0.1 <i>M</i> citric acid; 0.018 <i>M</i> NaOH	2.40	138
0.20	0.0013 <i>M</i> KH ₂ PO ₄ ; 0.0029 <i>M</i> Na ₂ HPO ₄ ; 0.1 <i>M</i> NaCl	7.10	158
0.24	0.017 <i>M</i> CH ₃ COONa; 0.003 <i>M</i> CH ₃ COOH	5.46	153
0.25	0.003 <i>M</i> CH ₃ COONa; 0.017 <i>M</i> CH ₃ COOH	3.99	151
0.25	0.004 <i>M</i> CH ₃ COONa; 0.016 <i>M</i> CH ₃ COOH	4.12	142
0.25	0.05 <i>M</i> H ₃ BO ₃ ; 0.014 <i>M</i> NaOH	8.70	141
0.26	0.006 <i>M</i> CH ₃ COONa; 0.014 <i>M</i> CH ₃ COOH	4.32	141
0.27	0.0005 <i>M</i> CH ₃ COONa; 0.0195 <i>M</i> CH ₃ COOH	3.40	143
0.29*	0.0013 <i>M</i> KH ₂ PO ₄ ; 0.0029 <i>M</i> Na ₂ HPO ₄ ; 0.1 <i>M</i> NaCl	7.10	145
0.34	0.01 <i>M</i> CH ₃ COONa; 0.01 <i>M</i> CH ₃ COOH	4.70	150

Mean $S_{W, 20} = 146 \times 10^{-13}$

* Electro dialysed prior to addition of buffer.

Fig. 1 shows a series of absorption pictures taken from one of the experiments in Table I, and typical of them all. The boundary is that of a perfectly homogeneous protein and shows a degree of sharpness which hitherto has only been obtained with some of the heavier haemocyanins [cf. Eriksson-Quensel & Svedberg, 1936, 1, 2]. One preparation which had been electro dialysed prior to the

addition of buffer showed the same perfect boundary sharpness as the others. The mean value of the sedimentation constant is 146×10^{-13} . The tobacco mosaic and cucumber virus proteins all give sedimentation constants higher than this, viz. $170\text{--}240 \times 10^{-13}$ [cf. Svedberg & Eriksson-Quensel, 1936; Wyckoff *et al.* 1937; Price & Wyckoff, 1938] but two plant viruses have smaller values, viz. latent potato mosaic (or "X" virus) for which $S_{W, 20} = 113$ [Loring & Wyckoff, 1937] and tobacco ring spot virus for which $S_{W, 20} = 115$ [Stanley & Wyckoff, 1937].

pH stability. From Table I it is seen that inside the range pH 2.4–8.7 the protein is homogeneous and unchanged in molecular size. This appears to be true for long periods at the hydrogen ion concentrations stated. After 1 hr. at pH 10 the protein consists of two components with sedimentation constants of 112 and 136×10^{-13} . Precisely the same splitting into components of 112 and 136 is found $\frac{1}{2}$ hr. after adjusting the pH to 1.3. After 48 hr. at this pH however only one boundary can be seen. This boundary is relatively sharp and sediments at a rate corresponding to $S_{W, 20} = 66 \times 10^{-13}$.

The pH stability range is thus a comparatively wide one, viz. pH 2.40–8.70. It is of interest that nowhere inside this range are any signs to be found of a minimum solubility zone in which molecular aggregation occurs such as is shown by some of the anisotropic virus proteins [cf. Bawden & Pirie, 1937].

Sedimentation equilibrium. Three determinations have been made at different protein concentrations. The height of fluid column used was approximately 2 mm. and the equilibrium centrifuge was run at the lowest speed available, viz. 1500 r.p.m., corresponding to a force of 150 times gravity at the centre of the fluid column. The scale method based on Lamm's refraction studies [Lamm,

Fig. 2. ○ After 48 hours. × After 96 hours.

1937] was used, and the scale photographs were taken with light of wave-length $366\text{m}\mu$. The superior accuracy of this method is especially apparent with the very steep equilibrium concentration gradients given by the heavier proteins. The temperature throughout was 20° and it was found that equilibrium was almost attained in 48 hr. and was complete after 96 hr. Fig. 2 shows the curves

obtained after 48 and 96 hr. in the same experiment. To obtain the concentration curve from this $x, dn/dx$ curve a procedure recently described by Pedersen [1937] was used with advantage. The fluid column is divided into layers 0.02 mm. in thickness and the concentration in the middle layer is given a hypothetical value (C_0), for example the value of the protein concentration at the beginning of the experiment. By means of the concentration (or refraction) increments obtainable from the $x, dn/dx$ curve the hypothetical mean concentration of an adjacent layer C_1 , equal to $C_0 + dc/dx \times 0.02$, is obtained and by repeating this procedure a series of mean concentrations corresponding to all the layers from the meniscus to the bottom of the cell is obtained. This enables a concentration curve to be constructed and the integral of this curve from the meniscus to the bottom of the cell should be equal to the total amount of protein in the cell. In order that this may be so a factor equal to the difference between the curve integral and the known amount of protein in the cell divided by the height of fluid column is then added (or subtracted) from the individual hypothetical layer concentrations, and a true concentration curve constructed. From the concentration values in each adjacent pair of fluid layers the molecular weight is calculated from the formula [Svedberg, 1926]

$$M_W = \frac{2RT \ln (C_2/C_1)}{(1 - V\rho) \omega^2 (x_2^2 - x_1^2)},$$

in which M_W is the molecular weight average and the other symbols have their customary significance.

The molecular weight may be determined by an alternative formula [cf. Pedersen, 1937], viz.

$$M_Z = \frac{2 RT \log_n \left(\frac{Z_2 x_1}{Z_1 x_2} \right)}{(1 - V\rho) \omega^2 (x_2^2 - x_1^2)},$$

in which the Z values are scale line displacements in arbitrary units.

Table II. *Results of a typical sedimentation equilibrium experiment. Concentration of protein at start 0.335% in 0.02M. Acetate buffer pH 5.17. Cell 4mm. thick. Speed 1530 r.p.m. Bottom of cell 5.95 cm. from axis of rotation $t = 20^\circ$*

x	z in μ	dc/dx	C %	Molecular weight $\times 10^6$	
				Weight-average (M_W)	Z-average (M_Z)
5.95	662	11.1	1.054		
5.93	515	8.64	0.962	7.22	7.55
5.91	412	6.91	0.807		
5.89	329	5.52	0.682	7.36	6.80
5.87	264	4.43	0.583		
5.85	211	3.55	0.503	7.70	6.72
5.83	168	2.83	0.440		
5.81	133	2.23	0.389	8.26	7.27
Mean				7.64	7.09

Table II shows the results of a typical experiment and it will be seen that the variation in M_W or M_Z values with height in the cell is comparatively small. This is a reliable criterion that equilibrium has been established and that we are concerned with a homogeneous protein.

In Table III are shown the mean M_W and M_Z values for three equilibrations. The mean molecular weight value of all the determinations is 7,600,000.

Table III. *Results of three sedimentation equilibrium determinations on Bushy Stunt virus protein*

Protein concentration	Buffer composition	pH	Mean molecular wt. $\times 10^6$	
			M_w	M_z
0.335	0.015 <i>M</i> CH ₃ COONa; 0.005 <i>M</i> CH ₃ COOH	5.17	7.64	7.09
0.220	0.015 <i>M</i> CH ₃ COONa; 0.005 <i>M</i> CH ₃ COOH	5.17	8.15	7.22
0.270	0.001 <i>M</i> CH ₃ COONa; 0.039 <i>M</i> CH ₃ COOH	3.40	8.01	7.42
			Mean 7.93	7.24

Size and weight of the molecule. The sedimentation constant is defined as the molecular velocity under unit force in water at 20°. Since the force of gravity on a spherical particle is $\frac{4}{3}\pi r^3 (\rho_v - \rho_s)$, the force required to produce unit velocity is

$$\frac{4\pi r^3 (\rho_v - \rho_s)}{3 \times S_{w, 20}},$$

where ρ_v is the density of the protein and ρ_s is the density of the solvent.

According to Stokes' law this force is equal to $6\pi\eta r$, where η is the viscosity of the medium.

$$6\pi\eta r = \frac{4\pi r^3 (\rho_v - \rho_s)}{3 S_{w, 20}},$$

$$r^2 = \frac{9\eta S_{w, 20}}{2 (\rho_v - \rho_s)}.$$

Substituting $S_{w, 20} = 146 \times 10^{-13}$ and $\rho_v = 1.353$ for Bushy Stunt protein we find the radius of the molecule to be $13.7 \text{ m}\mu$ and the weight 8,800,000.

The agreement between the molecular weight obtained from sedimentation equilibrium measurements and from sedimentation velocity measurements assuming Stokes' law indicates that the molecule does not depart markedly if at all from a symmetrical shape.

Electrophoretic mobility. Measurements of the electrophoretic mobility of the virus protein were made with the Tiselius apparatus [Tiselius, 1937], the migration of the protein boundary being followed photographically by the "Schlieren" method (Fig. 3). A sodium vapour lamp was used as a light source and exposures made on Ilford Panchromatic plates.

The mobility was measured at 0° in acetate buffers, total acetate 0.02 *M*, the protein concentration being maintained between the limits 0.25–0.35 g./100 ml. Corrections were applied for the viscosity effect of the buffer salts, and for the hydrostatic displacement [Tiselius, 1930]. Both of these corrections amounted to less than 1%.

The material showed the behaviour characteristic of a homogeneous substance, a single sharp Schlieren band being maintained throughout the experiments over the range of pH studied.

Details of the mobility experiments are given in Table IV and Fig. 4.

Table IV. *Electrophoretic mobility of Bushy Stunt virus protein*

pH	Mobility (<i>u</i>) cm. ⁻² V. ⁻¹ sec. ⁻¹ $\times 10^{-5}$	Direction
3.80	1.79	Cathodic
3.99	0.73	
4.12	0.15	Anodic
4.32	0.58	
4.70	1.73	
5.17	3.46	
5.47	4.59	

The isoelectric point of the virus protein was found to be $pH_0 = 4.11$ and the slope of the mobility curve at the isoelectric point, $du/d(pH_0) = 5.0 \times 10^{-5}$.

Fig. 3.

Fig. 3. Electrophoretic migration of Bushy Stunt virus protein in 0.02 *M* acetate pH 6.2. Potential gradient 9.5 V./cm. A single sharp boundary is present in all the exposures.

Fig. 4.

Fig. 4. Mobility- pH curve for Bushy Stunt virus protein in 0.02 *M* acetate buffer.

Specific refractive increment and optical dispersion. The refractive increment of a solution of the protein in 0.01 *M* NaCl at the isoelectric point was measured for three wave-lengths in the mercury spectrum using a photographic technique [cf. *Handbuch der Physik*, 18, 668, 1927]. The protein concentration was determined by drying to constant weight *in vacuo* at room temperature. The results are given in Table V.

Table V. *Specific refraction increment of Bushy Stunt virus protein at different wave-lengths. $T = 23^\circ$.*

Wave-length $m\mu$	Specific refraction increment
366	0.00178
436	0.00170
546	0.00164

SUMMARY

The partial specific volume, sedimentation constant, molecular weight, electrophoretic mobility, isoelectric point and specific refraction increment of the crystalline nucleoprotein causing Bushy Stunt disease in the tomato plant have been determined. The protein is exceptionally homogeneous both with respect to size and charge and no evidence of an asymmetrical molecular shape could be found.

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