Molecular Weights of Plant Virus Protein Subunits Determined by the Meniscus Depletion Method Using Solvents Containing Urea

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SUMMARY

The molecular weights of the protein subunits of *Odontoglossum* ringspot virus, belladonna mottle virus and brome mosaic virus were estimated by the equilibrium centrifugation method of Yphantis to be 17,300, 20,700 and 17,200, respectively.

Solutions of the virus protein subunit monomers were prepared by suspending the virus particles in neutral buffers containing 6 M-urea and Cleland's reagent; preliminary tests with well-characterized proteins showed that the urea solution decreased the partial specific volume of the proteins by about 0.02 to 0.03 g/ml.

In solvents without urea brome mosaic virus dimer subunits had a molecular weight of about 40,000.

INTRODUCTION

It is useful to know the molecular weights of virus protein subunits for many reasons: in studies of particle structure or as one of the characteristics for the classification of viruses, for example. The molecular weight can be estimated by several methods, of which the meniscus depletion method (equilibrium at high speeds; Yphantis, 1964) is a relatively simple procedure, provided the material is monomolecular. By contrast with SDS-polyacrylamide gel electrophoresis (Koenig et al. 1970), the meniscus depletion method gives a direct estimate of molecular weight and thus needs no markers. It is, however, more time-consuming. The precision of the method has been reported to be ± 2 % (Yphantis). In our own experiments the method was less accurate (about ± 5 %). In this paper we report experiments using the method to determine the molecular weight of protein subunit monomers in buffers containing urea and Cleland's reagent.

METHODS

The type and the cowpea strains of tobacco mosaic virus (TMV-N and TMV-C, respectively) and the closely related *Odontoglossum* ringspot virus (ORSV) were grown in tobacco (*Nicotiana tabacum*) cv. Samsun and purified as described by Paul et al. (1965). Turnip yellow mosaic (TYMV), and belladonna mottle viruses (BMV) were grown in Chinese cabbage (*Brassica chinensis* L.) and tobacco cv. Samsun, respectively. They were purified as described by Paul et al. (1968). Brome mosaic virus (BrMV) was grown and purified as described by Paul & Huth (1970). All purified viruses were lyophilized after exhaustive dialysis against distilled water and stored in the cold.

The protein subunit monomers of TMV, ORSV, TYMV and BMV were prepared in the following way. About 10 or 20 mg. lyophilized virus were dissolved in 5 ml. of a 0.1 M-tris-+
HCl buffer at pH 7.5 containing 6 M-urea and 6 mM-Cleland’s reagent and heated to 70° for 10 min.; in a few experiments with TMV-N and TYMV the protein subunits became degraded, presumably by enzymes, and heating stopped this. To the heated solution about 4 μg/ml. of ribonuclease were added, and the mixture was incubated at 37° for 1 hr. Then the mixture was cooled and dialysed against two changes of 0.1 M-tris buffer containing only 2 mM-Cleland’s reagent. The protein usually precipitated during dialysis, but if it did not, it was precipitated by adding a small amount of dilute acetic acid. The precipitate was washed twice with the same buffer and was then dissolved, depending on the amount of protein, in 2 or 4 ml. of tris buffer (0.1 M, pH 7.5) containing 6 M-urea and 2 mM-Cleland’s reagent. This solution was dialysed against a further 100 ml. of the solvent at 4° for 1 day to give what is referred to in Tables 1 and 2 as the undiluted protein solution. The protein monomers were stable in the solution for several days.

Protein subunit monomers of BrMV were prepared in the same way, but 0.1 M-acetate buffer at pH 4.9 was used instead of tris buffer. Protein subunit dimers of BrMV were prepared by the method described by Hiebert, Bancroft & Bracker (1968). In this case no urea was added.

The meniscus depletion method (Yphantis, 1964; Teller et al. 1969) was used to estimate protein molecular weights A Beckman model E ultracentrifuge with interference optics, symmetrical double slit aperture (slit width 0.73 mm.), 12 mm. Kel-F-coated double-sector or filled epon six-channel centrepieces, and sapphire windows was used for these determinations. The optical system was focused on the mid-plane of a quartz-windowed cell. Thus the focus for the sapphire-windowed cell was at 0.59 plane (Yphantis, 1964).

The subunits of each of the different viruses were centrifuged at different concentrations and speeds, for 21 to 27 hr, as indicated in the Tables. Protein solution and the proteinless solvent (0.1 ml. each) were put in the sectors in the six-channel or the normal double-sector cells together with either 0.01 ml. or 0.15 ml. Kel-F-oil (ρ = 1.8 g./ml.), respectively, to give the artificial baseline. At higher speeds, the six-channel centrepiece leaked, but this was stopped by greasing the sidewalls of the centrepiece with Kel-F-grease (ρ = 2 g./ml.). The conditions of the runs are given in the Tables.

The interference fringes were photographed on Kodak metallographic plates using exposure times of 5 to 9 min., and despite this long exposure the fringes were clear. The calculations were made according to Yphantis (1964; formula 13) using three equidistant points and values with a net fringe displacement of more than 0.15 mm. Graphs to show the relationship of σω(r) and net fringe displacement or \((b^2 - r^2)\) were made. Control runs with solvent in all sectors of the cells satisfied the criteria given by Yphantis (1964).

The specific gravity of the tris buffer containing 6 M-urea and 2 mM-Cleland’s reagent was determined pycnometrically to be 1.096. This value is in good agreement with data for similar solvents containing 6 M-urea (Kawahara & Tanford, 1966).

RESULTS

Measurement of the change of the partial specific volume (V) of virus proteins in 6 M-urea solutions

To interpret data from multicomponent systems centrifuged to equilibrium one must know how the macromolecular solute interacts with smaller solutes in the solution (e.g. Reisler & Eisenberg, 1969). Unfortunately, these data are lacking for virus proteins. Furthermore, this information is most important, as a small change in the partial specific volume \(V\) produces a great change in the estimate of the molecular weight; for example with
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\( \bar{V} = 0.74 \text{ ml./g.} \) and \( \rho \) about 1.1 g./ml., a change of \( \bar{V} \) of 2.5% will change the estimate of the molecular weight by 11%.

To determine the change of \( \bar{V} \) of the virus proteins in our solvent, the protein subunits of TMV-N, TMV-c and TYMV with the known molecular weights of about 18,000, 18,000 and 21,000, respectively were used. The \( \bar{V}s \) of these proteins are 0.73 to 0.74 ml./g. for TMV (Weber et al. 1963), and 0.734 ml./g. for TYMV (Kaper & Litjens, 1966). Table 1 shows the

<table>
<thead>
<tr>
<th>Subunits of</th>
<th>Number of experiments (at different concentrations)</th>
<th>Temperature (°)</th>
<th>Solvent density (g./ml.)</th>
<th>Speed (rev./min.)</th>
<th>( \sigma w(r) ) ( c \to 0 )</th>
<th>Known molecular weight* (ml./g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMV-N</td>
<td>3 (1/2, 1/4, 1/8)</td>
<td>23.0</td>
<td>1.094</td>
<td>39,460</td>
<td>2.80 †</td>
<td>( 18 \times 10^3 )</td>
</tr>
<tr>
<td></td>
<td>4 (1/4, 1/8, 1/16, 1/20)</td>
<td>23.0</td>
<td>1.094</td>
<td>50,740</td>
<td>5.21 ‡</td>
<td>( 18 \times 10^3 )</td>
</tr>
<tr>
<td>TMV-c</td>
<td>3 (1/2, 1/4, 1/8)</td>
<td>23.0</td>
<td>1.094</td>
<td>39,460</td>
<td>2.80 †</td>
<td>( 18 \times 10^3 )</td>
</tr>
<tr>
<td></td>
<td>3 (1/3, 1/5, 1/10)</td>
<td>23.0</td>
<td>1.094</td>
<td>50,740</td>
<td>4.00 †</td>
<td>( 18 \times 10^3 )</td>
</tr>
<tr>
<td></td>
<td>1 (1/4)</td>
<td>23.0</td>
<td>1.094</td>
<td>52,640</td>
<td>4.20 †</td>
<td>( 18 \times 10^3 )</td>
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<tr>
<td>TYMV</td>
<td>3 (1/4)</td>
<td>18.35</td>
<td>1.095</td>
<td>50,740</td>
<td>5.50 ‡</td>
<td>( 21 \times 10^3 )</td>
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<td>2 (1/10)</td>
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</table>

* For sources of information, see text.
† Fig. 1a; ‡ Fig. 1b; § Fig. 1c.

\( \bar{V}_{u} \), weighted mean 0.71

![Graph](image)

Fig. 1. Plots of \( \sigma w(r) \) v. net fringe displacement for protein subunits of (a) TMV-N at 39,460 rev./min.; (b) TMV-N at 50,740 rev./min.; (c) TYMV at 50,740 rev./min. Other experimental conditions, see Table 1.

The experimental data and the values of \( \sigma w(r) \) at zero concentration \( \sigma w(r) \). Fig. 1a to c are graphs showing the relationship between \( \sigma w(r) \) and net fringe displacement and indicate that there is little concentration dependence of \( \sigma w(r) \). From these data the \( \bar{V} \) of each of the proteins in our solvent could be calculated, and it was found that \( \bar{V} \) in the solvent containing urea (hereafter \( \bar{V}_{u} \)) was about 0.71 ml./g. in most experiments and only in two experiments with TMV-c was it larger (0.74 ml./g.); the weighted average of all experiments was 0.71 ml./g.
for $V_u$. Therefore the $V_u$ of these three proteins is 0.02 to 0.03 ml./g. smaller in the urea solutions than $V$ in the urea-free solvent. Similar changes of $V$ in concentrated salt solutions have been reported in the literature, although in most cases guanidine-HCl solutions were used as solvents (Kielley & Harrington, 1960; Marler, Nelson & Tanford, 1964; Gagen, 1966; Reisler & Eisenberg, 1969). As the amino acid compositions of all the virus proteins we used were similar, the value of 0.02 to 0.03 ml./g. was subtracted from the $V$ of all proteins to give $V_u$ for the calculation of the molecular weights (see also Ullmann et al. 1968).

**Determination of the molecular weights of ORSV-, BMV-, and BrMV-protein subunits**

ORSV is related to TMV, and their proteins apparently have the same number of amino acids (Paul et al. 1965). Table 2 shows the experimental conditions in the runs with ORSV subunits, and Fig. 2a to c gives the graphs showing the relationship between $\sigma w(r)$ and the net fringe displacement for these proteins. The straight line that best fitted all points at the different concentrations was calculated. The effect of concentration was small, and it can be assumed that the material was monomolecular. The experimental data gave a molecular weight of about $17.7 \times 10^3$ for the ORSV subunits, and this agrees well with the value of TMV protein subunit monomers. Thus, the subunits of ORSV and TMV seem to be of the same size.

BMV is related to the viruses of the Andean potato latent virus subgroup of the TYMV group (Jankulowa et al. 1968). The amino acid composition of BMV has been determined, but no direct estimate of the molecular weight of the protein subunit monomers has been made, though Gibbs & McIntyre (1970) estimated from the amino acid composition that the subunits probably contained 191 amino acid residues. In Table 2, 13 runs with BMV subunits are listed, and in Fig. 2d the $\sigma w(r)$ values of seven experiments at 39,460 rev./min. are plotted. The straight line in this figure represents, however, the least squares line for all 11 runs at this speed. In all experiments $\sigma w(r)$ was virtually independent of concentration. Using $\bar{V} = 0.75$ ml./g. and $\bar{V}_u = 0.72$ ml./g., the weighted average of the molecular weight is $20.3 \times 10^3$ (about 194 residues) for the BMV protein subunit. Thus, the subunits of BMV and TYMV are almost identical in size—another of the many properties they share.

Stubbs & Kaeberg (1964) estimated that the BrMV protein had a minimum chemical molecular weight of about $20.3 \times 10^3$, and they reported the amino acid composition, from
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which \( V \) could be calculated to be 0.744 ml./g. Furthermore, these authors determined the molecular weight of the subunit in dilute buffer by the Archibald method to be about \( 40 \times 10^3 \); twice the minimum chemical molecular weight. Using acetate buffer containing urea we found in seven experiments (Table 2) an average molecular weight of \( 17.2 \times 10^3 \); \( V_u \) was calculated in the same manner as for the other proteins. Graphs showing the relationship of \( \sigma w(r) \) and net fringe displacement or \( (b^2 - r^2) \) demonstrated the homogeneity of the preparations.

In dilute buffer without urea BrMV protein was present in dimers, with a molecular weight of \( 140 \times 10^3 \) (Table 2) which is in excellent agreement with the value found by Stubbs & Kaesberg (1964). In these experiments too, no concentration dependence of \( \sigma w(r) \) was found, indicating that no polymers were present.

![Graphs](image)

**Fig. 2.** Plots of \( \sigma w(r) \) v. Net fringe displacement for protein subunits of (a) ORSV at 50,740 rev./min.; (b) ORSV at 39,460 rev./min.; (c) ORSV at 44,770 rev./min.; (d) BMV at 39,460 rev./min. Other experimental conditions, see Table 2 and Results.

**DISCUSSION**

The viruses readily dissociated to give protein subunit monomers in dilute buffers containing 6 m-urea and Cleland's reagent. In all experiments the molecular weights we found were close to the reported minimum molecular weight of the chemical subunits. The major difficulty in evaluating the centrifugation experiments was that the protein solution was not a simple binary system, and the interaction parameters for our material were unknown. However, protein subunits of known molecular weights and partial specific volumes from viruses related to those under examination were used to study this interaction, and it was found that their \( V \) decreased by 0.02 to 0.03 ml./g. Since the two proteins used in these tests yielded similar results, despite being from unrelated viruses, it seemed justified to use this correction for all the viruses under examination. The accuracy of our assumption is shown
by the results for ORSV and BMV protein subunits. For BrMV protein monomers the estimated size was somewhat smaller than that calculated from the amino acid composition; perhaps the use of a different buffer and pH caused a different decrease of $V$.

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REFERENCES


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