VIROLOGY 41, 763–765 (1970)

Structure of Tomato Bushy Stunt Virus

A consistent set of molecular weights for tomato bushy stunt virus (TBSV) and its subunits has not hiterto been available despite the detail with which structural studies have revealed both the radial electron density distribution (*1*) and the arrangement of morphological units in the surface of the virus particle (*2*). This absence of chemical information has prevented the resolution of significant ambiguities in the structural information.

Both the BSV particle and that of the closely related turnip crinkle virus (TCV) have mean outer radii of about 155 Å and have structure units clustered on the two-fold positions of a T = 3 icosahedral surface lattice (*2*). Preparations of both viruses sometimes contain "small particles," 100 Å in radius, which have been shown to be a T = 1 polymorphic form of the outer-shell protein (*3–5*). On the basis of observations showing the RNA of TBSV concentrated in a spherical shell at about 110 Å, it has been suggested that the small particle corresponds to a core in the intact virus (*2, 6*). Measurements of the radial density distribution in TBSV showed matter in the interior of the virus particle with a mean density close to that expected for hydrated protein, in apparent agreement with the postulated two-shell structure (*1*).

The best estimate of the TBSV particle weight is 9×10^6 (see below) and the RNA content is 16.5% (*7*). A two-shell structure must have either two kinds of protein subunits or —if the core is the T = 1 small particle—240 subunits of a single species and of molecular weight 32,000. A simple T = 3 structure will have 180 identical subunits of molecular weight 43,000. It is therefore possible to distinguish among these alternatives if we know the number of different kinds of polypeptide chains and their molecular weights. Recently developed protein-chemical methods permit one to obtain this information from a few simple and rapid experiments. The results presented here indicate that there are 180 polypeptide chains in TBSV and that there is only one major protein component. We therefore infer that the small particle is not present as a core in the complete virus.

The molecular weight of virus proteins can be determined directly by disrupting virus particles with sodium dodecyl sulfate (SDS) and submitting the whole mixture to electrophoresis on polyacrylamide gel in the presence of SDS (*8, 9*). When TBSV at a concentration of 0.1% is thus treated (1% in SDS in 0.1 *M* sodium phosphate buffer, pH 7.0, 5 minutes at 98°), one major protein component (estimated to be at least 95% of the total) is found. From its electrophoretic mobility, the molecular weight of the polypeptide chain is between 42,000 and 44,000 (Fig. 1). No component with molecular weight 32,000 is found. Reduction and carboxymethylation of the protein (in SDS solution or in concentrated guanidine HCl) do not alter the mobility, nor does the presence of beta-mercaptoethanol (up to 0.2 *M*) during the SDS treatment. The observed species is therefore not a disulfide-linked dimer.

To check these results by an independent method, we have determined the molecular weight of the TBSV protein by gel filtration in 6 *M* guanidine-HCl. The viral protein was separated from RNA by phenol extraction of an SDS-treated virus solution. The protein was precipitated by addition of ethyl alcohol, dissolved in guanidine-HCl (8 *M*), and reduced and alkylated with iodoacetate by standard procedures (*10*). The distribution coefficient of this protein on an agarose column (Bio-Gel A 5M) in 6 *M* guanidine–HCl solution was determined by the procedure of Fish, Mann, and Tanford (*11*) with minor modifications (Rosenbusch and Weber, in preparation). The column was calibrated with proteins of known polypeptide-chain molecular weight. From the

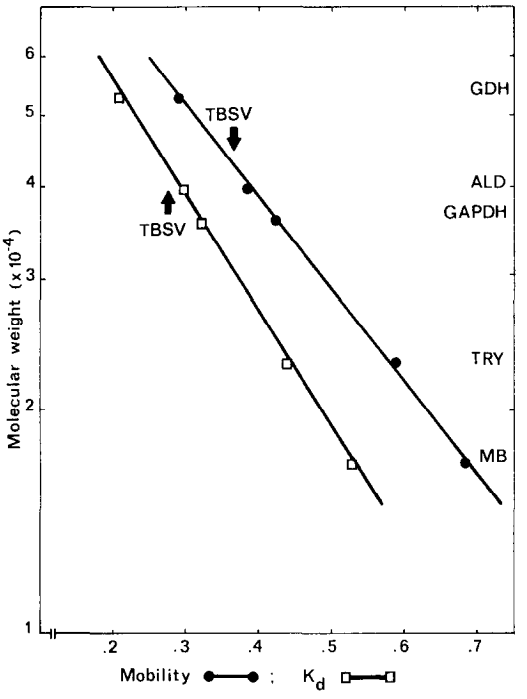

FIG. 1. Determination of the molecular weight of the polypeptide chain of tomato bushy stunt virus (TBSV). The marker proteins used were glutamic acid dehydrogenase (GDH), aldolase (ALD), glyceraldehyde phosphate dehydrogenase (GAPDH), trypsin (TRY), and myoglobin (MB). (For molecular weights cf. *9*.) The results of SDS electrophoresis on 10% polyacrylamide gels (*9*) are given by closed circles. The electrophoretic mobilities of the polypeptide chains are expressed relative to a marker dye. The results of gel filtration in 6 *M* guanidine-HCl on a 0.9 × 78 cm Agarose (Bio-Gel A 5M) column (*11*) are indicated by open squares. The elution position of the reduced and carboxymethylated proteins are expressed in K_d values. Mobilities and K_d values are plotted versus molecular weights.

elution profile, a weight of 42,000 could reproducibly be assigned to the TBSV protein (Fig. 1). Thus, two methods previously shown to yield accurate and reliable molecular weights (*9*, *11*) give, in excellent agreement, 42,000 ± 2,000 as the molecular weight of the TBSV polypeptide chain. Butler (*12*) and Michelin-Lausarot *et al.* (*13*) have independently obtained similar results since the present work was begun; Butler also reports a comparable weight for the polypeptide chain of TCV.

For the intact TBSV particle, reported molecular weights range from 7.8×10^6 to 10.7×10^6 (*14–16*). The most consistent values are derived from the hydrodynamic parameters. All recent measurements of $S^\circ_{20,w}$ fall between 131 and 135 S (*17*, *18*). We have performed diffusion experiments at two concentrations (1.2 and 2.5 mg/ml in 0.15 *M* NaCl, pH 7), using a 30-mm path length synthetic-boundary cell in the Model E ultracentrifuge. The diffusion constant obtained (1.26×10^{-7} cm²/sec) agrees with previous results (*17*) yielding a molecular weight[1] of 8.9×10^6. From the recently reported specific viscosity of TBSV solutions (*18*) we have computed an intrinsic viscosity of 3.94 cm³/g. This value together with the sedimentation coefficient gives a particle weight of 9.3×10^6.

From these molecular weights for the virus particle, the molecular weight of the polypeptide chain (42,000), and the RNA content of 16.5% (*7*), we calculate that the number of chains in TBSV is between 177 and 185. The structure therefore corresponds to a T = 3 design with 180 identical chains. A model with the small particle as a protein core (and hence 240 chains) is not consistent with the present data.[2] We therefore infer that the small particles are not preformed cores but are, as Leberman suggested (*4*), *in vitro* reaggregation products of outer shell protein. That a single kind of protein structure unit can pack into shells of different radii has previously been shown in the case of cowpea chlorotic mottle virus (*20*). The low intrinsic density of the TBSV particle at internal radii, assumed to represent protein (*1*), might also reflect the solvation of RNA in this region. Thus, the structural observations that suggested a two-shell design for TBSV are also compatible with the simpler structure indicated by our results.

ACKNOWLEDGMENTS

We are particularly grateful to Dr. C. A. Knight for the gift of bushy stunt virus used in this work. We are indebted to Dr. Susan Lowey for helpful discussions and to Drs. P. J. G. Butler

[1] The partial specific volume used in these calculations is 0.71 cm³/g (*17*, *19*).

[2] We cannot, however, exclude the presence of a small quantity of some additional protein species.

and M. E. Reichmann for communicating their results prior to publication. One of us (S. C. H.) is a Junior Fellow in the Society of Fellows, Harvard University. This research was supported in part by a grant from the National Institutes of Health, 5 R01 GM 16132-02.

REFERENCES

1. HARRISON, S. C., *J. Mol. Biol.* **42,** 457 (1969).
2. FINCH, J. T., KLUG, A., and LEBERMAN, R., *J. Mol. Biol.*, in press (1970).
3. HASELKORN, R., HILLS, G. J., MARKHAM, R., and Rees, M. W., *Abstr. Int. Biophys. Congr. Stockholm*, 293 (1961).
4. LEBERMAN, R., *Symp. Soc. Gen. Microbiol.* **18,** 183 (1968).
5. LEBERMAN, R., KLUG, A., and FINCH, J. T., *J. Mol. Biol.*, in press (1970).
6. KLUG, A., FINCH, J. T., LONGLEY, W., and LEBERMAN, R., *In* "Principles of Biomolecular Organization" (G. E. W. Wostenholme and M. O'Connor, eds.). Little, Brown, Boston, 1966.
7. DE FREMERY, D., and KNIGHT, C. A., *J. Biol. Chem.* **214,** 559 (1955).
8. SHAPIRO, A. L., VINUELA, E., and MAIZEL, J. V., *Biochem. Biophys. Res. Commun.* **28,** 815 (1967).
9. WEBER, K., and OSBORN, M., *J. Biol. Chem.* **244,** 4406 (1969).
10. WEBER, K., *Biochemistry* **6,** 3144 (1967).
11. FISH, W. W., MANN, K. G., and TANFORD, C., *J. Biol. Chem.* **244,** 4989 (1969).
12. BUTLER, P. J. G., *J. Mol. Biol.*, in press (1970).
13. MICHELIN-LAUSAROT, P., AMBROSINO, C., STEERE, R. L., and REICHMANN, M. E., *Virology* **41,** 160 (1970).
14. NEURATH, H., and COOPER, G. R., *J. Biol. Chem.* **135,** 455 (1940).
15. HEXNER, P. E., KUPKE, D. W., KIM, H. G., WEBER, F. N., BUNTING, R. F., and BEAMS, J. W., *J. Amer. Chem. Soc.* **84,** 2457 (1962).
16. OSTER, G., *Science* **103,** 306 (1946).
17. SCHACHMAN, H. K., and WILLIAMS, R. C. *In* "The Viruses" (F. M. Burnet and W. M. Stanley, eds.). Academic Press, New York, 1959.
18. DORNE, B., and HIRTH, L., *Biochemistry* **9,** 119 (1970).
19. KUPKE, D. W., *Proc. Fed. Amer. Soc. Exp. Biol.* **25,** 990 (1966).
20. BANCROFT, J. G., HILLS, G. J., and MARKHAM, R., *Virology* **31,** 354 (1966).

KLAUS WEBER
JURG ROSENBUSCH
The Biological Laboratories
Harvard University
Cambridge, Massachusetts

STEPHEN C. HARRISON
Children's Cancer Research Foundation
35 Binney Street
Boston, Massachusetts

Accepted May 14, 1970