The helical arrangement of the protein sub-units in tobacco mosaic virus

X-ray diffraction studies[1,2] have established that in the tobacco mosaic virus (TMV) particle the virus protein is in the form of structural sub-units set in helical array about the particle axis, and that there are (very nearly[3]) $3n+1$ such sub-units on three turns of the helix. The pitch of the helix is 23 A and the axial repeat period, therefore, 69 A. The value of n has, however, remained uncertain. It has been estimated as 10[1] or 12[2], giving 31 or 37 sub-units in the axial repeat period. We now know that both of these estimates are false, and believe the correct value of n to be 16, giving 49 sub-units in 3 turns of the helix.

The method used to establish this result was altogether more rigorous than that used to obtain the earlier estimates. It will be described in detail in *Acta Crystallographica*. It is based on a detailed quantitative comparison of the X-ray scattering by normal TMV and by a mercury-substituted TMV, (Hg-TMV), kindly prepared for us by Dr. FRAENKEL-CONRAT. In this preparation mercury was bound to the cystein residue of the virus protein, in the form $-Hg-CH_3$, to the extent of one Hg atom to about 20,000 molecular weight of virus. Since there is only one cystein residue in each chemical sub-unit of virus protein[4] it is to be expected that all mercury atoms occupy equivalent sites in Hg-TMV, and hence that they all lie at the same radial distance from the particle axis. Our X-ray diffraction measurements, on the equator and first five layer-lines of the fibre-diagrams of TMV and Hg-TMV, led us to conclude that all (or very nearly all) the mercury atoms lie on a helix of radius 57 ± 1 A, and, further, that in the axial repeat period of 69 A there are 3 turns of the helix and 49 equally spaced mercury sites.

Since there is only one mercury site on each chemical sub-unit, this indicates that there are 49 *chemical* sub-units on 3 turns of the helix. Using this result, it is found that a self-consistent interpretation of a substantial part of the X-ray diagram of TMV is obtained if it be supposed that

there are 49 *structural* sub-units in 3 turns of the helix. There is thus strong evidence that the sub-units determined by chemical methods on the one hand, by X-ray methods on the other, are identical.

We must now consider the implications of this conclusion with regard to the relationship between the molecular weights of the sub-unit and of the complete virus particle. Taking the particle length to be 3000 A,[5] there are $49 \times 3000/69 = 2130$ sub-units in the complete particle. If the molecular weight of a sub-unit is 17,000[4,6], this gives a total molecular weight of $36.2 \cdot 10^6$ for the protein or, assuming a nucleic acid content of 6%[4], a molecular weight of $38.4 \cdot 10^6$ for the virus particle. If the sub-unit weight is 18,000 the particle weight calculated in this way is $40.7 \cdot 10^6$.

Our results therefore suggest that the molecular weight of TMV is about $40 \cdot 10^6$. This is in good agreement with the values obtained by SCHRAMM AND BERGOLD[7] using sedimentation and diffusion measurements, and by OSTER, DOTY AND ZIMM[8] and OSTER[9], using light-scattering measurements. On the other hand WILLIAMS, BACKUS AND STEERE[10], using a direct method involving weighing and electron microscope measurements, found the particle weight to be $50 \cdot 10^6$.

We do not attempt, here, to decide between these conflicting values. We wish to stress, however, that, as far as the chemical and X-ray determination of the particle weight of TMV is concerned, the value, $40 \cdot 10^6$, arrived at above refers to the RNA together with the protein sub-units which follow the helical arrangement described. On the basis of the X-ray data alone we cannot exclude the possibility that the virus particle contains *small* amounts of protein organised in a different way. The combination of chemical and X-ray data, however, probably provides the most accurate method of determining the weight of that part of the virus which follows the regular helical arrangement.

This work is supported by the Agricultural Research Council.

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[1] J. D. WATSON, *Biochim. Biophys. Acta*, 13 (1954) 10.
[2] R. E. FRANKLIN, *Nature*, 175 (1955) 379.
[3] R. E. FRANKLIN AND A. KLUG, *Acta Cryst.*, 8 (1955) 777.
[4] C. A. KNIGHT, *Advances in Virus Research*, 2 (1954) 153.
[5] R. C. WILLIAMS AND R. L. STEERE, *J. Am. Chem. Soc.*, 73 (1951) 2057.
[6] J. I. HARRIS AND C. A. KNIGHT, *Nature*, 170 (1952) 613; *J. Biol. Chem.*, 214 (1955) 215.
G. SCHRAMM AND G. BRAUNITZER, *Z. Naturforsch.*, 8b (1953) 61.
G. BRAUNITZER, *Z. Naturforsch.*, 9b (1954) 675.
G. SCHRAMM, G. BRAUNITZER AND J. W. SCHNEIDER, *Z. Naturforsch.*, 9b (1954) 298.
C. I. NIU AND FRAENKEL-CONRAT, *Biochim. Biophys. Acta*, 16 (1955) 597.
C. I. NIU AND H. FRAENKEL-CONRAT, *J. Am. Chem. Soc.*, 77 (1955) 5882.
[7] G. SCHRAMM AND G. BERGOLD, *Z. Naturforsch.*, 2b (1947) 108.
[8] G. OSTER, P. H. DOTY AND B. H. ZIMM, *J. Am. Chem. Soc.*, 69 (1947) 193.
[9] G. OSTER, *J. Gen. Physiol.*, 33 (1950) 445.
[10] R. C. WILLIAMS, R. C. BACKUS AND R. L. STEERE, *J. Am. Chem. Soc.*, 73 (1951) 2062.

Received June 15th, 1956