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Aggregated Forms of the Satellite of Tobacco Necrosis Virus

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SUMMARY

The several kinds of the two- and three-dimensional crystalline forms produced by the satellite virus of tobacco necrosis virus are described and discussed.

INTRODUCTION

Purified preparations of the satellite virus of tobacco necrosis virus sometimes contain aggregates which consist of 12 particles of satellite virus. The aggregates can be seen in the electron microscope in negatively stained mounts, provided they are mounted in enough Na-phosphotungstate, for otherwise they collapse into disorganized but discrete groups of 12 particles. Neither the aggregates nor discrete groups of 12 particles are seen in shadowed mounts; probably the aggregates are not only destroyed by drying without the supporting film of Na-phosphotungstate but the particles are scattered over the mount in the remaining film (Kassanis & Nixon, 1961).

Our early preparations contained few aggregates but recent ones have contained very many, and not only aggregates of 12 particles. In addition to the previously recognized aggregates such preparations contain ones of half the size (six particles), dimers (two linked aggregates, having 23 particles), trimers (three linked aggregates, having 34 particles) and polymers. This paper describes these different forms and also the two-dimensional nets satellite virus particles sometimes form.

RESULTS

The shape of satellite virus particles. The schlieren pattern of an analytical centrifugation gives an idea of the amounts of the aggregates of various sizes present in a partially purified preparation (Pl. 1, fig. 2). It shows five peaks, with sedimentation constants (S_{20}W) of 49, 110, 169, 231 and 332, representing respectively individual satellite virus and tobacco necrosis virus particles, aggregates of six satellite virus particles, the previously recognized aggregates of 12 and dimers of these aggregates. Markham (1962) showed that the sedimentation constant of a dimer of an isometric virus is approximately $\sqrt{2}$ times the value of a monomer. This relationship fits very well the sedimentation constants of the three aggregated forms of satellite virus (169, 231 and 332 *S*). When the same virus preparation was examined in the electron microscope after staining with Na-phosphotungstate*, however, very few aggregates of 6 particles were seen compared with the many indicated by analytical centrifugation,

* All preparations were mixed with Na-phosphotungstate and were sprayed on to carbon films on seven hole platinum mounts.

whereas many trimers and polymers of aggregates showed in the micrographs (Pl. 1, fig. 1), for which there were no peaks formed during centrifugation. The most likely explanation for the differences between the two observations is that aggregated forms larger than dimers break during centrifugation into aggregates giving $169\,S$, some of which are rings of five particles and others with a sixth particle in the middle (Pl. 2, fig. 4*a*), depending on whether they came from the end or the middle of a polymer.

In electron micrographs most satellite virus particles appear angular and have an hexagonal outline (Pl. 2, fig. 5). No surface structure is visible although Reichmann (1964) claimed to have seen three morphological units along the edge in some particles, from which he concluded that the satellite virus particle has 42 morphological units. The fact that the particles form a two-dimensional net at 72° ($360^\circ/5$) (Pl. 2, fig. 5*a*), indicates that the particles probably have a fivefold axis of symmetry and Kassanis & Nixon (1961) suggested that satellite virus particles, like those of other spherical plant viruses, have an icosahedral symmetry (5–3–2). To help interpret the different aggregated forms seen in the electron microscope, we made models in which the individual particles are represented by icosahedra, but dodecahedra (Pl. 1, fig. 3*b*; Pl. 2, fig. 4*b*), rhombic triacontahedra, and possibly other more complicated shapes with icosahedral symmetry, could fit these forms. However, the well-defined hexagonal outline often shown by satellite virus particles can best be obtained by an icosahedron or somewhat similar shape, rather than by a dodecahedron and even less well by a rhombic triacontahedron, which has many more facets. A single dodecahedron is more likely to lie with its fivefold axis vertical, giving a dodecagonal outline (probably appearing circular at the resolution obtained), whereas the most common position for a single icosahedron is to have the threefold axis vertical, which gives an hexagonal outline. By joining icosahedra edge to edge, forms can be made closely resembling those seen in the micrographs. However, evidence is given below that the particles are probably joining in a much more complex way.

Three-dimensional forms. The aggregate of 12 particles is always an icosahedron whatever the shape of the individual particles might be. It is made up of two rings of five particles interpenetrating, with two particles closing the gaps, one in each side. In negatively stained mounts the individual aggregates appear in 3 different ways, depending on the axial orientation. Usually they lie with the twofold or threefold axis vertical, and very few with the fivefold axis vertical (Pl. 1, fig. 3*c*, *b* and *a* respectively). Paired aggregates of 12 particles (Pl. 2, fig. 4*b*) share one particle and therefore a polymer with x number of aggregates will contain $12x-(x-1)$ particles. The polymer sits with the twofold axis of the individual aggregates vertical. In a polymer the long axes of the rhombuses, outlined by the four particles in the upper surface about the twofold axis, are parallel (Pl. 1, fig. 1). This agrees with the assumption that the aggregate and the shared particle have fivefold symmetry and therefore all particles have this symmetry. When a trimer of a polymer is formed, the third aggregate can either fall in line with the other two or can join at an angle of $63{\cdot}4^\circ$, the angle between adjacent fivefold axes in the point group 5–3–2 (Pl. 2, fig. 4*c*). Regular icosahedra would join at $63{\cdot}4^\circ$, and the angles measured in micrographs ranged from 60° to 65°. The models of dimers and trimers have a gap between each aggregate that is larger than the distance between the two rings within an aggregate. The reason is that whereas the two rings within an aggregate interpenetrate, the rings of the neighbouring aggregates join by an intermediate particle. The gaps are visible in electron micrographs.

Two-dimensional nets. Just as the three-dimensional forms can be simulated if the individual model particles are icosahedra (or very similar shapes) joined edge to edge, so can the three kinds of two-dimensional nets that satellite virus particles sometimes form. Three arrangements are possible with the two-dimensional nets: (1) packing at 72° with fivefold axis vertical, (2) packing at 60° with threefold axis vertical, and (3) at 90° with twofold axis vertical (Pl. 2, fig. 5*a*, *b* and *c* respectively). The 90° packing seems less common than the other two, which was also the impression Wyckoff (1948) gained from examining the regular nets formed by a strain of satellite virus, which contained some small areas with rectangular arrangement.

Preparations of satellite virus crystallize to form rhombic plates when left at 4° with or without salt, and the crystalline pellets formed when preparations are ultracentrifuged also consist of minute thin rhombic plates (Plate 1 in Kassanis, 1962). The particles on the larger faces of the rhombic plates formed in either way are packed at 60°.

All the features observed in the micrographs are explained by packing icosahedra edge to edge, except for the rectangular net, which is presumably formed from particles lying with the twofold axis vertical. With regular icosahedra the 90° net is square (as in the model), but when the 90° nets of electron micrographs were measured in two directions at right angles the ratio of the two lengths was 1:1·13 (Pl. 2, fig. 5*c*) and in the direction of the larger repeat distance the particles seem staggered. Thus the particles are not strictly isometric. Keying of protuberances and hollows may account for the shorter distance, whereas the opposition of protuberances may account for the longer distance.

Conditions for the formation of aggregates. The forms so far described apply to the first strain of satellite virus isolated. Since 1961, when the 12-particle aggregates were first seen, two other strains of satellite virus have been isolated (SV2 and SV3) with only slight serological relationship to the first one (SV1) and to each other (Kassanis, 1966). So far aggregates have been seen only with SV1, although SV2 forms rectangular two-dimensional nets. Indeed, partially purified preparations of SV2 when ultracentrifuged give a crystalline pellet that consists of rectangular plates in which the particles are arranged at about 90° (Pl. 2, fig. 6).

The conditions needed for the aggregates to form are not known, but we possibly find more aggregates now because we have changed the purification method. Instead of two or three cycles of differential centrifugation, we now precipitate the virus repeatedly with ammonium sulphate. The aggregates appear after the first precipitation and become more plentiful after further precipitations when the virus is increasingly concentrated. When we purified SV1 and SV2 side by side, only SV1 formed aggregates. The aggregates in purified preparations survived prolonged dialysis against water and prolonged exposure to phosphate buffer at pH values of 6, 7 and 8. (The isoelectric point of satellite virus is about pH 7; Kassanis & Kleczkowski, 1965.) The aggregates also survive 4 hr in 0·1 M EDTA or 0·1 M-mercaptoethanol.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Electron micrograph of a partially purified preparation of satellite virus and tobacco necrosis virus, showing different aggregated forms of satellite virus. The arrows point to polymers of aggregates of satellite virus and a tobacco necrosis virus particle.

Fig. 2. Sedimentation pattern of the same virus preparation as in Fig. 1. The photograph was taken 12 min. after a speed of 23,150 rev./min. had been reached with a schlieren bar angle of 30°. The peaks from left to right have sedimentation constants of 49, 110, 169, 231 and 332 *S*.

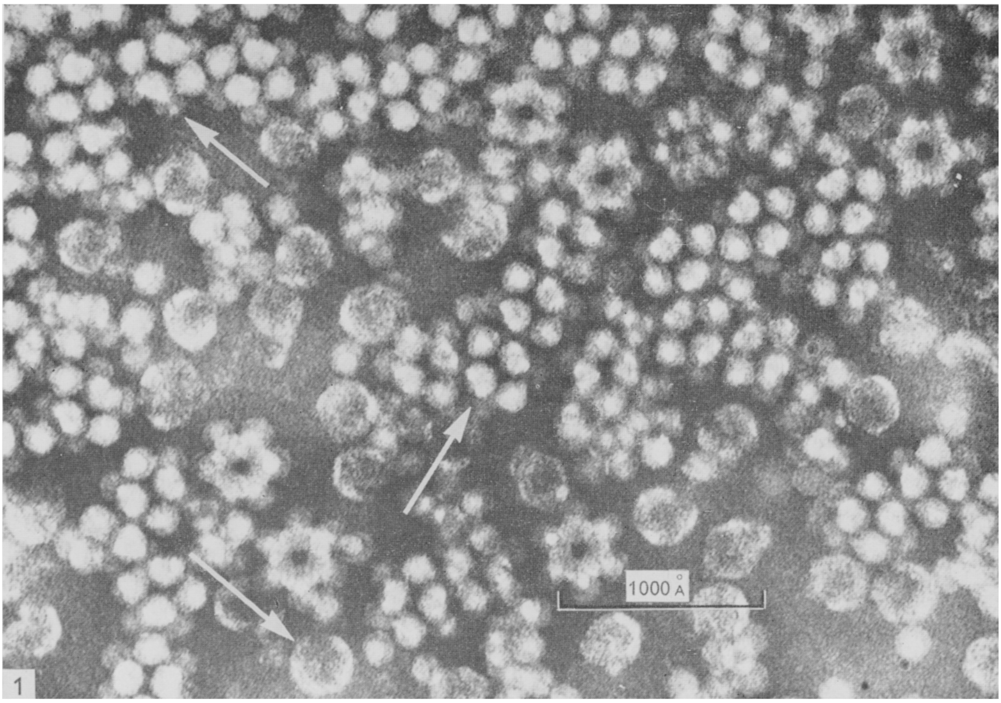
Fig. 3. Electron micrographs showing the three axial orientations of an aggregate consisting of 12 satellite virus particles, together with the respective models made up of 12 icosahedra; *a*, *b* and *c* show the aggregate respectively with the fivefold, threefold and twofold axes vertical; *b* also has a model made up of 12 dodecahedra.

PLATE 2

Fig. 4. Electron micrographs showing different aggregated forms of satellite virus together with the respective models made of icosahedra; *a* shows half an aggregate (six satellite virus particles), *b* a dimer (23 particles), *c* trimer (34 particles); *b* has also a model made up of 23 dodecahedra.

Fig. 5. Electron micrographs showing the three two-dimensional nets formed by satellite virus particles, together with the respective models of icosahedra; *a* shows the 72° packing, *b* the 60° packing and *c* the 90° packing.

Fig. 6. Electron micrographs of minute crystals produced when SV2 was ultracentrifuged, showing rectangular packing. This, like all the other micrographs, is of a mount stained with Na-phosphotungstate.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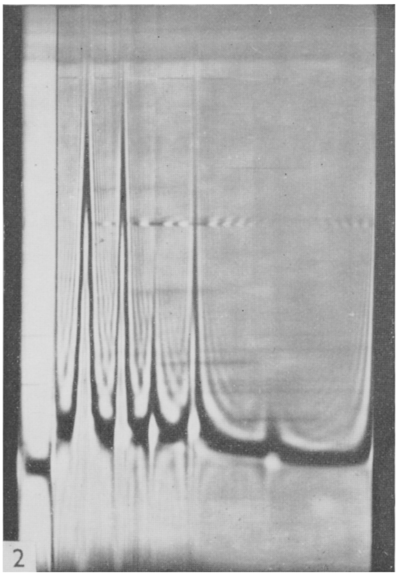
1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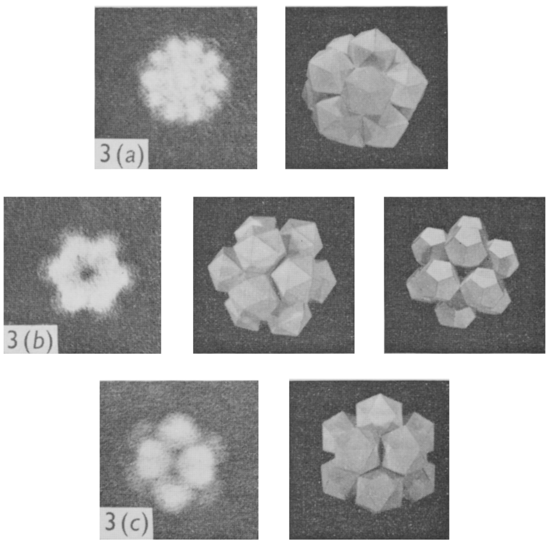
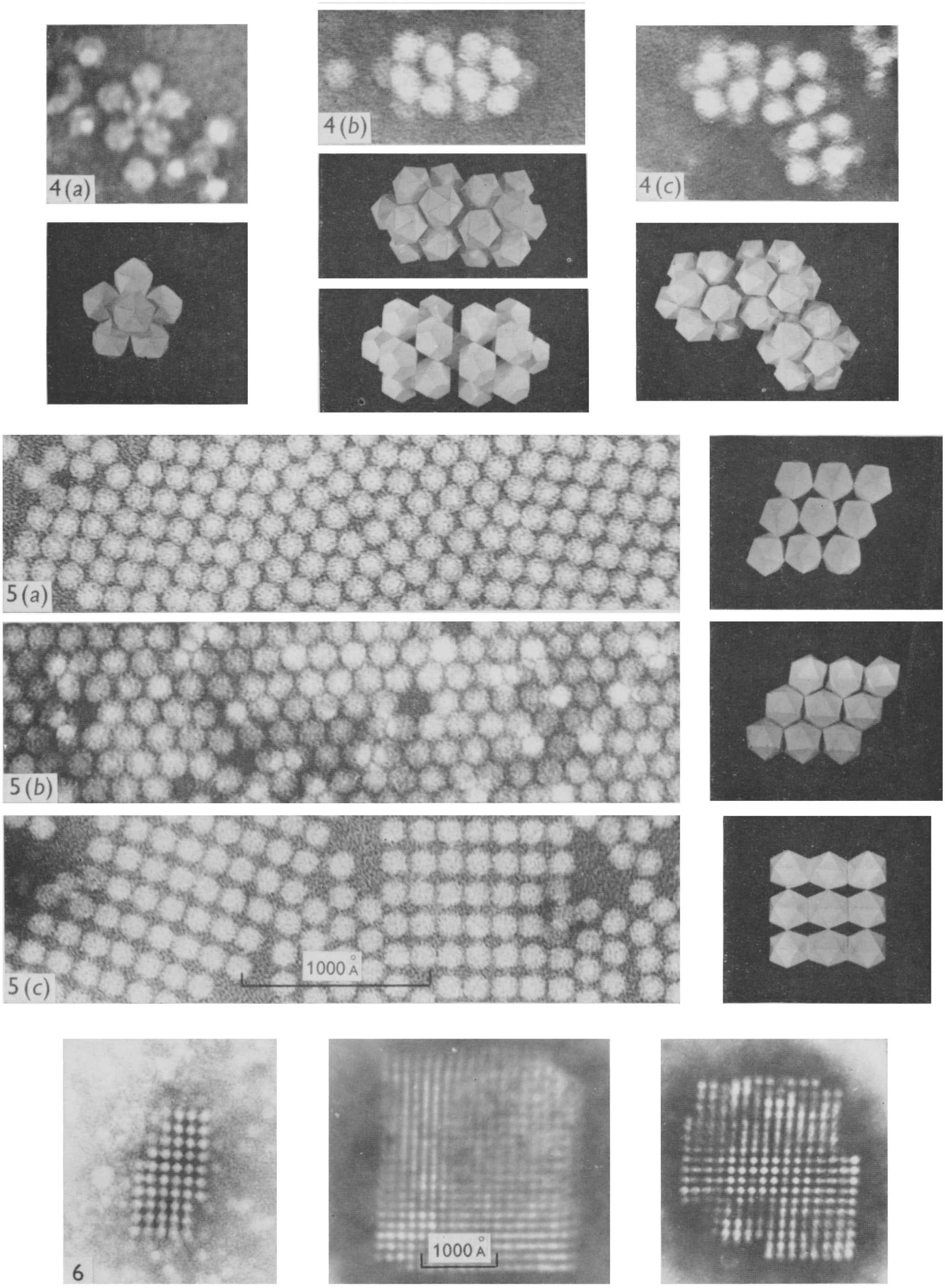

3 (*a*)
3 (*b*)
3 (*c*)



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