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Growth of Tobacco Mosaic Virus Particles

Abstract. Past studies have characterized the structure of tobacco mosaic virus particles by a variety of methods. In the present report the screw dislocation theory of crystal growth is applied to the formation of tobacco mosaic virus particles. The growth mechanism is shown to account for the rodlike morphology. It is also deduced that the biosynthetic process occurs at the growth step at the end of a particle.

The screw dislocation mechanism of crystal growth (*1*) has been proposed to account for gross discrepancies between observed and theoretically estimated growth rates. Since the inception of the dislocation theory of crystal growth, much activity has been stimulated in studies of growth phenomena, and understanding of these phenomena has been advanced markedly.

It is of interest to indicate the main features of the dislocation growth mechanism and to relate these to an important problem, the growth of tobacco mosaic virus particles. A crystal which is bounded by close-packed crystallographic planes can only grow by the addition of new atoms or molecules to steps at the edge of crystal layers partially covering the face. When a particle layer totally covers the surface, the bounding step grows out of existence and growth ceases. Growth can only continue through nucleation of a new patch of crystal plane. Nucleation will occur at an appreciable rate when the supersaturation exceeds a critical value. Thus, a close-packed face will not advance in slightly supersaturated solutions but will only grow above a critical supersaturation.

It has been pointed out that a permanent step exists (*1*) on a close-packed surface at which a screw dislocation terminates. The step extends from the dislocation to an edge or to a dislocation of opposite hand intersecting the same surface and may advance at low supersaturations. Unlike the edge of a partial layer, the step associated with a screw dislocation never grows out of existence, and the dislocated surface will grow at very low supersaturations.

If a crystal is formed which bears a screw dislocation or dislocations in a single direction, growth at low supersaturations (*2*) can only proceed in the direction of the screw axis. Such crystals have been grown of a variety of materials. They are thin rods of constant cross section and are called crystalline whiskers. The relevance of the preceding discussion to the growth of tobacco mosaic virus is immediate, because tobacco mosaic virus particles grow as crystalline whiskers.

Tobacco mosaic virus particles have a rodlike structure consisting of a thick-walled tube of protein surrounding a core of nucleic acid. The present discussion is concerned with the growth mechanism of the outer protein tube. Experiments on solution and reprecipitation of tobacco mosaic virus protein (*3*) demonstrate that the tubular habit is independent of the presence or absence of the nucleic acid core. X-ray diffraction (*4*) studies reveal that the tube walls are made up of structurally equivalent elements arranged on a helix having a pitch of 23 A. It has been estimated that there are $3n + 1$ elements in three helical turns where $n \sim 12$. The outer cylinder diameter is 150 A, and the inner diameter is 51 A.

The protein cylinder can be dissolved in water in the sense that native tobacco mosaic virus protein or elementary structural units are the solute molecules. The protein may be recrystallized at a *p*H of about 5 to form hollow cylinders, of varying length but arranged in a helical structure. At a *p*H of 6, cylinders do not form, but tiny washers do.

In the tubular form, 12⅓ structural elements are arranged on each turn of a spiral. Twelve elements enclose 350° of a circle, as shown in Fig. 1 (top). Either of two configurations might occur: The ring might deform in its own plane and form a bond (Fig. 1, middle) between elements *A* and *B*, or the ring might be elastically deformed into a lock washer (Fig. 1, bottom) so that addition of new structural elements could occur at either end of the lock washer, as shown in Fig. 2. As each element was added, its edge could serve as a further add-on site. The configuration of Fig. 2 corresponds to a crystal with a screw dislocation in which the unit cells are the structural elements. This growth behavior corresponds to that of a crystalline whisker.

A necessary condition for the operation of the growth mechanism is that the screw-dislocated configuration must be accessible to thermal fluctuations. The preceding condition may be written

$$E_s \leqq 20\ kT \qquad (1)$$

The strain energy E_s of an elastically deformed lock washer displaced by a distance τ is given (*5*) by

$$E_s = \frac{\mu \tau^2 b}{4\pi}\left[\ln \frac{r_1}{r_o} - 1\right] \qquad (2)$$

where μ is the elastic modulus, b is the thickness of the washer, and r_1 and r_o are the inner and outer radii, respectively. Substituting the numerical values $b = 2.3 \times 10^{-7}$ cm, $\tau = 2.3 \times 10^{-7}$ cm, $r_1 = 7.5 \times 10^{-7}$ cm, and $r_o = 2.5 \times 10^{-7}$ cm, the pertinent elastic modulus

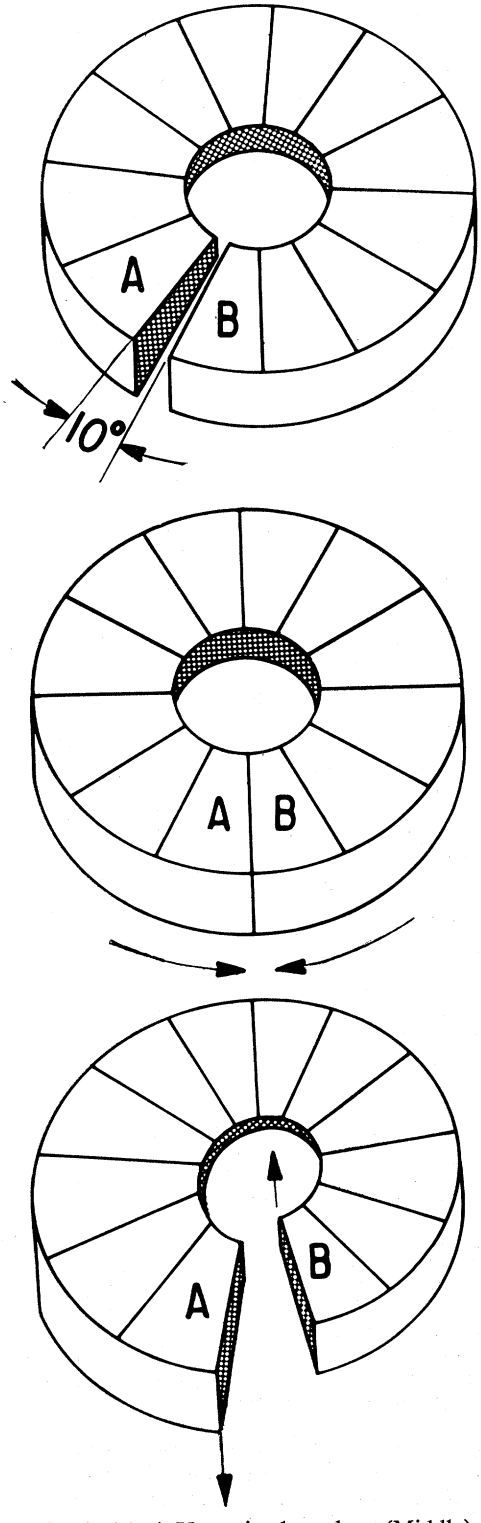

Fig. 1. (Top) Unstrained washer. (Middle) Ring with edge dislocation. (Bottom) Ring with screw dislocation.

μ can be estimated from Eq. 1 and 2. It is found that $\mu \leqq 10^{10}$ dyne/cm^2, which is a reasonable limit for a protein crystal.

When a structural element adds to the lock washer, as in Fig. 2, the configuration is stabilized by the amount of the binding energy. The stability continues to increase significantly until the length of the rod approximates the rod diameter. In the presence of the nucleic acid core, the stability would be further increased.

Qualitatively, the binding energy at the edges must be sufficient to give the screw-dislocated configuration a sufficient life-time to stabilize itself by further growth. Since the crystal ring with an edge dislocation offers no easy growth mechanism, it is only temporarily stable at a *p*H of 5, where tobacco mosaic virus rods grow.

If it is postulated that the dimensions of the structural elements are a function of *p*H, the formation of closed disks at a *p*H of 6 becomes understandable. If the closure gap in a single ring (Fig. 1, top) decreased, the elastic energy of forming an edge-dislocated structure would decrease as the square of the closure distance. As the gap decreased in width, or the circumferential dimension of the structural element increased, the strain energy would become unimportant with respect to bonding energy at the edges. The lifetime of the edge-dislocated structure would become very long, and almost all crystals should form as disks.

The characteristic rodlike morphology becomes a necessary consequence of the physical laws governing the growth behavior. It is of interest to proceed one step further—that is, to obtain a reasonable model by which the reproduction of tobacco mosaic virus rods in living plant cells can be accounted for by purely physical laws.

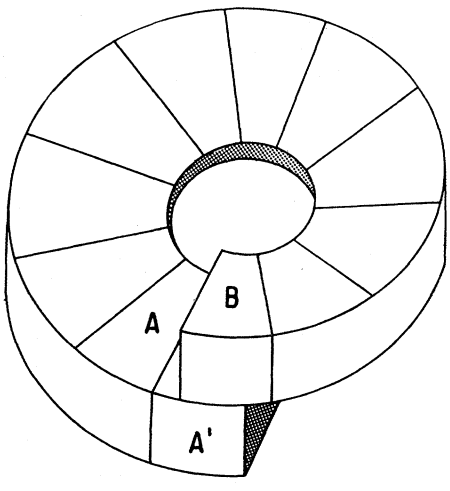

Fig. 2. Temporarily stabilized screw dislocation.

It is emphasized that this model is based on a simple and reasonable hypothesis, but that direct evidence as to its validity is unavailable.

It is well known that chemical reactions may either proceed heterogeneously or homogeneously. Recent studies of whisker growth (*6, 7*) have further clarified the role of surface structures in chemical reactions. It has been shown that aluminum oxide (*7*) can form from the reaction of water and a suboxide on a perfect surface of an alumina crystal but not in the vapor phase. On the other hand, copper (*6*) can form from the hydrogen reduction of cuprous iodide only at growth steps on a copper surface. It seems a fair extrapolation to suggest that in many organic reactions a preferential yield of a desired product may be obtained only in the presence of the crystalline product phase.

To account for the synthesis of tobacco mosaic virus in plant tissue, it is postulated that native tobacco mosaic virus elements are synthesized from simpler molecules only at the growth steps of the original rods. If the new structural units are synthesized faster than the core is built in, the solution becomes saturated with respect to the unstabilized tobacco mosaic virus rod phase. Since this solution would be supersaturated with respect to nucleic acid-stabilized rods, it is proposed that nucleation of new stabilized rods could occur at the available supersaturation.

Since tobacco mosaic virus particles are only synthesized in plant cells in the presence of infecting particles, it must be concluded that the structural elements are not synthesized homogeneously in the cell solution. The reproduction process must involve nucleation. Nucleation requires a finite critical supersaturation of the cell solution with respect to nucleating phase. The production of the critically supersaturated solution must be thermodynamically possible. Kinetically this reaction only occurs in the presence of infecting particles. Thus, it does not occur homogeneously.

A detailed mechanism (*8*) has been described for the growth of tobacco mosaic virus particles involving the screw dislocation growth mechanism. A reasonable mechanism has been offered to account for the reproduction of tobacco mosaic virus particles in living plant cells. These mechanisms are based on the physical chemical behavior of strictly nonliving substances.

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References and Notes

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8. It has been called to my attention that it has been previously proposed from biochemical considerations by Barry Commoner that virus replication involves linear extension of the ribonucleic acid fiber and of the protein sub-unit helix with the initial synthesis of the protein sub-unit occurring at the growth step [B. Commoner, "The biochemical basis of tobacco mosaic virus infectivity," *Proc. Intern. Congr. Biochem. 4th Congr.* (1958); ———, in *Plant Pathology—Problems and Progress, 1908–1958* (American Phytopathological Society, in press)].

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Induced Synthesis of Liver Microsomal Enzymes Which Metabolize Foreign Compounds

Abstract. The administration of 3,4-benzpyrene to rats markedly increases the activities of certain liver microsomal enzymes which metabolize foreign compounds. Evidence based on studies of enzyme induction is presented which suggests the presence in liver microsomes of several enzymes which can catalyze the same type of reaction.

Previous studies have shown that a variety of polycyclic aromatic hydrocarbons induce the synthesis of certain liver microsomal enzymes by a mechanism not involving the adrenal gland, the pituitary gland, or the hormone testosterone (*1,2*). For instance, the intraperitoneal injection of rats with 3,4-benzpyrene (BP), 3-methylcholanthrene (MC), or 1,2,5,6-dibenzanthracene (DBA) induces marked increases within 24 hours in the activities of the hepatic microsomal enzymes which reduce the azo linkage and N-demethylate aminoazo dyes (*1*), hydroxylate 3,4-benzpyrene (*2*), or N-2-fluorenylacetamide (*3*) and conjugate *o*-aminophenol as the glucuronide (*4*). These increases in enzyme activity are paralleled by marked increases in total liver protein (*1*). The purpose of the study reported here (*5*) was to determine the effect of 3,4-benzpyrene on various other liver microsomal enzymes which metabolize foreign compounds.

The results shown in Table 1 are typical of four similar experiments in each of which pooled livers from 10 to 30 animals were used. The intraperitoneal administration of 1 mg of BP to weanling rats resulted in increased activity of a variety of enzymes within 24 hours. The hydroxylation of 3,4-