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Polymerization-Depolymerization of Tobacco Mosaic Virus Protein. VII. A Model*

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ABSTRACT: A model is presented which imitates the major aspects of the endothermic polymerization of tobacco mosaic virus protein. The model involves the assumption that the increase in entropy necessary to drive the polymerization comes from the liberation of water from different kinds of water binding centers located at different positions on the surface of the monomeric unit. Each center is assumed to have its

own ΔH° and ΔS° for water "binding" and its own "melting temperature," defined as the temperature at which $\Delta H^\circ - T\Delta S^\circ$ is 0. Below its "melting temperature," contact with water will be a thermodynamically favorable environment for a center, above, isolation from water. Change from an aqueous to a nonaqueous environment can in some cases be achieved by rotation of monomeric centers and in others by polymerization.

The following facts which bear on the mechanism of polymerization of tobacco mosaic virus (TMV) protein, first described in detail by Schramm (1943), have been established: (1) Polymerization is endothermic (Lauffer *et al.*, 1958). (2) The starting material, commonly known as TMV "A" protein, because it was first obtained by alkaline degradation of the virus, is a stable trimer of the ultimate protein subunit (Banerjee and Lauffer, 1966). (3) The ultimate protein subunit with a molecular weight of 17,500 is obtained at pH 6.5 at very low concentration (Ansevin and Lauffer, 1959), in 67% acetic acid (Banerjee and Lauffer, 1966; Anderer *et al.*, 1964), and in solution at pH 13 (Anderer, 1959; Wittmann, 1959). (4) At temperatures between 4 and 14°, in 0.1 ionic strength buffer at pH 6.5, the reaction follows the mathematics

of condensation polymerization with values of ΔH° and ΔS° of 30,000 cal/mole and 124 eu, respectively (Banerjee and Lauffer, 1966). (5) In the temperature range 14–ca. 20°, under the same conditions, the reaction also follows the pattern of condensation polymerization, but with much higher values of ΔH° and ΔS° , approximately 190,000 cal/mole and 682 eu, respectively (Smith, 1961; Lauffer, 1962, 1964b). (6) A double disk consisting of 32 ultimate protein subunits is a stable intermediate, certainly in the dry state (Markham *et al.*, 1963) and possibly in the wet state, because a sedimenting boundary with approximately the right sedimentation coefficient, 20–25 S, can be observed (Schramm and Zillig, 1955; Lauffer *et al.*, 1958; Caspar, 1963). (7) Further polymerization leads to stacked disks (Markham *et al.*, 1964) which rearrange to form helical structures resembling the protein in TMV (Franklin, 1955, 1956; Markham *et al.*, 1964). At temperatures of 25–30° in 0.1 ionic strength buffer at pH 6.5, these rodlike polymers can be about the size of TMV particles (Lauffer *et al.*, 1958). (8) The polymerization is accompanied by the release of "bound" water molecules and by an increase in volume (Stevens and Lauffer, 1965; Lauffer, 1964a). (9) Polymerization involves a decrease in negative charge of 2–3 f/mole of trimer, but this is accompanied by a

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decrease in entropy and thus does not provide the large increase required for polymerization (Ansevin *et al.*, 1964). (10) Polymerization is favored by lowering the pH and by increasing the ionic strength, thereby probably indicating that electrostatic repulsion opposes polymerization and that "salting out" may have something to do with the process (Lauffer *et al.*, 1958; Smith, 1961; Lauffer, 1962, 1964b). A model is presented which can imitate the first nine aspects of the behavior of TMV A protein described above.

Theory

The model is a slightly deformable ellipsoid of revolution, with major and minor semiaxes of 35 and 12.25 Å, which is incapable of forming covalent bonds, hydrogen bonds, or salt linkages with neighboring ellipsoids. The basic postulate is that the controlling factor in the polymerization-depolymerization of the model is the "binding" or releasing of water molecules. It is visualized that the surface contains chemical centers which interact with or "bind" water when in contact with water and do not when in contact with the surfaces of other model units. The binding might be by the mechanism suggested by the work of Frank and Evans (1945), by electrostriction, by hydrogen bonding, or by some other mechanism. A particular kind of center will "bind" water with definite negative enthalpy and entropy. When, for a particular binding center, $\Delta H^\circ - T\Delta S^\circ$ for water "binding" is negative, it will be in a thermodynamically favorable environment if in contact with water, *i.e.*, it will be hydrophilic and depolymerization will be favored. When, at a higher temperature, $\Delta H^\circ - T\Delta S^\circ$ is positive for water "binding," the binding center will be in a thermodynamically favorable environment when isolated from water, *i.e.*, it will be hydrophobic and polymerization will be favored. The temperature at which $\Delta H^\circ - T\Delta S^\circ$ is 0 for a particular binding center is called, for convenience, the "melting temperature" for this binding center. If ΔH° and ΔS° are known for some particular temperature, the melting temperature can be calculated from the above relationship if ΔS° and ΔH° do not change appreciably over the temperature range involved. Thus, when the temperature is below the "melting temperature" of a particular binding center, that center will tend to be in water, and when the temperature is above the "melting temperature," that center will tend to be in contact with other model units. It is possible that a center can come in contact with water or model substance merely by rotation of the monomeric subunit. However, under other circumstances, polymerization or depolymerization will be required (Lauffer, 1966a).

An important secondary assumption is that the surface of the model monomer has different kinds of water binding center at various locations, each with its own "melting temperature." By this postulate, at a temperature below the "melting temperature" of all binding centers, all surfaces of a monomer would find water a thermodynamically favorable environment.

At such a temperature, monomers should be found at all concentrations. At intermediate temperature above the "melting temperature" of some of the centers, contact with the surface of other model subunits will be thermodynamically advantageous for those centers. However, those centers with "melting temperatures" above that intermediate temperature will find water a favorable environment. This situation should favor a stable intermediate. At temperatures above the "melting points" of all water binding centers, the thermodynamically favorable environment of all water binding centers will be contact with the model units; then the maximum degree of polymerization should be favored.

To imitate the first nine phenomena listed in the introduction, it is necessary to postulate the existence of at least four kinds of water binding center on a model subunit. Centers of Type I must be strongly hydrophobic at all temperatures above 0°. These are assumed to be responsible for stable trimers. It is probable that they have a "melting temperature" equal to or lower than that of type II centers. Centers of type II are assumed to be responsible for the early stages of polymerization at temperatures between 4 and 14°. Centers of type III are assumed to be responsible, along with those of type II, for polymerization at temperatures between 14 and about 20°. Type IV centers are assumed to be responsible for later stages of polymerization.

Figure 1 provides a highly schematic illustration of what is meant by the assumption that the various types of water binding center are located at different positions on the monomer. The model is an ellipsoid of revolution 70 Å long and 24.5 Å in maximum diameter (Figure 1a). In the direction of the long semiaxis, the inner end and the outer end can be distinguished on the basis of the way the ellipsoids are oriented in the completely polymerized state. It is assumed that in all states of polymerization, the major semiaxes are more nearly parallel than antiparallel, but that rotation about the major semiaxis of as much as 180° and some rotation about a minor semiaxis are possible in going from one intermediate to another. For example, let the large circles in Figure 1 represent a zone on the surface of the ellipsoid near the center, and let the small circles represent a zone on the surface near the inner end of the major semiaxis. It is visualized that type I centers occupy 60° of arc near the maximum diameter (large circle) in a position which will arbitrarily be referred to as the bottom (with respect to the ultimate orientation in a helical polymer standing on end). Type II centers are visualized as occupying two 60° segments, one on each side of the type I center, on the bottom half of the ellipsoid near the maximum diameter. Type III centers are visualized as occupying three 60° segments on the bottom half of the ellipsoid near the inner end (small circle) and type IV centers are pictured as occupying six 60° segments on the top half of the ellipsoid (three each on the large and the small circles).

At a temperature below the "melting point" of type I centers, monomer should be stable at all concentrations

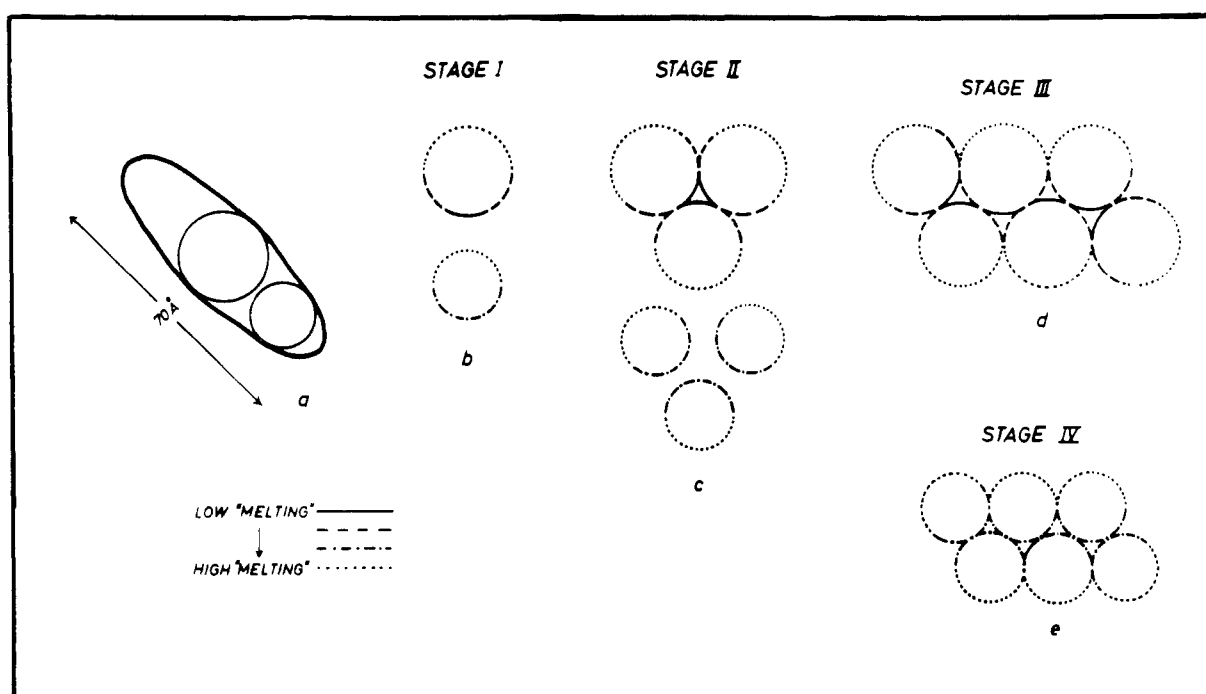


FIGURE 1: Diagram illustrating polymerization of model. For description see text.

(Figure 1b). However, with TMV protein, at temperatures slightly higher than the melting point of ice, monomer can be obtained only at extremely low protein concentrations, or at pH values corresponding to high electrostatic charge. Thus, for the model to resemble TMV protein, type I centers must be strongly hydrophobic at approximately 3°.

In the trimer, designated as A' to differentiate it from TMV A protein, type I centers can get away from water in the way illustrated in Figure 1c. If the major semiaxes of the three ellipsoids are approximately parallel, then all types II-IV centers can be exposed to water as shown in Figure 2, upper left. For TMV protein, the magnitude at 3° of ΔF° in 0.1 ionic strength buffer at pH 6.5 can be estimated for the transition, monomer to trimer, from the data of Ansevin and Lauffer (1959). For the reaction $3P \rightleftharpoons A$, $K = [A]/[P]^3 = 0.33/[P_0]^2(1-f)^3$, where $[P_0]$, $[P]$, and $[A]$ are the molality of total protein calculated as monomeric unit, the actual concentration of monomer, and the actual concentration of trimer, respectively, and f is the fraction of monomeric units combined. When f is 0.2, $3K[P_0]^2 = 0.4$ and when $f = 0.8$, $3K[P_0]^2 = 100$. Thus, a 250-fold change in $[P_0]^2$ or a 16-fold change in $[P_0]$ should correspond to changing from a system which is largely monomer to one which is largely trimer. The data of Ansevin and Lauffer (1959) are more or less in agreement with this calculation. If $f = 0.8$ when $[P_0] = 0.175\% = 10^{-4} m$, $K = 0.33 \times 10^{10}$ and $-\Delta F^\circ$ for trimer formation is 12,000 cal/3 moles of $[P]$ or 4000 cal/mole of $[P]$. Therefore, for the model to simulate the behavior of TMV A protein, $-\Delta F^\circ$ for

polymerization must be *ca.* 4000 cal/mole of monomeric unit at 3°.

Figure 1d and Figure 2, upper right, illustrate how type II centers can account for early stages of polymerization. This takes place at temperatures below the "melting points" of type III and type IV centers. If the long axes are approximately parallel, type III centers can still be in contact with water. The data of Banerjee and Lauffer (1966) show that $+\Delta H^\circ$ and $+\Delta S^\circ$ for polymerization of TMV A protein are 30,000 cal and 124 eu/mole, respectively. At 3° and at 14°, respectively, $-\Delta F^\circ$ for polymerization is 4300 and 5600 cal/mole of A, respectively. It is apparent from Figures 1c and d that two molecules of trimer each have six exposed type II centers and the hexamer formed when they react has six exposed type II centers. This type of equivalence between monomer and polymer is a necessary condition for the application of the equations of condensation polymerization. When further polymerization takes place, two particles, each with six type II centers exposed, will polymerize to form a larger particle with six such centers exposed. Thus, the formation of a "bond" involves the isolation of six type II centers from water. Values of $-\Delta F^\circ$ for polymerization per type II center are, therefore, 716 and 933 cal, respectively, at 3 and 14°. As is shown in detail in the Discussion, type II centers are much less strongly hydrophobic at 3° than type I centers. It is for this reason assumed that the monomeric units at the two ends of polymers are so oriented that a total of six type II centers, rather than four type II and two type I centers, are exposed. Rotation about

the long semiaxis must occur on subsequent polymerization so that all monomeric units are either top or bottom side down, except one at either end of the polymer.

When the temperature is raised above the "melting point" of type III centers, there will be rotation of monomeric units about minor semiaxes in all existing trimer particles and polymers thereof, so as to bring type III surfaces into contact as much as is possible, thereby excluding more water. This is illustrated by the difference between the upper left and lower left drawings in Figure 2. Polymers will now have the shape of a segment of a circle, as required ultimately for double disks and helices and as illustrated in Figure 2, lower right. Furthermore, subsequent polymerization will involve removal from water of type III plus type II centers. It is assumed that, after the internal rotation mentioned above, the number of type III centers exposed to water in each particle equals the number of type II centers thus exposed. The subsequent formation of a "bond" will then involve the isolation of six type II plus six type III centers. ΔH° and ΔS° for polymerization of TMV A protein in 0.1 ionic strength buffer at pH 6.5 at temperatures between 14 and *ca.* 20° were found to be about 190,000 cal and 682 eu, respectively/mole of "bond" formed (Smith, 1961; Lauffer, 1962, 1964b). The values attributable to type III centers on the model are these minus those attributable to type II centers, or 160,000 cal and 558 eu/mole of "bond" or 26,667 cal and 93 eu/type III center. This corresponds to a "melting temperature" of 14°.

The mechanism described above permits condensation polymerization to take place until 11 trimers have joined together. The mathematics of condensation polymerization, which gives a simple relationship between turbidity and equilibrium constant, should be valid until an appreciable amount of material is in the undecamer. One can imagine that at this stage the following reaction takes place, where A'_{11} is the undecamer and D' is a double disk with 32 monomeric units. $3A'_{11} \rightleftharpoons 3D' + A'$. The only compact structure of A'_{11} in the model which would be consistent with the known habits of TMV protein would be a helix of slightly more than two turns. This would require a few type IV centers to be excluded from water in each A'_{11} , a thermodynamically unfavorable situation at temperatures below the "melting point" of type IV centers. The net change in exposure to water of type II and type III centers is zero for the above reaction. Thus, ΔF° for the reaction will be negative. The resulting A' molecules can enter into further condensation polymerization with an appreciable decrease of free energy and thus will be depleted, contributing further to the above reaction. This can account, therefore, for the existence of double disks as a stable intermediate.

It is assumed that at some temperature above 20° type IV centers are also above their "melting point." These can escape from water by stacking. Furthermore, after stacking, monomeric units can rotate about their major semiaxis without change of "hydration" free energy. If steric or other factors lead to a lower free

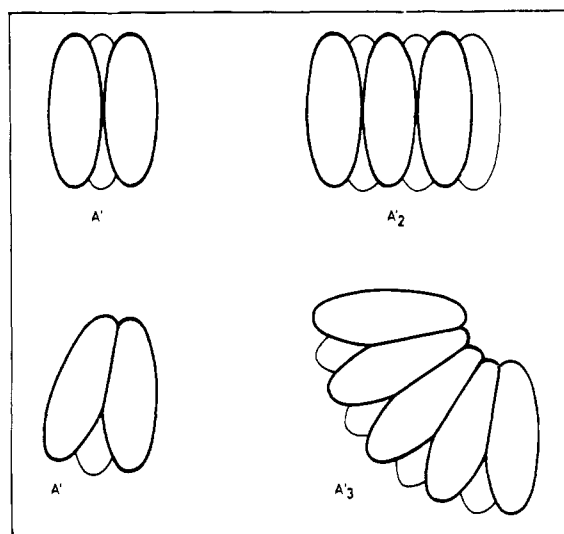


FIGURE 2: Diagram illustrating polymerization of model. For description see text.

energy when all are oriented top side up, that will be the stable orientation. A small shift in position of monomers will transform stacked disks into a giant helix resembling TMV and polymerized TMV protein.

Discussion

Kauzmann (1959) was apparently the first to emphasize the importance in protein chemistry of the type of interaction here invoked. "Hydrophobic bond" is the name frequently applied. "Hydrophobic," as pointed out by Tanford (1961), is a misnomer. In the present model at least, there are no forces between monomeric units. Thus, the word "bond" is misleading. Polymers are stable only because certain surfaces find contact with water thermodynamically unfavorable. When they can be isolated from water, in some instances by polymerization, there is a decrease in free energy attributable to an increase in entropy. It would seem, therefore, that "entropic union" would describe the actual situation better than "hydrophobic bond."

A water "binding" center as used herein is not necessarily a region of uniform surface chemistry. The ΔF° , ΔH° , and ΔS° for any one center can be the sum of several terms contributed by smaller surface elements in the center. It is not to be thought that the "entropic union" involves only hydrocarbon and similar residues. For example, when a proton moves from an NH_2 group to a COO^- group, there is large increase in entropy attributable to the release of water molecules from the two charged centers. In aqueous solution, the proton will be found on the NH_2 group because of strongly negative enthalpy. However, if a pair of charged amino and carboxyl groups is brought into a medium of sufficiently low dielectric constant, the proton will migrate to the carboxyl group. For the reaction acetate ion plus ethylammonium ion yield

acetic acid plus ethylamine at 25°, ΔF° , ΔH° , and ΔS° per mole are approximately 8280 cal, 13,400 cal, and 17.2 eu, respectively (Edsall, 1943). The same values of ΔF , ΔH , and ΔS per mole obtain when this reaction is carried out with all reactants at the same concentration, low enough for each to be ideal. The values of the parameters for transferring acetic acid and ethylamine from an ideal aqueous solution to a solution in ether with the same mole fractions can be estimated from the distribution between water and ethyl ether at different temperatures of the two solutes at very low concentrations (International Critical Tables, 1928). ΔF is not significantly different from 0 for ethylamine. For acetic acid it has a value of -565 cal/mole at both 0 and 25°. Thus, for the transfer of 1 mole of each, ΔF , ΔH , and ΔS at 25° are approximately -565 cal, -565 cal, and 0 eu, respectively. For the transfer of 1 mole of methane from a dilute aqueous solution (ideal) to a solution of the same mole fraction in ether, ΔF , ΔH , and ΔS are -3300 cal, $+2400$ cal, and $+19$ eu (Kauzmann, 1959). The simultaneous deionization and transfer of 1 mole each of acetate ion and ethylammonium ion and transfer of 4 moles of methane from water to ether without change in mole fractions involves, therefore, ΔF , ΔH , and ΔS values of -5485 cal, $+22,435$ cal, and $+93$ eu, respectively. From data cited by Kauzmann (1959) and by Edsall (1943) one can estimate an increase in volume of 108 ml for this total operation. This should be somewhat analogous to transferring a water binding center consisting of four methyl groups, a carboxyl ion, and an amino ion, widely separated, from an aqueous to an organic environment. A similar result could be obtained by transferring simultaneously appropriate amounts of alcohol or phenol and methane from water to an organic medium. These values of ΔH and ΔS resemble somewhat those required for a type III center.

In order to speculate concerning the possible molecular equivalents of type I and type II centers, one must first find a way of dealing with the translational and rotational components of the entropy change when two or more ellipsoids come together to form a single particle. The Sackur-Tetrode equation is frequently used to make such calculations for proteins (Sackur, 1913; Tetrode, 1913). Because the entropy calculated in this manner depends on \ln molecular weight and \ln moment of inertia, the value for the process, $2E \rightarrow E_2$, is not very sensitive to molecular weight or model chosen and is always close to -100 eu. The calculated values for the process $3E \rightarrow E_3$ are always close to -200 eu. Since the Sackur-Tetrode equation is really intended for diatomic molecules, it is hardly to be expected that it will yield anything more than a rough approximation of the entropy change involved in reaction between particles composed of thousands of atoms. Accordingly, we will use -100 and -200 eu as representative values for the processes indicated above.

For the model to resemble TMV protein, ΔF° at 3° for formation of A' from three monomers must be about -4000 cal/mole of monomer. The entropy change for translation and rotation is *ca.* $-200/3$

$= -67$ eu/mole of monomer. Transfer of 5 moles of toluene from water to organic solvent has a ΔH° of zero and a unitary entropy change of $5 \times 16 = 80$ eu (Kauzmann, 1959). The sum is $+13$ eu. For ΔH° of zero this corresponds to $\Delta F^\circ = -3600$ at 3°. This is close to the value required of a type I center.

For the model to imitate the polymerization of TMV A protein at temperatures below 14°, there must be a Sackur-Tetrode entropy change of -100 eu/mole of "bond" formed. Furthermore, Ansevin *et al.* (1964) found that *ca.* 5 moles of hydrogen ion was bound/100,000 g of A protein upon polymerization at pH 6.5, 0.1 ionic strength. This corresponds to about 3 moles/mole of A protein. Because this is coupled with the conversion of $3H_2PO_4^-$ to $3HPO_4^{2-}$, the over-all standard enthalpy and standard entropy changes at pH 6.5 are -369 cal and -26 eu/mole of A protein. If the model is given groups which imitate this aspect of TMV protein polymerization, these enthalpy and entropy values must apply to the model also. Transfer of 12 moles of methane (corresponding to 2 moles for each type II center) from water to organic medium involves ΔH° of $+28,800$ cal and $+228$ eu. The totals turn out to be $+28,400$ cal for the enthalpy and $+102$ eu for the entropy. These compare with ΔH° of 30,000 cal and ΔS° of 124 eu for polymerization of TMV A protein at temperatures below 14°. Thus, it can be seen that different combinations of organic residues and pairs of ions could give the variety required by the present model in its water binding centers.

The model here presented has many features in common with the earlier theory of Caspar (1963) and was strongly influenced by it. Caspar considered the various "faces" of the monomer to be approximately (not exactly) equivalent in their "bond" forming potentiality. He attributed stability to geometric or quantum mechanical considerations. His theory scored the major success of predicting a stable trimer, since established experimentally (Banerjee and Lauffer, 1966).

A stable cyclical heptamer is also predicted by Caspar's theory. However, it would be hard to rationalize such an intermediate with condensation polymerization mathematics. The evidence in favor of condensation polymerization mathematics is not only that the equilibrium variations of turbidity and of osmotic pressure with temperature fit this postulate and no other tried thus far, but also that the rate of the polymerization reaction in the early stages fits this mechanism (Ansevin and Lauffer, 1963). The only experimental evidence adduced in favor of the heptamer is the occasional finding of appropriate sedimentation coefficients. The type of sedimentation pattern one should get when an equilibrium mixture is centrifuged depends on the rates of sedimentation and of reequilibration of the various components. The theory is unfortunately not well developed. Therefore, the finding of a particular sedimentation coefficient is not strong evidence for the existence of a corresponding stable intermediate. For the purpose of the present discussion, a stable intermediate is assumed to exist only when there is strong experimental evidence in addition to that afforded by an

appropriate sedimenting boundary. Therefore, the stable cyclical heptamer called for in Caspar's theory seems to be a disadvantage.

A further disadvantage of Caspar's theory is that the reaction $3A_{11} \rightleftharpoons 3D + A$ should result in no change in the number of nearly identical "faces" exposed to water and should therefore have a "hydration" ΔF° of zero. The model presented here obviates all of the difficulties mentioned above, and, in addition, accounts for the fact, discovered since Caspar's work was published, that polymerization of TMV A protein at temperatures above 14° (0.1 ionic strength buffer at pH 6.5) proceeds with much higher ΔH° and ΔS° than at lower temperatures.

The novel features of the present model are the assumptions that the various positions on the surface of a monomer are radically different, that different regions "bind" water with different free energy changes, and that whether a center is hydrophobic or hydrophilic depends on temperature. This simple model can imitate the most important aspects of the polymerization and dissociation of TMV A protein. To simulate all of the known properties of TMV protein, a more complex model is required. First, there must be a center near the inner end capable of interacting with ribonucleic acid (RNA) to produce a copolymer (virus particle) with greater stability than polymerized protein. Second, there must be additional acid and base binding groups at least on the outer half of the ellipsoid. In accord with the views expressed earlier by Ansevin *et al.* (1964), only those nearest the outer end will contribute to the electrophoretic mobility of polymerized protein and virus, but both completely exposed ionizable groups and those found in the crevices in completely polymerized protein (nearer the center of the ellipsoid) are involved in the titration curve of virus or polymerized protein. Electrostatic repulsion between charged units will oppose polymerization, accounting, qualitatively at least, for the relationship between pH and polymerization (Ansevin *et al.*, 1964). The effect of electrolyte concentration on TMV A protein polymerization might involve changes in charge resulting from ion binding, changes in potential between charged particles in accordance with the Debye-Hückel theory, and alterations in the structure of water which will affect the entropy and enthalpy changes involved in release of "bound" water. Finally, the outer halves of the ellipsoids must have properties which account for the crystallization of protein helices and virus.

The success of the model in imitating the polymerizing ability of TMV protein must not obscure the fact that it differs from the protein. One important difference is that the model, aside from a minor degree of elasticity needed to make surface fit, is essentially fixed in shape and surface constitution. Protein in general can undergo conformational changes. Thus, while water "binding" centers on the model can be isolated from water by rotation or polymerization only, in a protein molecule conformational change offers a third possibility. The contribution, if any, that conformational changes make to polymerization of protein is at present not

known. It is sufficient to say that a model incapable of conformational change can imitate the gross features of TMV A protein polymerization.

Endothermic aggregation reactions, and, therefore, presumably entropic unions, are observed in many biological and biochemical processes. Included are pseudopodia formation in amoeba, protoplasmic streaming, division of fertilized eggs, spindle formation in the nucleus during mitosis, polymerization of several proteins, ion binding, certain antigen-antibody reactions, etc. Details are provided in two review articles (Kauzmann, 1959; Lauffer, 1964b). Thus, water, by virtue of its ability to force entropic unions, participates actively in many structure-forming processes in living systems.

Acknowledgments

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Dye-Catalyzed Photoinactivation of Tobacco Mosaic Virus Ribonucleic Acid*

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ABSTRACT: Tobacco mosaic virus ribonucleic acid (TMV RNA) loses infectivity upon illumination in the presence of thiopyronin and proflavin. To reach the same level of inactivation requires about 1000 times as much proflavin as thiopyronin. Both dyes cause loss of guanine and no other base. Similar levels of inactivation by the two classes of dye are characterized by similar extents of guanine alteration, but the number

of guanines lost is greatly in excess of the lethal events, as measured by infectivity. The integrity of the macromolecule is unaffected at a stage when many guanines have been destroyed. Virus grown in the presence of these dyes is both qualitatively and quantitatively affected. Dye illumination of both the RNA and of the infected leaf tissue leads to increased frequency of mutants.

Methylene blue has been reported to catalyze the photodecomposition of guanine residues in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Simon and Von Vunakis, 1962, 1964). Thiopyronin (in which the ring N is replaced by CH) showed the same behavior, but was effective at lower concentrations (Wacker *et al.*, 1963, 1964). The interactions of acridine dyes and nucleic acids have been very extensively studied, but less work has been done concerning their photocatalytic effects, particularly in regard to RNA. A recent report by Sastry and Gordon (1966) showed that this class of dyes also catalyzed the photodecomposition of guanosine, but reported no data on any changes in the base composition of photoinactivated tobacco mosaic virus ribonucleic acid (TMV RNA).

The present study is concerned with a comparative investigation of proflavin and thiopyronin as catalysts of photoinactivation and photodecomposition of TMV RNA. It appears that the two dyes differ greatly in their catalytic efficiency, but that both cause proportional losses of infectivity and of guanine residues. Other bases do not decompose at measurable rates. The number of guanines affected greatly exceeds the

number of lethal hits, as determined by infectivity tests.

Methods and Materials

TMV RNA and ¹⁴C-labeled TMV RNA were isolated by standard procedures (Fraenkel-Conrat *et al.*, 1961; Sugiyama and Fraenkel-Conrat, 1961). The dyes were commercial samples used without further purification. The light source was a Hanovia fluorolamp (31,300,125 w). For most experiments it was placed 10 cm from the samples, the light being filtered and cooled by passage through a thick glass plate and a Petri disk containing about 1% CuSO₄ solution. The samples, usually containing about 1 mg/ml of RNA and 10⁻² M pH 7.0 EDTA or pH 9.0 borate, were in stoppered test tubes laid into Petri dishes containing crushed ice.

The RNA was precipitated with 2.5 volumes of ethanol with pH 7 acetate added to about 0.05 M. At times the RNA was repeatedly redissolved and reprecipitated in the same manner. Reconstitution of virus, with or without prior alcohol precipitation of the RNA, was done by addition of excess protein (usually a 500-fold) and pH 7.3 pyrophosphate to a final concentration of 1 mg/ml and 0.1 M, respectively, followed by incubation at 30° for 6 hr.

Base analysis was done after 1 N HCl hydrolysis (1 hr at 100°) according to Smith and Markham (1950), with chromatographic separation of adenine,

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