# Kinetics and mechanism of tobacco mosaic virus assembly: Direct measurement of relative rates of incorporation of 4S and 20S protein

(protein–RNA interactions/initiation/elongation/reconstitution)

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ABSTRACT    The mechanism of assembly of tobacco mosaic virus has been investigated under conditions in which the rates of incorporation of the 4S and 20S proteins can each be directly measured by analytical centrifugation. Under these conditions, pH 6.5, 6.5°C, 0.10 M ionic strength potassium orthophosphate, the protein can be made to exist as a metastable 20S aggregate that is necessary for efficient reconstitution. The overall assembly process consists of an initiation (nucleation) reaction that requires two to three 20S disk aggregates per RNA molecule and is followed by an elongation (growth) reaction. In the elongation phase of assembly the 4S protein is incorporated 50 to 70 times faster than the 20S disk, calculated on the basis of a steady-state kinetic analysis. Therefore, under these conditions, in which the rate of assembly is about 0.06 of that at pH 7, 20°C, 0.10 M ionic strength orthophosphate, the 4S protein preferentially participates in the elongation phase. At this slow reconstitution rate intermediate assembly states (about 70–168 S) can be observed. The kinetics of both protein incorporation and nucleoprotein formation suggest that the elongation process is composed of at least two different, possibly sequential, rate-limiting reactions.

Although it has been known for some time that the isolated coat protein (TMVP) of tobacco mosaic virus (TMV) can exist in a variety of polymerization states (1, 2), it has only recently become evident that this structural polymorphism is relevant to understanding the mechanism of assembly of the virus. In the early experiments to demonstrate the self-assembly of TMV from the isolated TMVP and TMV RNA, at 3°C, pH 6, it was necessary to incubate the mixture for 24 hr to produce virus particles (3). Subsequent systematic studies of optimum conditions for TMV assembly revealed that the reaction was quite sensitive to pH, ionic strength, buffer type, and temperature (4). However, it was not until 1971 that it was recognized that for a given TMVP concentration the rate of virus assembly at pH 7 and 20°C depends strongly on the relative amounts of TMVP in the boundaries sedimenting at 4 S and 20 S (5). At 4–6°C TMVP is in the 4S form, whereas at 20°C there can be as much as 90% in the 20S form at pH 7, depending on the protein concentration and buffer (6). The rate at which this equilibrium is achieved after warming to 20°C is slow at pH 7, 20°C, 0.10 M ionic strength potassium hydrogen orthophosphate [K(H)PO₄], 5 mg ml⁻¹ TMVP, where the equilibrium ratio of 20S to 4S forms is about 70:30 by weight. The half-time for 20S formation from 4S protein is 4–6 hr (7–9). The 4S material has been shown to be a rapidly self-associating system with an average molecular weight, under the above conditions, corresponding to about a trimer of the 17,500 $M_r$ fundamental subunit (2, 7, 8). The 20S sedimenting boundary represents a two-layer disklike structure containing 17 subunits in each layer with the two layers stacked in a polar fashion (10–12). After Butler and Klug (13) found that virus assembly in vitro could be achieved from TMVP and TMV RNA within

10 min, at pH 7, 20°C, 0.10 M ionic strength K(H)PO₄, if the protein were first incubated at 20°C for many hours to allow 20S to be preformed, they concluded that the 20S disk is an obligate reactant for the initial (nucleation) step in virus assembly. This central role of the 20S disk has been confirmed in three other laboratories (14–16). Butler and Klug (13) concluded that the slow rate of assembly observed in the original work of Fraenkel-Conrat and Williams (3) resulted from the rate-limiting formation of the 20S disk nucleating species.

However, the role of the 20S disk in the subsequent stages of the assembly reaction (rod elongation) has been the subject of considerable controversy. Under scrutiny here is the question of whether or not the assembly of TMV proceeds by the direct incorporation of preassembled protein aggregates, as is the case in bacterial virus assembly (see ref. 17 for a review). Evidence has been presented by Butler and Klug (5, 13), by Butler (18–20), and by Butler and Finch (21), who used partially assembled (prenucleated) rods, indicating that at pH 7 and 20°C the 20S disk is preferentially incorporated in the elongation phase, and, although 4S protein can also participate, it does so at a slower rate. Contrary evidence was obtained by Richards and Williams (14, 22), who found the rate of elongation to be about the same with 4S or with equilibrium mixtures of 4S and 20S protein. A similar conclusion was arrived at by Okada and Ohno (15). An unambiguous characterization of the adding species has not been made because all investigations to date have been with "20S protein" that is an equilibrium mixture of 4S and 20S. Richards and Williams (22) suggested that the adding species may actually be the monomer, which appears to be in rapid equilibrium with both 4S and 20S protein. More detailed discussions and criticisms of the experimental methods and the interpretations of results from various laboratories are presented in recent reviews by Richards and Williams (22), Butler (23), and Butler and Durham (24).

The experiments reported here were undertaken in an effort to measure directly the relative rates of incorporation of 20S and 4S protein by using sedimentation analysis. Because all previous experiments have used either measurements of turbidity changes or quantitative electron microscopy, they were based upon measurements of the sizes and rates of appearance of the reconstituted nucleoprotein *products* of the assembly reaction (5, 13–16, 18–21). We describe here experiments that were designed to provide direct measurements of the rates of disappearance of *reactants* (4S and 20S protein) in an effort to resolve this controversy. Furthermore, because much of the criticism of the experimental methods (22–24) centers on the homogeneity and stability of the partially assembled rods used for studying the elongation phase, we chose to use protein-free TMV RNA and investigate the entire course of the reaction.

Because previous results at pH 7 and 20°C indicated a rapid

Abbreviations: TMV, tobacco mosaic virus; TMVP, tobacco mosaic virus coat protein; K(H)PO₄, potassium hydrogen orthophosphate at a given pH and ionic strength; PAR, partially assembled rod.

assembly rate and some kind of rapid subunit exchange between the 4S and 20S protein species (22–24), it appeared unlikely that sedimentation analysis would be useful. After the virus assembly reaction had been studied under other conditions this view proved to be incorrect. These "other" conditions were suggested by TMVP self-association results obtained at pH 6.5 (25–30). These studies revealed that when a solution of TMVP at pH 6.5 and 20°C is cooled to 6.5°C a metastable 20S species (presumably disk protein) is formed; this species slowly depolymerizes to the equilibrium distribution with a half-time of about 12 days. Because the reverse reaction does not occur under these conditions, this slow 20S to 4S reaction appeared to provide ideal conditions for measuring directly by sedimentation the relative rates of disappearance of the 4S and 20S species during TMV assembly. In addition, the reduced overall rate of assembly at pH 6.5 and 6.5°C, compared to that at pH 7 and 20°C, makes it possible to observe assembly intermediates during the elongation phase of the reconstitution reaction.

## MATERIALS AND METHODS

TMV (common strain) was isolated and purified from infected tobacco leaves (provided by C. A. Knight) by the method of Paglini and Lauffer (31). Residual plant proteins and pigments were removed from the virus in a manner adapted from Marcinka (32). The virus, suspended in 0.01 M EDTA, pH 7.5, was passed over an uncoated porous glass bead (Nucleonics, CPG-10; 375-Å pore size) chromatographic column (2.8 × 40 cm) at 4°C with the EDTA buffer as the eluant. The purified virus was stored at 4°C in the EDTA eluant at a final concentration of 15–20 mg ml⁻¹. TMVP was prepared by the modified acetic acid degradation method of Scheele and Lauffer (33), dialyzed against the reconstitution buffer for 48 hr, and stored at 4°C. The single-phase phenol method of Diener and Schneider (34) was modified as follows to obtain TMV RNA. To the RNA pellet, resuspended in pH 7, 0.02 M K(H)PO₄, were added 2 vol of cold 100% ethanol and a few drops of 3 M sodium acetate. The solution was stored at 0°C for ½ hr and the RNA was pelleted by centrifugation at 5°C for 15 min at 5000 × *g*. The pellet was resuspended in 1–2 ml of 20–50 mg ml⁻¹ sodium bentonite in pH 7, 0.01 M K(H)PO₄/0.01 M EDTA for 1 hr at about 23°C and dialyzed against the reconstitution buffer at 4°C (35). The bentonite was removed by centrifugation at 4°C at 12,000 × *g* for 30 min to yield TMV RNA that, after dilution in water, had a 258 nm:230 nm absorbance ratio of 2.7. In the preparation of the TMVP and TMV RNA, techniques to avoid protease and nuclease contamination were employed (36). We found that by avoiding bacterial contamination the extent of irreversible TMVP aggregation in pH 6.5 phosphate buffer was made negligible and samples were stable for up to 3–4 weeks (30). Sedimentation velocity measurements at 25°C revealed that 70–80% of the RNA sedimented with $s_{20,w}$ values of 28–31 S in pH 6.5, 0.10 M ionic strength K(H)PO₄ buffer. The remainder of the RNA sedimented with a broad trailing boundary. Similar sedimentation coefficients have been reported by Boedtker (36, 37). Concentrations of TMV, TMVP, and TMV RNA were determined from light-scattering-corrected (38) ultraviolet spectra taken on Cary 15 or 118 instruments. The following values were used for determining concentrations: TMV, $A_{260}$ (0.1%) = 2.7 at pH 7, 0.05 M K(H)PO₄, 23–25°C (ref. 3); TMVP, $A_{281}$ (0.1%) = 1.30 at pH 8, 0.10 M K(H)PO₄, 4–5°C (ref. 3); TMV RNA, $A_{258}$ (0.1%) = 25.5 in H₂O, 20–25°C (ref. 37). Only TMVP preparations having a 281 nm:250 nm absorbance ratio of 2.5 or greater were used.

## RESULTS

The kinetics of virus assembly at pH 7.0 and 20°C and at pH 6.5, 6.5°C, 0.10 M ionic strength K(H)PO₄ are shown in Fig. 1. The reconstitution reaction at 20°C and pH 7.0 was found to proceed with an initial rate of 0.05 OD min⁻¹, which compares favorably with the initial rates previously determined (5, 39) under similar conditions. The initial rate of the reconstitution reaction at 6.5°C and pH 6.5 was 0.003 OD min⁻¹, about 0.06 of that at 20°C and pH 7.0. The difference in protein concentration (≈1 mg ml⁻¹) was not sufficient (18) to account fully for this difference in rates.

Similar reconstitution experiments at pH 6.5 and 6.5°C using protein that was previously at 0°C for ≥ 2 hr, to depolymerize all 20S protein to 4S, failed to exhibit turbidity increases over several hours. Analysis by sedimentation of such mixtures, at pH 6.5 and 6.5°C, of 100% 4S protein and RNA did not reveal any fast-sedimenting boundaries corresponding to partially or fully reconstituted TMV. These results show that the metastable 20S protein aggregate produced by cooling polymerized TMVP at pH 6.5 from 20°C to 6.5°C acts as an obligatory nucleus for initiation of reconstitution as it does at pH 7.0 and 20°C (5, 14, 15, 39). The reconstituted product formed under metastable 20S conditions is quite similar in appearance and behavior to TMV reconstituted at pH 7.0 and 20°C as judged by electron microscopy and analytical ultracentrifugation. Fig. 2 is an electron micrograph of the product of the reconstitution from metastable protein and RNA, showing many virus rods between 2500 and 3000 Å in length. Similar pictures were obtained after the reconstituted nucleoprotein rods were cooled to 0°C for 2 days, conditions that lead to depolymerization of helical rods composed of protein alone. TMV reconstituted at pH 7.0 and 20°C similarly resisted depolymerization upon cooling or raising of the solution pH. Moreover, the TMV rods formed at pH 6.5 and 6.5°C were not sensitive to RNase degradation at pH 7.0 and 20°C. The sedimentation pattern of the nucleoprotein complex reconstituted at pH 6.5, 6.5°C is shown in Fig. 3 *right*. The material giving the sharp single schlieren peak sedimented with an $s_{20,w}$ value of 174 S at a concentration of 2.2 mg ml⁻¹ of reconstituted material, which is in agreement
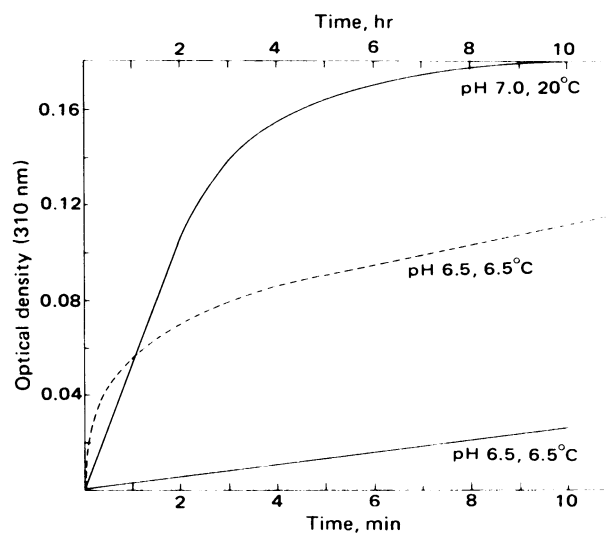


Fig. 1. Comparison by turbidity measurements at 310 nm of the TMV assembly rate at pH 6.5 and 6.5°C with that at pH 7.0 and 20°C, both at 0.10 M ionic strength K(H)PO₄. Protein concentrations were 3.0 and 3.9 mg ml⁻¹ for the pH 6.5 and 7.0 samples, respectively. RNA was 0.06 mg ml⁻¹ in each solution. The overall kinetics at pH 6.5 (- - -) are plotted on the upper time axis (hours). The pH 7.0 and early pH 6.5 (——) reconstitution kinetics are plotted on the lower time axis (minutes).
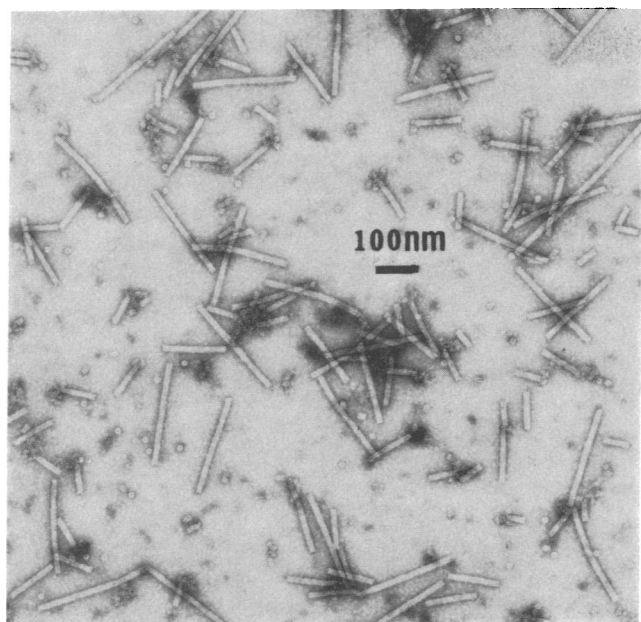
FIG. 2. Reconstituted tobacco mosaic virus after 2-day reaction of 0.20 mg ml⁻¹ TMV RNA and 5.0 mg ml⁻¹ TMV metastable protein at pH 6.53, 6.5°C, 0.10 M ionic strength K(H)PO₄, and subsequent cooling 1 hr at 0°C. Electron microscope grids were prepared at ≈23°C after 1:10 dilution with reconstitution buffer. (Negatively stained with 1% uranyl acetate, pH 4.5; primary magnification ×19,800.)

with the value obtained by Boedtker and Simmons (40) for monomeric TMV at the same concentration. The final yield of reconstituted nucleoprotein complex at pH 6.5 and 6.5°C, based on total initial RNA concentration, was 75%. This is consistent with the results of sedimentation analysis of the RNA, which showed that about 20% of the nucleic acid was substantially smaller than native TMV RNA.

Fig. 4 summarizes the kinetics of the reconstitution reaction under these conditions as measured by analytical ultracentrifugation. The $s_{20,w}$ values and concentrations, obtained from corrected schlieren peak areas, of the protein species and reconstituted TMV as a function of time are given in Table 1. Fig. 4A shows that the rate of formation of reconstituted nucleoprotein is the same for the two protein concentrations used here, 4.3 and 10.0 mg ml⁻¹. Butler (18), using turbidity measurements, has observed a similar protein concentration independence of the rate at pH 7.0 and 20°C. Although the yield and rate of formation of nucleoprotein complex ($s_{20,w}$ > 100 S) were
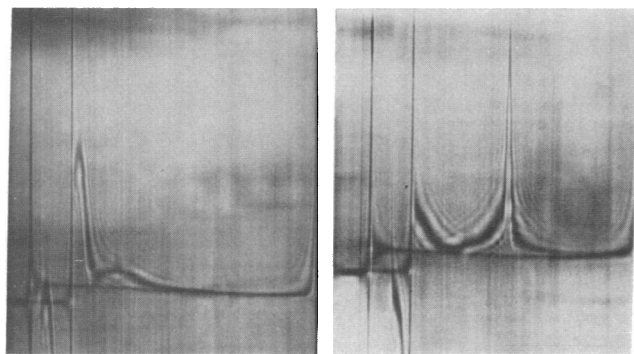


FIG. 3. Schlieren sedimentation patterns of reconstituted TMV, taken in a Beckman model E. Sedimentation temperature, 6.5°C; An-D rotor speed, 20,000 rpm. (*Left*) Intermediate species, $s_{20,w}$ = 91 S, after 0.5 hr reaction time. Conditions: 4.3 mg ml⁻¹ sample in Table 1. (*Right*) Same sample used for electron microscopy in Fig. 2, $s_{20,w}$ = 174 S. In both cases there is an excess of TMV protein, seen at the meniscus of each cell.
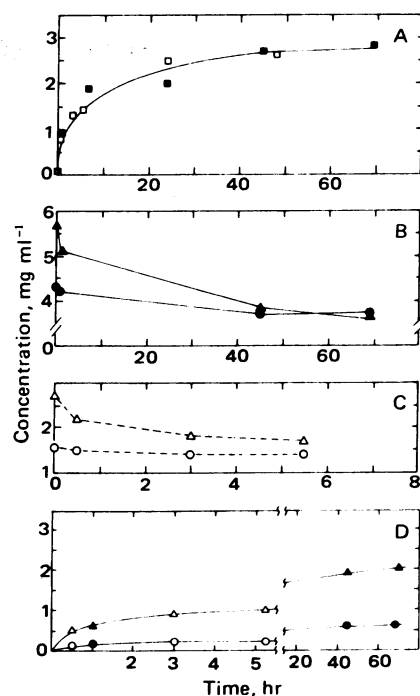


FIG. 4. Kinetics of TMV reconstitution. 4S and 20S protein incorporation determined by schlieren pattern sedimentation analysis. Conditions as for Table 1. All runs were in a Beckman model E ultracentrifuge and An-D rotor at 6.5°C in 12-mm double sector charcoal-filled Epon cells. Early part of each run was at 20,000 rpm to sediment reconstituted material (A) followed by acceleration to 52,000 rpm to sediment unreacted 4S and 20S protein (B, C, D). Times refer to reaction time. Concentrations were obtained by integrating schlieren peak areas. Sedimentation coefficients and concentration data are given in Table 1. (A) Time course of total nucleoprotein rod formation. Initial TMVP concentration 4.3 mg ml⁻¹ (□) or 10.0 mg ml⁻¹ (■). (B) Time course of disappearance of 4S (▲) and 20S (●) protein at 10.0 mg ml⁻¹ initial TMVP concentration. (C) Time course of disappearance of 4S (△) and 20S (○) protein at 4.3 mg ml⁻¹ initial TMVP concentration. (D) Time course of 4S and 20S incorporation into nucleoprotein rods. Symbols same as for B and C.

the same in the two experiments (Fig. 4A and Table 1) with different protein and RNA preparations in each case, the yield of full virus-size rods, 164–174S, was 55% at the higher protein concentration and 65% at 4.3 mg ml⁻¹ (see Table 1). The concentration of intermediates at 10 mg ml⁻¹ protein is higher than that seen at 4.3 mg ml⁻¹ and may result from additional nucleation sites in nicked RNA or may reflect weaker nucleation sites to which disks bind at the higher protein concentration (4.3 versus 1.6 mg ml⁻¹ at zero time). Further experiments with highly fractionated RNA will be required to understand this complex kinetic pattern.

Fig. 4D shows the rate of incorporation into reconstituted rods of 4S and 20S protein at *both* protein concentrations.

The rates of 4S and 20S incorporation shown in Fig. 4 B, C, and D suggest three kinetic phases. The first phase takes place within the 30 min "dead time" of the centrifuge method and reveals a small decrease in 20S concentration and about a 4- to 6-fold greater decrease in 4S concentration. During this initial phase the nucleoprotein complex is considerably smaller than TMV, 70–90S versus 174S for the whole virus (90S corresponds to rods about 600 Å long) as shown in Fig. 3 and Table 1. Fig. 3 *left* shows the sedimentation pattern at an intermediate extent of TMV reconstitution. Under these conditions nucleation appears to be rapid and the elongation phase rate limiting. If the opposite were true, we would not expect to observe reconstituted material with $s_{20,w}$ values as small as 70–90 S. If instead

Table 1.    Kinetics of TMV reconstitution

| TMVP 4.3 mg ml⁻¹ | | | TMVP 10.0 mg ml⁻¹ | | |
|---|---|---|---|---|---|
| Time, hr | $s_{20,w}$, S | Conc., mg ml⁻¹ | Time, hr | $s_{20,w}$, S | Conc., mg ml⁻¹ |
| 0 | 4.3 | 2.7 | 0 | 5.0 | 5.7 |
|  | 17.9 | 1.6 |  | 18.3 | 4.3 |
| 0.5 | 4.2 | 2.2 | 1.0 | 5.2 | 5.1 |
|  | 17.7 | 1.5 |  | 18.2 | 4.2 |
|  | 91 | 0.70 |  | 69 | 0.90 |
| 3.0 | 3.7 | 1.8 | 45 | 4.9 | 3.8 |
|  | 18.0 | 1.4 |  | 17.9 | 3.7 |
|  | 81 & 122 | 0.73 |  | 99 & 146 | 1.3 |
|  | 168 | 0.52 |  | 166 | 1.4 |
| 5.5 | 3.7 | 1.7 | 69 | 4.8 | 3.7 |
|  | 17.6 | 1.4 |  | 17.9 | 3.7 |
|  | 94 & 125 | 0.77 |  | 96 & 144 | 1.2 |
|  | 169 | 0.60 |  | 164 | 1.5 |
| 24 | 3.7 | 1.5 | 119 | 3.9 | 3.8 |
|  | 17.2 | 0.50 |  | 18.0 | 3.7 |
|  | 107 & 145 | 0.82 |  | 98 & 157 | 1.0 |
|  | 173 | 1.7 |  | 166 | 1.8 |
| 48 | 3.8 | 1.6 | 144 | 3.7 | 3.8 |
|  | 16.2 | 0.3 |  | 18.2 | 3.4 |
|  | 108 & 162 | 0.3 |  | 97 & 147 | 1.4 |
|  | 174 | 2.3 |  | 164 | 1.6 |
| 120 | 3.7 | 1.5 | 168 | 4.5 | 3.8 |
|  | 104 | 0.39 |  | 18.4 | 3.4 |
|  | 172 | 2.6 |  | 100 & 154 | 1.3 |
|  |  |  |  | 166 | 1.7 |
|  |  |  | 408 | 4.5 | 4.4 |
|  |  |  |  | 18.4 | 2.8 |
|  |  |  |  | 112 | 0.80 |
|  |  |  |  | 164 | 2.2 |

TMV reconstitution was at pH 6.53, 6.5°C, in 0.10 M ionic strength K(H)PO₄; total TMVP concentrations of 4.3 and 10.0 mg ml⁻¹, TMV RNA concentration 0.20 mg ml⁻¹ in both cases. Protein species concentrations and $s_{20,w}$ values were obtained from schlieren sedimentation patterns. Zero time values were obtained from samples that did not contain RNA. Metastable 20S TMVP used in reconstitution was prepared by cooling to 6.5°C protein solutions that were previously warmed to 20°C at 0.1°C min⁻¹. Solutions prepared in this way yield 35–40% protein having the same intrinsic sedimentation coefficient, 20.3 S, as the two-turn disk at pH 7, 20°C (30). Values of 3.7–5.0 S for 4S protein result from both concentration-dependent sedimentation and concentration dependent self-association. The $s_{20,w}$ values of these broad boundaries are estimated to have a 5% uncertainty. A similar uncertainty is estimated for the area of each schlieren boundary. The RNA was assumed to have the same refractive increment as TMVP (36, 37).

the nucleation reaction were much slower than elongation, one would expect a lag phase followed by the rapid appearance of much longer nucleoprotein rods (25).

After this initial fast phase, which is complete within 0.5–1.0 hr, there is about a 10% decrease in the concentration of 20S protein and a 20% decrease in 4S protein after 45 hr in the 10 mg ml⁻¹ protein sample. During this phase of the reaction the reconstituted material increases in both amount and size. At longer times, 69 hr, the rates of incorporation of 4S and 20S are both small and nearly equal. However, during this period the reconstituted material increases in size and homogeneity. These results demonstrate a rapid nucleation and elongation reaction (up to about 90S) followed by a further, slow, elongation process.

Over the time course of these reconstitution reactions the metastable 20S protein did not depolymerize to the equilibrium distribution of nearly 100% 4S. Further details of the metastable

20S protein are given by Shire *et al.* (30). We found that the stability of the metastable 20S protein is concentration dependent. When the total concentration of nonreconstituted protein fell below about 3 mg ml⁻¹ there was an abrupt micelle-like concentration-dependent depolymerization of the 20S species (unpublished results). We have therefore excluded the long time data at 4.3 mg ml⁻¹ TMVP from our kinetic analysis. This depolymerization at low protein concentrations suggests the existence of a highly concentration-dependent rapid subunit exchange reaction with 20S protein, as has been observed by others (22, 24) at pH 7 and 20°C.

## DISCUSSION

Although there is agreement (5, 39, 41) that the 20S disk is an obligatory nucleus for TMV reconstitution, there is disagreement over the rate of the nucleation event. Estimates of the time for nucleation range from seconds (20) to 3–4 min (39) at pH 7.0 and 20°C. Because the overall rate of the reconstitution reaction at pH 6.5 and 6.5°C is approximately 0.06 of that at pH 7.0 and 20°C we assume that nucleation at 6.5°C is substantially complete in 0.5–1 hr. This assumption is supported by the appearance within 1 hr of an amount of 70–90S nucleoprotein complex that can account for 90% of the RNA that is capable of reconstituting >100S nucleoprotein rods. These results, along with the amount of 20S incorporation after 1 hr (Fig. 4), allow an estimate of the number of disks per nucleation event. We find 2.2 and 2.6 20S disks per RNA molecule for the low and high TMVP concentrations, respectively. This is, of course, the maximum number because it was assumed that all of the 20S protein was incorporated in the nucleation reaction. However, it has been found by nuclease protection experiments (42) that the average size of the RNA initiation site is 250 nucleotides, which corresponds to between 2 and 3 disks, in good agreement with these kinetic results. It thus appears that in the initial phase of reconstitution (70–90S) all of the 20S incorporation can be reasonably ascribed to nucleation. During the same period of reconstitution, about 6 times as much 4S (on a weight basis) is incorporated into the growing virus (Table 1).

In a study of the initial rates of TMV reconstitution at pH 7 and 20°C by turbidimetry, Butler (18) found that a given disk protein concentration the overall rates varied linearly with RNA concentration and at a given RNA concentration increased with disk protein concentration up to 4–5 mg ml⁻¹, at which a saturation plateau was reached. Butler's interpretation of this result was that an initial rapid binding of protein to nucleic acid precedes a slower rearrangement of the protein along the RNA. In terms of a disk elongation model it was assumed that the observed Michaelis–Menten kinetics are due to a subunit rearrangement process similar to that observed in TMVP self-association at low pH (10). However, the observation of saturation kinetics is not inconsistent with a subunit elongation model, because a rearrangement of subunits after rapid binding to RNA may also take place. Such a model has been proposed (43) on the basis of electron micrographs that reveal a clustering of protein on the nucleic acid strand that, with time, forms helical rods. It is especially interesting to note that at both protein concentrations in the present study the sedimentation values and the amounts of the various reconstitution intermediates changed during the later course of the reaction, although the total amount of reconstituted material increased only slowly.

If the 20S disk incorporates preferentially during the elongation phase the rate of disappearance of free 20S TMVP is given by:

$$-\frac{d[20S]}{dt} = k_i[\text{RNA}][20S] + k_d[20S] + k_g'\frac{[\text{PAR}][20S]}{k_m' + [20S]}, \quad [1]$$

in which $k_i$ is the rate constant for initiation (nucleation), $k_d$ is

the rate constant for depolymerization of metastable 20S disk to 4S component, $k_g'$ is the rate constant for the forward rate-limiting process in elongation, $k_m'$ is the disk protein concentration at half-maximal rate, and [PAR] is the molar concentration of reconstituted material (partially assembled rods).

Conversely, if it is assumed that only 4S protein incorporates during the elongation phase (but not at all during nucleation) then:

$$- \frac{d[4S]}{dt} = k_g \frac{[PAR][4S]}{k_m + [4S]} - k_d[20S], \qquad [2]$$

in which the parameters are for 4S protein as defined previously. The observed rate of 20S depolymerization to 4S protein is quite slow under these conditions, with a half-time of about 12 days (30) and thus at early times one can neglect $k_d[20S]$. If we also assume that the protein concentrations are at saturation levels ($>4$ mg ml$^{-1}$), then $[20S] > k_m'$ and $[4S] > k_m$ and Eqs. 1 and 2 reduce to:

$$- \frac{d[20S]}{dt} = k_i[RNA][20S] + k_g' [PAR] \qquad [3]$$

$$- \frac{d[4S]}{dt} = k_g[PAR]. \qquad [4]$$

In the elongation model $k_i[RNA][20S]$ is smaller than the second term in Eq. 3 and one can estimate $k_g/k_g'$ at early times in the reconstitution reaction. The 4.3 mg ml$^{-1}$ protein data yield $k_g/k_g' = 49$ over the first $\frac{1}{2}$ hr if the 4S material is assumed to be a trimer. Similarly, data from the first hour of reconstitution at 10 mg ml$^{-1}$ yield a value of 70 for $k_g/k_g'$. If the adding kinetic unit were the TMVP monomer rather than the trimer, these ratios would each be 3 times as great. It can be concluded from these results that under these conditions the 4S protein is initially incorporated at least 50–70 times more rapidly than the 20S aggregate during the early elongation phase if the mechanism follows Michaelis–Menten kinetics, as suggested by Butler (18). This difference in rates may even be an underestimate, because the initial decrease in 20S concentration is probably due to nucleation but has been included in this calculation.

At later times during the elongation phase the rates of 4S and 20S incorporation appear to become more similar (Fig. 4D, Table 1). This biphasic character of the elongation phase suggests that previously observed differences between relative rates of 4S and 20S incorporation at pH 7 and 20°C (14, 20) may result in part from small differences in the lengths of the starting PARs. Preliminary results of similar experiments performed at pH 7 and 20°C show that both 4S and 20S protein components participate in the elongation reaction under these conditions (44, 45). If the recently proposed bidirectional model for TMV assembly (46–48) is correct, one would expect different rates for the early 3′-to-5′ and later 5′-to-3′ elongation processes because the mechanism by which RNA folds into the growing rod is stereochemically quite different in each direction. The biphasic elongation rates observed here may correspond to the predictions of this model.

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