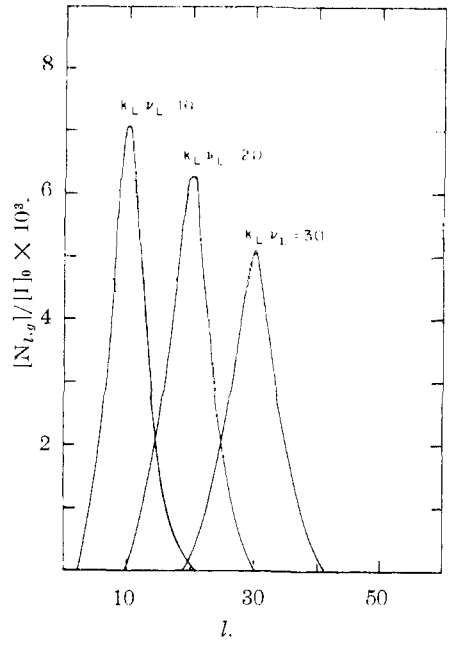

Fig. 9.—The relative number of chains containing l lysine residues and g glutamate residues $[N_{l,g}]/[I]_0$ ($l + g = 60$), in copolymers obtained by the copolymerization of L and G at a monomer to initiator ratio $([L]_0 + [G]_0)/[I]_0 = 60$. The given curves were calculated according to eq. 17 for $k_L\nu_L$ = 10, 20 and 30.

(see their eqs. 1 to 9) for the molecular weight distribution of homopolyamino acids, it can readily be shown that the concentration $[N_{l,g}]$ of copolymer molecules composed of l residues of the amino acid L and g residues of the amino acid G is given by

$$[N_{l,g}] = [I]_0 \frac{(k_L\nu_L)^l}{l!} \frac{[k_G\nu_G]^g}{g!} \exp(-k_L\nu_L - k_G\nu_G) \quad (17)$$

where

$$\nu_L = \int_0^t [L]dt \text{ and } \nu_G = \int_0^t [G]dt$$

The expression obtained for $N_{l,g}$ may also be derived by successive solution of the following set of kinetic equations describing the rate of formation of peptide chains composed of l lysine residues and g glutamic acid residues.

$$\frac{d[N_{l,g}]}{dt} = k_L[N_{(l-1),g}][L] + k_G[N_{l,(g-1)}][G] - k_L[N_{l,g}][L] - k_G[N_{l,g}][G] \quad (18)$$

The total concentration $[N_j]$ of copolymer chains composed of j amino acid residues may be derived from eq. (17) on summation over l values from 0 to j, for the case $j = l+g$. The expression thus obtained is identical with that given in eq. 15.

The concentration $[N_l]$ of molecules containing l residues of amino acid L, irrespective of the number of residues of amino acid G, is given by

$$[N_l] = \sum_{g=0}^{\infty} [N_{l,g}] = [I]_0 \frac{(k_L\nu_L)^l}{l!} e^{-k_L\nu_L} \quad (19)$$

Equation 19 shows that the Poisson distribution of N_l is the same as that of the corresponding homopolymer.

The distribution of $N_{l,g}$ as a function of l for the case $j = 60$, $g = 60 - l$, and $k_L\nu_L = 10$, 20 or 30 is represented in Fig. 9. The curves given in the figure show that a copolymer fraction with a well defined chain length has a sharp distribution with respect to component amino acid composition. The maximum of $N_{l,g}$ occurs at a value of l at which $l/g = k_L\nu_L/k_G\nu_G$.

The formulas given above for the molecular weight distribution and the compositional distribution derived for the Lys–Glu copolymers obviously apply to the copolymers derived from the other pairs of NCAs which undergo copolymerization at a rate which equals the sum of the rates of the homopolymerization of the corresponding component monomers under similar conditions.

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REHOVOTH, ISRAEL

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF PITTSBURGH]

Action of Urea on Tobacco Mosaic Virus[1]

BY ANNE BUZZELL

RECEIVED AUGUST 7, 1959

Six molar urea at 0° degrades tobacco mosaic virus within 20 minutes into apparently intact RNA having sedimentation constant, S_{20}^w, 30 and into the smallest subunit of protein with S_{20}^w about 2. Intact monomer virus is less stable than fragments. The monomer is degraded in 2 minutes to rods, measured in electron micrographs, ranging from $^2/_3$ to $^1/_2$ the original length. After 6 minutes these stable intermediates begin to degrade further. Fragments do not degrade appreciably in 6 minutes but are mainly gone in 20.

Introduction

The mechanism of urea action on tobacco mosaic virus (TMV) has appeared paradoxical in two respects. One paradox is that infectivity of the virus is destroyed by urea[2,3] whereas infectivity of phenol extracted RNA is not.[4] The other paradox is that the virus protein appeared to be

(1) Publication No. 69 of the Department of Biophysics. This research was supported by contract no. AT(30-1)913, Atomic Energy Commission.

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(3) M. A. Lauffer and W. M. Stanley, *Arch. Biochem.*, **2**, 413 (1943).

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grossly altered by urea, since on dilution in 0.1 *M* neutral phosphate buffer, the protein precipitates and cannot be redissolved save in concentrated urea or alkali solutions.[3,5] By contrast serum albumin appears to undergo a predominantly reversible change, and many enzymes retain activity in concentrated urea.[6-8]

This paper contains a first report of experiments undertaken to resolve the paradoxes. It is primarily concerned with the kinetics of the initial stages of urea degradation of the virus rod followed by the techniques of ultracentrifugation and electron microscopy. These kinetics appear qualitatively similar to the initial kinetics of alkali degradation since both involve the formation of stable intermediate products.[9-11] A surprising finding is that broken virus rods appear more stable against urea action than intact ones. It appears possible, on reviewing, that fragments may also be more stable to alkali.[9]

Materials and Methods

Virus.—The common strain of TMV was grown on Turkish tobacco plants and isolated by the method of Simmons,[12] a simplified modification of the method of Boedtker and Simmons[13] which yields preparations of predominantly monomer virus.[14] Briefly the procedure is as follows. The frozen infected leaves are homogenized in an "Osterizer" and then stirred with an acid exchange resin (IRC50), the *p*H being maintained at 7 by small additions of strongly basic resin (Dowex 1-X8). Resin and leaf pulp are removed by low speed centrifugation and versene is added. High speed centrifugation yields a colorless pellet of virus covered by a residuum of dark debris which can be shaken off. The pellet is dissolved in *p*H 7 10^{-3} *M* versene solution. One or two cycles of low and high speed centrifugation then generally suffice to remove the last traces of plant pigment and debris. Two stocks of virus, A and B, were prepared. Analytical ultracentrifugation showed the stocks had nearly equal monomer fractions (Table I). Preparation A was used exclusively for centrifugation studies on the totally degraded virus and no electron micrograph data was obtained for it.

Ultracentrifugation.—The Spinco Model E ultracentrifuge equipped with ultraviolet absorption optics was used throughout. Runs with whole and partially degraded virus were made at 27,690 r.p.m. and runs with totally degraded virus, at 59,780 r.p.m. All runs were done with total virus concentrations of 0.03 wt.%. To minimize convection[15] the runs were made in a 4 degree sector cell and at about 4°. Rotor temperature was controlled by chamber refrigeration and the total drift in temperature during the 40 minute runs was never more than 0.2°. Pictures of the absorption profile were taken at four minute intervals after the first picture, taken during acceleration. The Kodak "commercial" film was developed with continuous brushing for precisely 2 minutes at 20° in 1:1 Kodak DK 50 developer. Film optical density was found to vary linearly with the solution optical density over a wide range. In only a few cases were small corrections for non-linearity required. Exposure times of 40 seconds were required to achieve sufficient film blackening. Photodensitometric traces were made with a modified Spinco Analytrol calibrated with neutral density filters. Typical traces are shown in Fig. 1. The symmetrical outer peaks correspond to the reference holes. Within the cell, from left to right are first the air space and the sharp minimum due to light scattering at the meniscus. For the trace corresponding to whole virus there is next a plateau, at air space level, due to virus free buffer. At the bottom of the cell the low plateau corresponds to the region of uniform

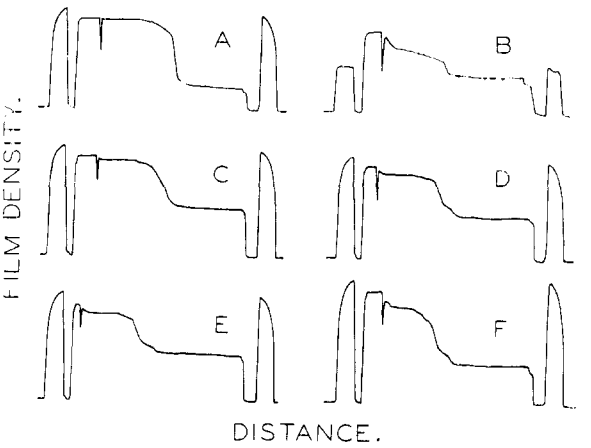

Fig. 1.—Typical absorption profiles for sedimentation runs with: A, control TMV; B, TMV totally degraded into RNA and protein; C, TMV after 1 minute in 6 molar urea at 0°; D, after 2 minutes; E, after 4 minutes; F, after 6 minutes. The differences in total trace height reflect differences in lamp intensity.

TABLE I

ULTRACENTRIFUGE DATA

Reaction time, min.	Degraded material: Degraded virus, % (obsd.)	Degraded material: Virus degraded, % (calcd.)	S_{20^w}	Residual material larger than degraded: %	Residual material larger than degraded: S_{20^w}	Free RNA, % [b]	Free protein, % [b]
1	39		158	39	192	5	14
1[a]	36		159	45	186	5	16
1	33		160	45	188	5	20
1[a]	36		158	47	186	5	12
2	47	61	155	26	186	11	24
2[a]	46	55	160	32	191	5	20
2	39	56	155	35	191	14	25
2[a]	43	53	157	30	188	6	23
3	48	65	151	22	193	12	29
3[a]	45	63	154	24	195	11	29
3	46	57	158	28	192	6	25
3[a]	49	58	153	28	188	5	26
4	44	61	154	26	194	11	26
4[a]	41	53	151	28	188	8	23
4[a]	45	58	155	29	189	11	20
5	47	63	153	22	188	11	28
5[a]	44	59	157	23	189	9	30
6	30		155	25	188	13	29
6[a]	28		158	30	195	9	23

Virus Stock	Monomer %	"Dimer"	S_{20^w}
A	64	17	192
B	68	8	189

[a] Solution dialyzed. Others 1 *M* urea. [b] Percentage of total RNA (or protein).

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virus concentration. The general procedure for calculating the percentage of material corresponding to a given component is as follows. Total concentration is taken as the height difference between the air or free buffer plateau and the final lowest plateau. Concentration of components is taken as proportional to the trace rise corresponding to the trailing boundary.

Electron Microscopy.—An RCA model B microscope was used. The virus preparations, with sufficient number of polystyrene latex (PSL) particles of 132 mμ diameter added, were sprayed onto collodion covered grids, air dried and lightly shadowed with platinum at an angle of about 5 to 1. Enlargement prints were made of the photographs to facilitate the measurement of virus rod lengths. Corrections for electron optical aberrations were estimated by measuring diameters of the PSL particles.

Procedure for the Kinetic Experiments.—All degradation reactions were done at 0° in 6 *M* urea solution buffered with 0.06 *M* phosphate. The buffer alone had a *p*H of 6.9 b[illegible] in the presence of urea the apparent *p*H,[5] measured with a Beckman glass electrode *p*H meter, was 7.6.

To start the reaction 0.1 ml. of the 1% virus stock solution (10^{-3} *M* in versene) was pipetted into 0.4 ml. of 7.5 *M* urea solution. To stop the reaction 1 ml. of 0.02 *M* *p*H 6.9 phosphate buffer was pipetted in. Estimates of extent of reaction were found quite reproducible for reaction times as short as a minute if care was taken to prechill solutions to 0° and operations were performed in a cold room.

The reaction mixture was then divided, and 0.5 ml. was further diluted 1:1 in buffer immediately and analyzed in the ultracentrifuge. The remainder was dialyzed at 0° for a total of 1.5 hours against two changes of 400 ml. 0.002 *M* phosphate *p*H 6.9 buffer with 10^{-3} *M* versene. Virtually complete removal of urea to less than 0.006 *M* measured by urease action[16] was achieved if the small dialysis bag was sufficiently bounced around in the vortex caused by a rapidly spinning magnetic stirrer. The dialyzed reaction mixture was divided, and one portion diluted 1:1 in 0.04 *M* phosphate buffer for centrifugation analysis. The other portion was diluted 1:20 in distilled water for electron microscopic analysis.

Results

Complete Degradation.—First attempts made to prepare TMV RNA free from protein for centrifugation analysis proved fruitless. Although a heavy, predominantly protein, precipitate forms when the virus–urea reaction mixture is diluted in 0.1 *M* phosphate buffer, too much protein remains soluble and too much RNA is lost in the precipitate. Investigation showed that no precipitate at all occurs if the reaction mixture is diluted in 0.02 *M* phosphate. Furthermore, after removal of the urea by dialysis, the protein is once more soluble in 0.1 *M* phosphate. Centrifugal analyses were thereafter made on the RNA protein mixtures, diluted 1:6 in 0.02 *M* phosphate buffer and the absorption profile for a typical extraction is shown in Fig. 1B. A sharp boundary can be seen corresponding to a substantial fraction of the material. Analysis shows that the sedimentation constant S_{20^w} for this fraction is 30 or that to be expected for intact TMV RNA.[17,18] The slow component has a sedimentation constant of about 2 or the expected value for the TMV protein subunit.[19] This value also agrees with that obtained by Lauffer and Stanley[3] for the protein degradation product by centrifugal analysis of undiluted virus urea solutions.

Since virus infectivity is destroyed by urea action, it was expected that the intact fraction of RNA would not survive long at room temperature. Results of the study of RNA stability have so far proved quite erratic. On some occasions the RNA survives for hours at 20° and on others appreciable degradation occurs at 0°. Only a few general observations seem warranted at present. It appears fairly certain that the RNA is less stable in the presence of Mg^{++} ions and more stable in the presence of versene. It is also quite clear that the RNA remains intact for 2 hr. at 0° in the undiluted reaction mixture. The data so far obtained suggest the possibility of a ribonuclease role for the urea altered protein such as has been recently described for other systems.[20,21] Test of this possibility, however, cannot be made until the various factors controlling RNA stability have been sorted out.

In the course of study of the RNA moiety, it became evident that the virus particles were not identical in their susceptibility to urea action. Although 20 minutes treatment at 0° sufficed for total disassembly of most of the virus rods, about 5% resisted the urea for 2 hr. and a few rods were visible in the electron micrographs after much longer treatments. This observation led to the study of the initial stages of the reaction, the principal subject of this paper.

Partial Degradation.—The procedure for the kinetics experiments is given in the Materials and Methods section. However, it should be mentioned here that an ultracentrifuge run was made immediately with the diluted reaction mixture and another as a control with the same mixture after the dialysis needed to remove urea for electron microscopic analysis No change in the mixture was apparent after dialysis. The data obtained by ultracentrifugation analysis are summarized in Table I. Typical absorption profiles are shown in Fig. 1C-F. Data obtained by electron microscopy are given in Figs. 2 and 3 and Table II. The general features of the reaction can be seen on direct inspection.

For convenience virus rods shortened by urea treatment will be called degraded rods; broken rods will be called fragments and understood to include both rods shorter than monomer and the longer ones which presumably are dimer fragments. (There were very few intact dimers.) After one minute of urea action more than half of the monomers had begun to degrade, some being reduced to $^2/_3$ the monomer length. After two minutes almost all the monomers had been reduced to lengths between $^2/_3$ and $^1/_2$ the original. Then for three more minutes no further change occurs. After six minutes the degraded rods had begun to degrade further. It is very striking that the fragments do not degrade appreciably in this time. Even after six minutes the fragments can be seen to persist in essentially their initial frequency distribution. By 20 minutes most of the fragments are degraded, but as mentioned earlier, a few rods of widely varying lengths persist for hours.

Electron micrographs have shown that some at least of the urea degraded rods are "firecrackers"

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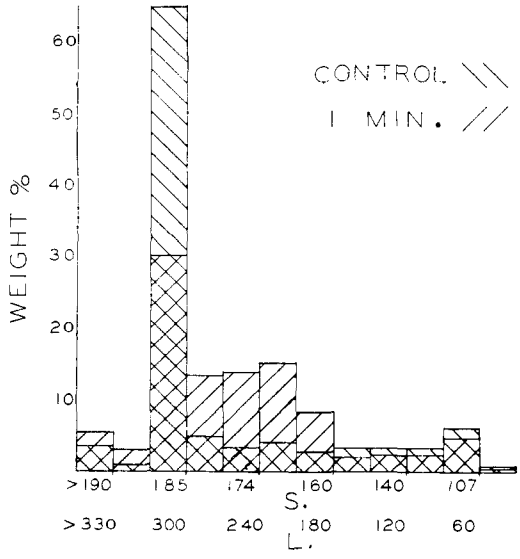

Fig. 2.—Weight percentage distribution, based on electron microscope data, for control TMV and TMV after 1 minute in 6 molar urea at 0°. On the abscissa is plotted measured rod length, L, in mμ; and calculated sedimentation constant, S, with the monomer value set at 185.[29]

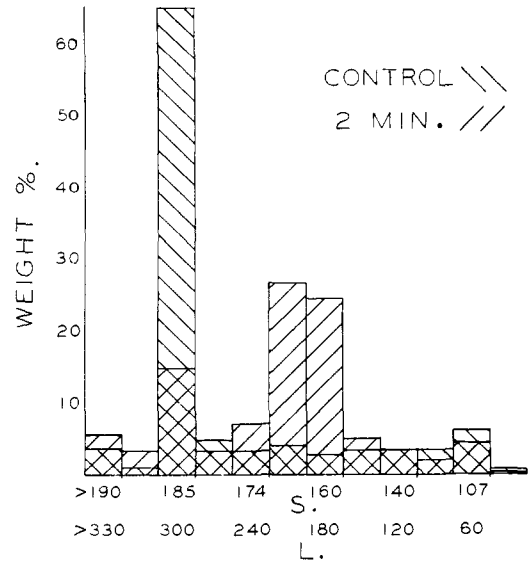

Fig. 3.—Weight percentage distribution based on electron microscope data, for control TMV and TMV after 2 minutes in 6 molar urea at 0°. L is rod length in mμ, and S is calculated sedimentation constant.

with visible tails, like those found by Hart[22] after partial detergent degradation of TMV. The RCA model B electron microscope unfortunately does not reliably produce the high quality micrographs needed for systematic observation of these tails. It appeared likely however that the majority of the rods had lost their tails, which would not be surprising in view of the erratic survival behavior of the free RNA. "Doughnut" particles[9] with clearly visible central hole and the diameter of the virus rod were seen on occasion and particles of doughnut size were fairly numerous. Systematic count did not seem warranted since the particles tended to cluster.

Discussion

For the purpose of obtaining quantitatively accurate data on virus particle degradation both techniques, ultracentrifugation and electron microscopy are open to some question. The difficulty with electron microscopy is primarily the possibility that some particles may break when the virus solution is dried.[23] Recent work, however, indicates that most of the particles can survive intact.[14] There are several difficulties with ultracentrifugation. For TMV concentrations well above 0.03% the Johnston–Ogston effect can seriously enhance the apparent concentration of the slower moving degradation products.[24,25] At concentrations as low as the 0.03% used here, convection becomes a problem both in destroying real boundaries and creating spurious ones.[15] Recent studies indicate that essentially convection free sedimentation can occur at concentrations of 0.004% or lower for nucleic acid[15] and at concentrations of 0.01% for Southern bean mosaic virus.[19] Precautions, described earlier, were taken to minimize convection, and the internal consistency of the data described below indicate that no convection occurred serious enough to appreciably effect the principal boundaries. In one centrifugation run with virus treated 6 minutes (Fig. 1F), a second degradation component appeared to be present having a sedimentation constant of 115 and comprising about 5% of the mass. The electron microscopy data suggest the boundary was real. If the boundary was real, the corresponding boundary in the companion centrifuge run was destroyed by convection.

The hypothesis that degraded rods originate only from monomer requires that the sum of degraded plus monomer fractions be constant and equal to the initial monomer fraction. The raw data indicate that this requirement is only approximately fulfilled. Detailed analysis shows that a number of corrections should be applied to the raw data. Since the rationale for the various corrections may not be apparent, they are described in detail in an appendix. To summarize the appendix: first the real concordance of the various independent estimates of the initial monomer fraction, 65–68%, is demonstrated. Then the method is described for converting the observed fraction of degraded rods to fraction of virus degraded. It can be seen in Tables I and II that the calculated fraction of virus degraded is in fact closely equal to the initial monomer fraction for reaction times of two minutes or greater.

Degradation by alkali and by urea, while qualitatively similar, appear to differ in certain respects. Perhaps the most significant difference is that alkali action yields only the larger "A protein" particle, comprising six of the ultimate subunits. Also it appears that central as well as terminal protein can be removed.[9] Urea for the most part removes

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TABLE II

ELECTRON MICROSCOPE DATA–WEIGHT PER CENT. DISTRIBUTION

React. time (min.)	Rod length (mμ) >330	330	300	270	240	210	180	150	120	90	60	30	Total length (μ)	Total no.
0	3.6	0.9	64.8	4.7	3.2	4.0	2.7	3.2	3.2	3.2	6.0	0.7	151.3	745
1	5.2	3.0	30.0	13.3	13.7	15.1	8.2	1.9	2.3	2.4	4.6	.3	199.3	967
	4.8[a]	**2.8**	**27.9**		**41.8 + 12.0**			**1·8**	**2.1**	**2.2**	**4.3**	**.3**		
2	5.5	3.2	15.6	3.2	6.9	26.4	24.3	4.9	3.4	1.9	4.2	.3	122.2	659
	4.0	**2.4**	**11.9**	**2.4**	**5.3**		**57.5 + 9.0**		**2.6**	**1.4**	**3.2**	**.2**		
3	3.1	1.7	15.7	3.3	3.4	17.0	32.3	10.3	4.2	3.7	4.6	.3	130.8	758
	2.3	**1.3**	**11.7**	**2.5**	**2.5**		**61.0 + 9.0**		**3.1**	**2.8**	**3.4**	**.2**		
4	3.8	0.4	13.5	0.9	2.3	19.8	34.4	8.3	4.3	5.4	6.2	.7	155.7	964
	2.8	**.3**	**9.9**	**.7**	**1.7**		**63.6 + 9.0**		**3.2**	**4.0**	**4.6**	**.5**		
5	7.0	1.2	7.3	1.8	4.3	21.5	29.5	13.3	3.7	4.7	5.3	.5	77.1	467
	5.1	**0.9**	**5.3**	**1.3**	**3.1**		**65.0 + 9.0**		**2.7**	**3.4**	**3.9**	**.4**		
6	4.8	6.1	11.3	3.7	3.6	15.2	23.2	2.8	6.6	9.9	6.7	.9	150.0	977
	3.3	**4.2**	**7.8**	**2.6**	**2.5**		**39.5 + 9.0**			**21.4 + 9·0**		**.6**		
S_{20}^{w}		190	185	180	174	167	160	151	140	126	107			

[a] Data in bold faced type have been corrected according to procedure outlined in Appendix B.

the subunits one at a time and apparently only terminal units. The few protein doughnuts, seen in the electron micrographs, could have been reaggregation products.[10] Conditions in the reaction mixture were not favorable for reaggregation,[26] but conditions during drying might have been.

The different mechanism for protein removal may account for the difference in location of the regions stable to alkali and to urea. Alkali action is impeded after $^2/_3$ of the rod is degraded. Other degradation products have been reported, having multiples of $^1/_6$ the monomer length,[9,11] but they may be reaggregation products.[10] Degradation products of urea action appear to have a range of lengths from $^2/_3$ to $^1/_2$ the monomer length.

The reason for the stability of intermediate degradation products has been and must remain subject for speculation. It has been suggested that the protein subunits may not all be identical.[27,28] The present finding that fragments of all sizes are more resistant than monomer virus to urea action cannot very well be explained by variant subunits. Another idea was that, owing to RNA continuity, optimum RNA–protein and protein–protein bonding could not always be achieved simultaneously, so that periodic variation in stability along the rod might be expected.[28] This idea is in better accord with fragment stability. Fragment stability for instance might result when the RNA anchor at the broken end was loosened or degraded and the outer protein layer was free to form stronger bonds with the underlying protein layer. Unfortunately, the same line of reasoning could be used to predict stability for the monomer virus. Further *ad hoc* assumptions could be made to circumvent the difficulty but they would render the whole idea less plausible, if not less possible.

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Appendix

A. Estimation of Initial Monomer Fraction.—The sedimentation constant of highly asymmetric rods is not sensitive to small changes in rod length.[29] It follows that the monomer fraction, 68%, estimated from centrifugation data for the control TMV undoubtedly includes some fragments. However, the method which was used to determine the boundary height on the photodensitometric traces should minimize the non-monomer contribution. The upper and lower limits of the boundary rise were set where the curve first deviates perceptibly from the straight line tangent. Four estimates of the height, on traces corresponding to different sedimentation times, were averaged after correcting for the dilution experienced by all particles sedimentating in a sector shaped cell.[30] The individual corrected estimates always were in close agreement. Apparent sedimentation constants calculated for the boundary limits were 8 svedbergs above and below the constant for the boundary midpoint. The theoretical sedimentation constants listed with corresponding rod lengths in Table II indicate that such a boundary spread would correspond to a rod length range of 45 mμ above and below the monomer length of 300 mμ. The actual range in lengths is considerably smaller, however, since at least half of the boundary spread can be accounted for by diffusion and by the particle migration during the 40 seconds exposure time.

Electron microscope data gives a value for the monomer weight fraction in excellent agreement with the 68% just cited. The monomer wt. %, w_m, is related to monomer count, n_m

$$w_m = \frac{l_m n_m 100}{\Sigma l_i n_i + l_n n_m} = \frac{l_m n_m 100}{L}$$

where l_m is monomer length and L, total length of all rods. Statistical fluctuation in n_m and the various n_i contribute to the over-all standard deviation, σ, of w_m. Using the relation[31]

$$\sigma^2 = \left(\frac{\partial w_m}{\partial n_m}\right)^2 \sigma^2_m + \Sigma \left(\frac{\partial w_m}{\partial n_i}\right)^2 \sigma^2_i$$

and the Poisson relation $\sigma_i^2 = n_i$, it can be shown that

$$\sigma^2 < \frac{100 n_m}{(L/l_m)^4} [(L/l_m)^2 - n_m^2]$$

Therefore, from the data in Table II, it follows that $w_m = 65 \pm 3\%$. The particles included in this fraction lie approximately in the size range 315 to 285 mμ.

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A third estimation for the virus monomer fraction is given by centrifugation data for the extracted RNA. The fraction of intact RNA in a phenol extract was 65%. The apparent range for the sedimentation coefficients was ± 0.8 svedbergs about the median of 30.1. Using the Mandelkern–Flory relation for a random coil,[32] the probable form for RNA in 0.02 *M* phosphate buffer,[33] the corresponding range in molecular weights is ± 5% of the median. The true weight range should be less since at least half of the boundary spread can be accounted for by diffusion. For five samples of TMV totally degraded by urea, the average fraction of intact RNA was calculated to be 64.9 ± 1.6%. This value, however, depends on the value used for the ratio of RNA to protein optical density in the mixture. The two boundaries, presumably RNA and protein, are well separated (see Fig. 1B), but the protein migrates so slowly that it was necessary to use the trace height for the airspace above the meniscus, to calculate protein and total optical density. This procedure is valid so long as the solution contains no low molecular weight ultraviolet absorbing contaminants. The five values for the optical density ratio agreed well, the average being 1.61 ± 0.05 to one. Absorption spectra given by Fraenkel-Conrat and Williams[34] predict an optical density ratio of 1.34:1 for a wave length of 265 mμ and of 2.41:1 for λ = 254. The ratio of 1.6:1 suggests that 75% of the incident light had λ = 265 which seems reasonable.[30]

B. Calculation of Fraction of Virus Degraded.—Clearly the apparent fraction of degraded virus does not correspond directly to the fraction of virus reacted, since the particles have lost mass. Also, presumably, the apparent degraded virus fraction includes some fragments. Considering first the electron microscope data, both the mass of degraded rods and the total mass must be corrected for the mass of material rendered too small to see. Assuming, for the case of 2 to 5 minutes reaction, the average degraded rod is $^7/_{12}$ of monomer length, the average length lost is $^5/_7$ of the length remaining. Then if the fragment fraction in the size range 210–150 mμ is taken as 9% it follows that

$$L_c - L = \frac{5}{700}(w_o L - 9L_c)$$

and $$w_c = \frac{12}{7}\frac{(w_o L - 9L_c)}{L_c}$$

where L_c and L are calculated and observed total length; w_o is observed wt. % of degraded virus and w_c is calculated wt. % of virus degraded. Also, the wt. % for the remaining classes of rods must be multiplied by L/L_c. Analogous corrections were applied to the data for 1 and 6 minute reaction times and the corrected data is given in bold faced type in Table II. If the wt. % for degraded rods and residual monomer are added the total is 71 ± 3%. Statistical analysis of the wt. % for all classes of fragments show that calculated and observed standard deviations agree and that therefore no detectable decrease of fragments occurs with treatment times up to 6 minutes.

To allow for the mass loss in the case of the ultracentrifugation data, the optical density of the two slowest sedimenting components was added to that of the degraded component. For the slower of the two, presumably free protein, which appears motionless at 27,690 r.p.m. the optical density estimate was based on the trace height of the air space above the meniscus. The calculated percentages of free protein (total protein being taken as total optical density × 1/2.6) are close to expectation. The faster component, which appears to vary erratically in magnitude, is probably free RNA. If virus fragments are impervious to 6 minute urea treatment, the only source of free RNA is the firecracker tails of the degraded rods. As noted earlier survival of exposed RNA strands is likely to be erratic.

The average fraction, so calculated, for the virus degraded in reaction times from 2 to 5 minutes, is 54 ± 4%. This value neglects the scattering contribution to optical density. An estimate of the scattering correction was made assuming the optical density would increase by 40%[34] if totally degraded material were assembled into monomers and that the scattering contribution of degraded virus is $^7/_{12}$ that of monomers.[35] The corrected values for the fraction of virus degraded are given in Table I. The average value is 59 ± 4%. (It should be noted that similar correction for the control virus data leaves the 68% value for the monomer fraction virtually unchanged but reduces the "dimer" fraction, closer to the 4% estimated from electron microscope data.) The apparent sedimentation constants for the upper and lower boundary limits were, on the average, 11 svedbergs above and below the constant for the boundary mid point, indicating a range of particle sizes. Allowing for diffusion, the range in sedimentation constants should be about ± 6 svedbergs.

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Convenient Syntheses of L-Isoglutamine and L-Isoasparagine through Derivatives Commonly Useful for Peptide Synthesis

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A new route to L-isoglutamine and a simplified route leading to L-isoasparagine are described. Carbobenzoxy-L-glutamic acid was converted through preferential activation of its α-carboxyl group followed by treatment with NH_3 to carbobenzoxy-L-isoglutamine, from which L-isoglutamine was obtained through hydrogenolysis. Direct amidation of *p*-toluenesulfonyl-L-aspartic anhydride yielded *p*-toluenesulfonyl-L-isoasparagine, which, after purification, was converted with sodium in NH_3 to L-isoasparagine. The preparation of *p*-toluenesulfonyl-L-asparagine is also given.

Since glutamine and asparagine occur so widely in nature, both free and combined as constituents of polypeptides and proteins of biological importance, a study of the activity and properties of polypeptides containing the isomeric isoglutamine and isoasparagine residues is of interest. For example, isoglutamine-oxytocin, a synthetic, isoglutamine-containing isomer of oxytocin, has been found to possess the interesting property of inhibiting some of the physiological activities of vasopressin.[2] Synthetic methods have therefore been sought which would provide in reasonable yield and purity sources of L-isoglutamine and L-isoasparagine for such purposes.

Many of the syntheses of isoglutamine and iso-

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