Auxiliary Infectious Nucleoprotein from Plants Infected with Tobacco Mosaic Virus

Tung-Yue Wang and Barry Commoner

The Henry Shaw School of Botany, Washington University, St. Louis, Missouri

PLANTS infected with tobacco mosaic virus (TMV) synthesize massive quantities of infectious material. When this material is extracted from long-infected plants and compared with the inoculum, it appears to be a replica of the latter with respect to biological, chemical, and physical properties. Such an observation implies a rigid relationship between the chemical composition of TMV and its biological specificity. It suggests that virus reduplication is achieved by replication of the specific nucleoprotein introduced into the cell by inoculation.

The experimental evidence for the apparent identity between the TMV inoculum and the final product of the reduplication process depends on analyses of the nucleoprotein extracted by common neutral buffers from infected leaf, for TMV is readily soluble in such buffers. Our attention was directed to the leaf proteins *not* found in such extracts by the observation that TMV-infected leaf synthesizes an excess (as compared with otherwise identical healthy tissue) of buffer-insoluble nucleoprotein. The excess reaches a maximum in advance of the appearance of TMV and declines in amount as the virus accumulates in the tissue (*1*).

This evidence of a close relationship between insoluble nucleoprotein and TMV has led us to compare the insoluble nucleoprotein components of infected and uninfected leaf. The present paper summarizes the initial results of this investigation (*2*). It describes the isolation and partial characterization of a hitherto unknown buffer-insoluble nucleoprotein which is consistently associated with TMV infection. This protein is infectious, and appears to possess biological properties indistinguishable from those of the virus. However, the new nucleoprotein, although similar to TMV in composition, differs significantly from ordinary virus with respect to amino acid composition and certain physical properties.

When tobacco leaf is homogenized in *p*H 7.0 phosphate buffer (0.05*M*) and the homogenate is centrifuged at about 2500 *g*, the soluble proteins of the supernate, which include TMV, represent about one-half of the total protein content of the leaf. If the thoroughly washed residue is then extracted in 10-percent NaCl for 18 hr at 4°C, about 15 percent of the previously insoluble protein and almost all of the nucleic acid become solubilized. A number of components can be isolated from this extract by suitable procedures, such as dilution, salting-out, and ultracentrifugation. When such extracts of comparable infected and uninfected tobacco leaf are prepared and the high-molecular weight components are isolated by ultracentrifugation (1 hr at 104,500 *g*) a prominent component not found in extracts of normal tissue is observed in infected material. Results of such parallel fractionation are shown in Fig. 1. The ultracentrifuge pellet containing the high molecular weight material from uninfected tissue, which is readily redissolved in *p*H 7.0 phosphate buffer, on electrophoretic analysis shows the presence of a fast-moving component (mobility at *p*H 7.0 $= -18 \times 10^{-5}$ cm^2/v/sec) and poorly resolved slow-moving components with mobilities at *p*H 7.0 ranging from -4 to -7. These components are present in comparable extracts prepared from infected tissue, but the infected material also contains an additional component of mobility $= -8.5$, which has been given the designation I8.

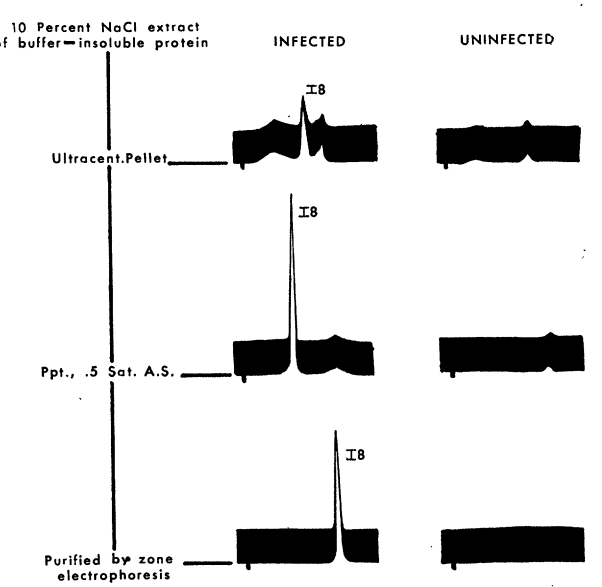

Fig. 1. Descending electrophoresis patterns of comparable infected and uninfected material. Migration is to the right from the marked starting boundaries. Times and currents for all but the lower left-hand pattern were 51 to 60 min and 7.7 to 7.8 ma. In the latter pattern, migration time was 83 min at a current of 7.8 ma. The crude 10-percent NaCl extract is ultracentrifuged and the resulting pellet dissolved in *p*H 7.0 phosphate buffer. The precipitate obtained from this solution by salting out with 0.5 saturated ammonium sulfate is finally purified by zone electrophoresis.

Component I8 can be isolated from the crude high molecular weight fraction in an electrophoretically homogeneous form by salting out in 0.5 saturated ammonium sulfate (which precipitates I8, but fails to precipitate the normal slow components), followed by zone electrophoresis on a starch column (*3*), which separates I8 from the fast-moving normal component. When this procedure (outlined in Fig. 1) is applied to the extracts of infected tissue, electrophoretically homogeneous preparations of I8 are obtained. Systemically infected tobacco leaf yields 10 to 50 μg of I8 per gram wet weight. The average yield of TMV is about 2500 μg/g. The new protein has been found consistently in a series of eight preparations from infected leaf and has never been detected in uninfected tissue. We conclude therefore that I8 is a specific component uniquely present in the insoluble residue of TMV-infected leaf.

Several different preparations of I8 have been purified as described in the preceding paragraph and partially characterized with the following results.

1) *Size and shape.* Electron micrographs of I8 and of the TMV isolated from the same tissue are shown in Fig. 2. Both are rods averaging about 450 mμ in length. The frequency distribution of the lengths of I8 and TMV rods obtained from such photographs show no significant differences between the two components.

2) *Electrophoretic mobility.*. Preparations of I8 and of TMV from the same tissue were dialyzed against a series of common-ion buffers (*4*) and electrophoretic mobilities were determined at various *p*H's. The results, shown in Fig. 3, indicate that the two components are distinctly different. TMV is isoelectric at *p*H 3.4, precipitating as characteristic microneedles. I8 is isoelectric at *p*H 2.9, precipitating in an amorphous form. The two mobility curves also differ qualitatively; the mobility of I8 becomes more negative when the *p*H is dropped below 7.0. This anomalous behavior indicates that the lower *p*H induces a marked change in the ionic configuration of I8. This change is also reflected in the physical character of I8; at *p*H's below 5.0, I8 tends to form viscous gels. In these respects I8 is qualitatively different from TMV, for the virus shows neither electrophoretic anomalies nor

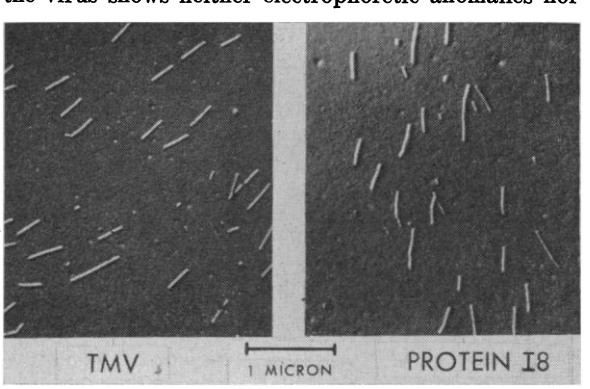

Fig. 2. Electron micrographs (Palladium-platinum shadowed) of concurrently isolated preparations of I8 and TMV.

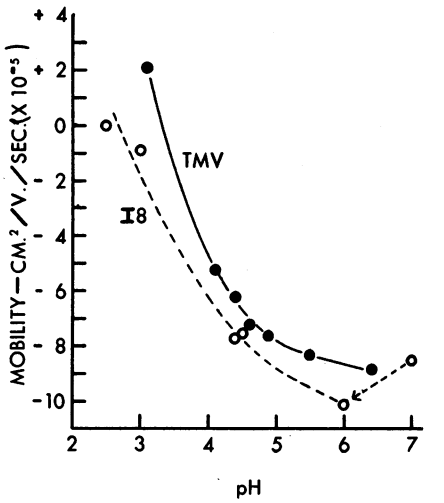

Fig. 3. Electrophoretic mobility of concurrently isolated preparations of I8 and TMV at various *p*H's. All samples were dissolved in buffers of ionic strength 0.1, which contained NaCl to provide common ions (*4*).

gel formation at low *p*H's. When a mixture of equal amounts of TMV and I8 is brought to *p*H 5, the resulting gel contains the TMV as well as I8. This suggests that I8 and TMV tend to combine under these conditions. One result of this phenomenon is that mixtures of the two proteins cannot be resolved electrophoretically.

3) *Chemical composition.* Several samples of electrophoretically homogeneous preparations of I8 and concurrently isolated TMV have been hydrolyzed and analyzed for amino acid composition by the method of Fischer and Dörfel (*5*). Table 1 shows the results obtained from quadruplicate analyses of identical amounts of I8 and TMV based on chromatogram sheets carrying hydrolyzates of both proteins and six samples of a standard calibration mixture of amino acids. The analyses reveal an over-all general similarity between I8 and TMV. However, the proteins are not identical. Most apparent is the relatively large difference in the tyrosine contents of the two proteins. This difference has been confirmed by three additional parallel partial analyses of I8 and TMV for this amino acid. The results, which are presented in Table 2, show a consistently lower tyrosine content in I8 than in TMV, the average difference being about 50 percent of the larger value. Table 1 suggests that the amino acid composition of I8 and TMV may also differ with respect to other components, notably serine and glycine, but these possible differences need to be confirmed. Nevertheless, at least on the basis of the tyrosine values, we conclude that the protein moieties of I8 and TMV are not identical in amino acid composition.

Samples of I8 and concurrently isolated TMV were analyzed for nucleic acid content by extraction with hot 0.5*M* perchloric acid. The average nucleic acid contents of I8 and TMV calculated from Kjeldahl analysis of the samples and the optical densities of perchloric acid extracts at 260 mμ were 5.2 and 5.1

Table 1. Percentage composition of I8 and TMV. Amino acid analysis is based on acid hydrolyzate and therefore does not include tryptophane which represents 2.1 percent of ordinary TMV protein.

Amino acid	I8	TMV	Average deviation of mean
Alanine	5.6	5.6	0.2
Aspartic acid	9.9	11.3	.6
Arginine	13.4	14.4	*
Cystine	0.6	0.5	.1
Glutamic acid	12.4	11.4	.3
Glycine	2.0	1.5	.1
Lysine	2.7	2.5	.5
Phenylalanine	7.5	8.7	1.2
Serine	6.6	7.6	0.2
Threonine	9.0	9.1	.5
Tyrosine	1.8	3.3	.5
Valine	6.8	7.2	.8
Leucine, isoleucine, and proline	17.0	14.2	1.4
Total protein	95.3	97.3	
Nucleic acid	5.2	5.1	
Total nucleoprotein	100.5	102.4	

* Only one value obtained.

Table 2. Percentage tyrosine content of I8 and TMV.

Preparation	I8	TMV	Average deviation of mean
1	2.1	3.4	0.2
2	2.1	2.8	.03
3	1.8	3.5	.1
4*	1.8	3.3	.5
Avg.	2.0	3.5	

* These values obtained from the analysis shown in Table 1.

percent, respectively. There appears to be no significant gross difference in nucleic acid content. Limited amounts of I8 nucleic acid were available for analysis with respect to nitrogen base content. Hydrolysis in 70-percent perchloric acid for 1 hr, followed by paper chromatography (*6*) yielded four ultraviolet-absorbing spots that were identified as adenine, guanine, cytosine, and uracil. Quantitative analysis of these chromatograms, although insufficient to give final results, suggests that the molar ratios of the four bases in I8 may be different from the ratios given by the nucleic acid of concurrently isolated TMV.

These results show that I8 is a pentosenucleoprotein that differs from TMV with respect to amino acid composition and, possibly also, with respect to nitrogen base ratios.

4) *Immunochemical properties.* Figure 4 compares the precipitin curves obtained when various amounts of I8 and concurrently isolated TMV are reacted with 0.001 ml of anti-TMV rabbit serum according to the method of Heidelberger and Kendall (*7*). The two

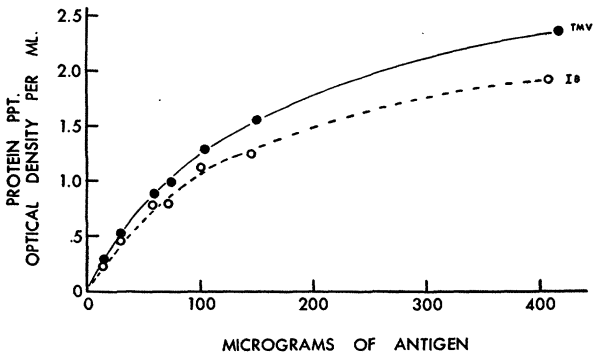

Fig. 4. Precipitin curves yielded by various amounts of concurrently isolated preparations of I8 and TMV. In each case the antigen was reacted with 0.001 ml of anti-TMV rabbit serum. Protein contents of precipitates determined by the Folin phenol method and reported as optical density at 750 mμ.

curves are essentially identical. This observation is evidence of a close immunochemical relationship between I8 and TMV.

5) *Biological properties.* The infectivity of I8 and concurrently isolated TMV have been compared by local-lesion tests on leaves of *Nicotiana glutinosa.* A series of comparable concentrations of the two nucleoproteins were rubbed on opposite halves of *N. glutinosa* leaves. Lesions were examined and counted after 3 days. Both I8 and TMV produced lesions that were indistinguishable in appearance and emerged on the leaf at the same time after inoculation. Lesion counts given by various concentrations of I8 and TMV (Table 3) show that there is no significant difference in the specific infectivity of the two nucleoproteins.

When I8 is rubbed on leaves of *N. tabacum,* symptoms typical of TMV-infected plants become evident in the newly formed leaves. Analysis of leaves infected in this way shows that they contain ordinary buffer-soluble TMV. Parallel groups of plants inoculated with I8 and TMV were found after 13 days to contain 1.1 and 1.3 mg/g of ordinary soluble TMV, respectively. Thus, plants inoculated with I8 produce TMV and show the typical symptoms of infection with TMV. With respect to these biological properties, I8 and TMV do not appear to differ.

Table 3. Comparative infectivity of I8 and TMV.

Preparation	No. of lesions per 4 half-leaves of *N. glutinosa**					
	0.020 mg/ml		0.050 mg/ml		0.100 mg/ml	
	I8	TMV	I8	TMV	I8	TMV
1	20	18	32	39	130	63
2	16	12	40	51	54	25
3	28	22	29	56	25	104

* In each case, parallel numbers represent lesions given by comparable concentrations of I8 and TMV inoculated on opposite halves of the same four leaves.

In sum, we find that plants infected with TMV also produce small but consistent amounts of an auxiliary nucleoprotein, I8. Although apparently possessing biological properties identical with those of TMV, I8 is different from the latter with respect to chemical composition and certain physical properties. Both proteins are rods of similar size and shape, contain about the same amount of nucleic acid, show certain similarities in amino acid composition, and are close immunochemical relatives.

Several possible explanations of these observations need to be considered (*8*):

1) *That I8 is a strain of TMV different from the common form used as inoculum.* This proposal would imply either that I8 was present as a contaminant in the original inoculum, or that it originated by mutation in the infected leaf. However, the biological properties of TMV and I8 appear to be identical. Furthermore, no TMV-related virus strain is known that is not initially soluble in buffer. It is unlikely, therefore, that I8 represents a virus strain different from the common TMV used as inoculum.

2) *That I8 represents an intermediate or alternative product of the specific biosynthesis of ordinary TMV.* This proposal coincides most closely with the information on hand at present. The two proteins are equally characteristic products of plants infected with TMV. They appear to be biologically identical and are very similar in composition. These facts indicate that I8 and TMV are common products of the specific biosynthetic processes induced in the host by the entering TMV inoculum.

The distinct differences that we find in the composition and physical properties of I8 and TMV lead to the further conclusion that the biological specificity of the virus is not rigidly dependent on the particular chemical structure ascribed to ordinary TMV. This conclusion is subject to two alternative interpretations: (i) In the reduplication of the virus, a certain range of latitude in the chemical composition of the product is tolerated without discernible alterations in biological specificity. In this view, I8 would represent one of the allowed alternative structures of the virus, its distinguishing physical properties and cellular location being a consequence of slight, biologically inconsequential chemical differences from ordinary TMV. (ii) Alternatively, the foregoing results may be construed as evidence that the biological specificity of the virus is determined by only part of the chemical structure of ordinary TMV, and that this determinative subunit is present in both I8 and TMV.

Within either interpretation, consideration must also be given to the possibility that I8 is the initial form in which the virus is synthesized, being subsequently converted to the soluble TMV commonly found in the infected plant. Further investigations of these relationships should help to illuminate the biochemistry of TMV reduplication.

References and Notes

1. B. Commoner *et al.*, *Science* **118**, 529 (1953).
2. Aided by a grant from the National Foundation for Infantile Paralysis. We are Grateful to Kathryn Baker and Betty Read for assistance in carrying out some of this work.
3. H. G. Kunkel and R. J. Slater, *Proc. Soc. Exptl. Biol. Med.* **80**, 42 (1952).
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6. G. R. Wyatt, *J. Gen. Physiol.* **36**, 201 (1950).
7. M. Heidelberger and F. E. Kendall, *J. Exptl. Med.* **62**, 697 (1935).
8. Bawden [F. C. Bawden and N. W. Pirie, *Brit. J. Exptl. Pathol.* **26**, 294 (1945)] has reported that when TMV is removed from infected leaf by expression of sap from minced tissue, a considerable amount of infectious material remains in the residue from which it can be released by enzymatic treatment or milling. This residual virus does not appear to differ from the initially extracted virus with respect to physical and chemical properties; it represents about one-third of the total TMV content; we find that it can be extracted by buffer from homogenized leaf. In all these respects Bawden's residual virus differs from I8; the two components are therefore not identical.

The "Atomic" Rivals

Francis Simon*

Clarendon Laboratory, University of Oxford, England

DEVELOPMENTS in the domain of atomic energy are going to affect our future profoundly. Already it is clear that all our ideas about defense and the role of conventional weapons will soon have to undergo a radical change. (Even though the general public does not yet seem to have realized all the implications of the latest development, the "cheap" hydrogen bomb.)

* Reprinted by permission from the *Financial Times* (London), 6 Aug. 1954, with the thought that our readers may be interested in seeing how the position looks from the other side of the Atlantic. Sir Francis is professor of thermodynamics at Oxford.

The development of atomic power for industry is a comparatively long-term project, but its consequences will be almost as far-reaching as in the weapons field. Probably the first really important changes will be seen in those underdeveloped countries where a relatively small-scale provision of power would make a great difference. Nuclear fuels will not have such a marked impact on the more highly industrialized countries which now depend on coal and oil for their power, if only because of the much longer time needed to change over their enormous power systems to nuclear energy. As I have discussed elsewhere [in