NEW NUCLEOPROTEIN COMPONENTS WITH PROPERTIES OF TOBACCO MOSAIC VIRUS *

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In further attempts to determine the significance of the trace metals found recently in purified preparations of tobacco mosaic virus, TMV, (Loring and Waritz, 1957; Loring, Al-Rawi, and Fujimoto, 1958), we have treated the original sedimented virus with sodium ethylenediaminetetraacetate, EDTA, and subjected it to extensive washing in the ultracentrifuge with deionized water. In contrast to the usual preparations in phosphate buffer, a relatively large amount of the previously sedimented virus, e.g. 20 to 50 per cent, failed to form a pellet during the third, fourth and fifth ultracentrifugal cycles and remained in the lower portion of the supernatant liquid as concentrated but non-compacted virus. Like the non-compacted components described previously (Bawden and Pirie, 1945; Sigurgeirsson and Stanley, 1947), the present preparations also gave relatively low sedimentation rates in the analytical ultracentrifuge. Unlike the former, however, those now described have proved comparable in infectivity to the virus sedimented during the same ultracentrifugal cycle or to the usual virus preparations in phosphate buffer. The procedures used for the preparation of these new TMV components and preliminary data on their sedimentation characteristics, infectivity, and chemical composition are presented in this communication.

The virus was sedimented from cold clarified infectious juice

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(extracted from previously-frozen diseased plants) by centrifugation in an average centrifugal field of 78,400 x g for 90 min. at 4°. The virus pellets and aggregated materials were concentrated three or four fold by resuspension in deionized water (deionized water from a commercial deionizer passed through a mixed bed of Duolite C-20 and A-101*), and the suspension was brought to pH 6.5 in the cold and made about 0.005 molar with EDTA. The insoluble components were removed by centrifugation at about 1200 x g and the virus in the supernatant liquid sedimented as mentioned before. The amber-colored nucleoprotein components usually associated with the virus prepared in water (Ginoza, Atkinson, and Wildman, 1954) were largely removed in the EDTA supernatant liquid. This fraction represented from 25 to 30 per cent of the absorbance at 260 mμ of the first clarified pellet and like the fractions described by Bawden and Pirie and Sigurgeirsson and Stanley contained only small amounts of virus activity. The pellets resulting after ultracentrifugation of the EDTA solution were redissolved in deionized water and again ultracentrifuged. From 30 to 40 per cent of the previously-sedimented virus formed a non-compacted fraction above the sedimented pellet and could be readily separated both from the clear supernatant liquid and the pellet. Continued washing of the pelleted virus in the ultracentrifuge for one or two more cycles gave additional amounts of non-compacted virus corresponding to from 20 to 30 per cent of each previously-pelleted sample, but only relatively small amounts of ultraviolet-absorbing components, e.g. 2 to 3 per cent of the previous pellet, were found in the supernatant fraction after a sixth ultracentrifugal cycle, even at concentrations comparable to those used in the initial cycles. Similarly no appreciable amounts of non-compacted virus resulted from an additional treatment with EDTA followed by a second course of repeated washings in the ultracentrifuge. As would be expected from the large amounts of non-compacted virus found, the yield

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of the more-readily sedimentable component after four or five cycles of purification was of the order of one-third to one-half that obtained when 0.1 M phosphate buffer at pH 7 was used.

Sedimentation rates were determined in a Spinco Model E Ultracentrifuge in Kel-F, 12 mm cells, using quartz optical windows so that ultraviolet absorption and Schlieren records, could be made simultaneously. The non-compacted virus from the third, fourth and fifth ultracentrifugal cycles was sedimented in deionized water at several concentrations and gave sedimentation rates ranging from 100 S to 121 S. Extrapolation to zero concentration gave a value close to 130 S. Ionic strength had little effect on sedimentation rate for similar values were found for one preparation examined in 0.1 M tris(hydroxymethyl)aminomethane-acetate buffer at pH 7.5, in 0.1 M NaCl and in 0.1 M KCl. The pelleted virus after EDTA treatment contained a fraction giving a sedimentation coefficient of 175 S as well as typical virus with 190 S. It could be freed from the 190 S component, however, by further treatment with EDTA and washing with deionized water.

The infectivities of the uncompacted and sedimented virus fractions were compared by the half-leaf method on Nicotiana glutinosa (Loring, 1937) and in several tests 130 S or 175 S virus was compared with that prepared either in deionized water alone or in 0.1 M phosphate buffer. The total numbers of lesions found for the 130 S preparation from the third, fourth and fifth ultracentrifugal cycles in one experiment where solutions of 0.01 absorbance at 265 mμ in 0.01 M phosphate were applied to 11 to 15 half-leaves in each test were 565, 453 and 307 in the order mentioned in comparison with 614, 356 and 250 for the sedimented virus from the same cycles. Similar activity results were found when 130 S or 175 S virus was compared with typical virus preparations.

The nitrogen (Ma and Zuazaga, 1942), phosphorus (Loring et al., 1952), and protein contents (Gornall et al., 1949) of solutions of both non-compacted and pelleted virus were determined and nitrogen to phos-

phorus ratios and biuret color (absorbance at 540 mμ) per unit of absorbance at 265 mμ calculated. The N/P ratios of both non-compacted and compacted virus, 29 to 36 and 30 respectively, were in the range published for purified TMV, 25 to 43. Values for protein were slightly higher for the 130 S component from the third and fourth cycles, 0.028 and 0.024, as compared to a value of 0.020 for both fifth cycle 130 S and 175 S virus or for that prepared in phosphate buffer.

It is clear from the data presented that treatment of the original virus pellet with EDTA and washing in deionized water have led to new types of infectious TMV preparations. While the 130 S component mentioned has been best characterized so far, evidence has also been found in the uncompacted fraction from virus preparations washed in deionized water alone for other highly infectious components of even lower sedimentation rate, e.g. 80 S. The extent to which such components represent either smaller virus units sedimented originally or the dissociation of the typical virus at the concentrations of divalent metals which resulted after EDTA treatment has not been established. The relatively large yield of the 130 S component, however, indicates that dissociation is at least partly responsible for the occurrence of the smaller particles. The isolation of highly infectious nucleoproteins of relatively low sedimentation rate is in agreement with other recent evidence (e.g. Shalla, 1959) that the primary infectious nucleoprotein unit in the tobacco mosaic disease is smaller than the usually accepted rod-shaped particle of 300 mμ x 15 mμ.

REFERENCES

Bawden, F. C., and Pirie, N. W., Brit. J. exp. Path. 26, 294 (1945).

Ginoza, W., Atkinson, D. E., and Wildman, S. G., Science 119, 269 (1954).

Gornall, A. G., Bardawill, C. J., and Maxima, D. M., J. Biol. Chem. 177, 751 (1949).

Loring, H. S., J. Biol. Chem. 121, 637 (1937).

Loring, H. S., Al-Rawi, S., and Fujimoto, Y., J. Biol. Chem. 233, 1415 (1958).

Loring, H. S., Bortner, H. W., Levy, L. W., and Hammell, M. L., J. Biol. Chem. 196, 807 (1952).

Loring, H. S., and Waritz, R. S., Science 125, 646 (1957).

Ma, T. S., and Zuazaga, G., Ind. and Eng. Chem. Anal. Ed. 14, 280 (1942).

Shalla, T. A., Virology, 7, 193 (1959).

Sigurgeirsson, T., and Stanley, W. M., Phytopathology 37, 26 (1947).