Particle-Length Variability of the Pea Seedborne Mosaic Virus

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Cooperative investigations of the Western Region, Agricultural Research Service, U.S. Department of Agriculture, and the Oregon and Washington Agricultural Experiment Stations, Corvallis 97331 and Prosser 99350, respectively. Technical paper No. 3662, Oregon Agricultural Experiment Station.

Accepted for publication 24 May 1974.

ABSTRACT

Particle lengths of pea seedborne mosaic virus (PSbMV) were significantly shorter in leaf-dip and in partially purified preparations fixed with formalin, than those derived from either preparation when fixed with glutaraldehyde. Inherent structural properties, possibly unique to our strains of this virus, and unfavorable preparatory procedures caused particle breakage and anomalous particle-length modes. Preparations obtained by a sequence of differential, rate-zonal density-gradient-, and sucrose-polyethylene glycol ultracentrifugation were highly infectious and showed virus particle-length modes of 750 nm to 770 nm when fixed with glutaraldehyde.

Phytopathology 64:1358-1363.

Length measurements of filamentous virus particles have been considered more reliable when derived by the "leaf-dip" method than when derived by use of purified virus preparations, because of various artifacts (4, 5), and because of demonstrable particle breakage and/or linear aggregation (7) during purification. Such effects appear

to be common among viruses of the potato virus Y group. Potato virus Y (PVY) and turnip mosaic virus particles were found to be 6% and 9% shorter, respectively, in purified preparations (6, 15) than in leaf-dip preparations (6). Use of leaf-dip preparations may also fail to establish true particle length. Taylor and Smith (18) and Bos (1) determined that normal lengths of bean yellow mosaic virus particles were 6% to 7% greater in leaf dips from *Chenopodium amaranticolor* Coste and Reyn. than from *Pisum sativum* L.

Pea seedborne mosaic virus (PSbMV) clearly belongs in the PVY group (10, 13). Yet particle lengths determined for this virus from both leaf-dip and partially purified preparations (9) were significantly shorter than those ultimately concluded to be representative for this virus (10, 13). Indeed, serological relatedness of our isolates to the seedborne virus reported by Inouye (11) was the basis for intensively evaluating particle length and factors affecting PSbMV particle variability. Factors predisposing PSbMV particles to fragmentation and the resultant aberrent particle-length distributions are examined in this paper.

MATERIALS AND METHODS.—PSbMV isolate PS-2 originated from a field-infected pea plant, *Pisum sativum* L. cultivar 447 (9), collected in 1968. Isolates W-1 (ATCC No. PV 184) and S-1(10) originated from infected pea seed. All isolates were maintained by serial passage in greenhouse-grown plants of pea cultivar 447 or 187. Solutions containing PSbMV, partially purified by various procedures (Table 1), were mixed with equal volumes of diluted formalin or glutaraldehyde to provide desired fixative concns. Suspensions of fixed particles were placed on electron-microscope grids, allowed to set for 3 min, blotted free of excess liquid, and negatively stained with neutralized phosphotungstate (PTA) or shadow-cast with platinum-palladium.

At least 100 particles were measured for the histogram of each virus preparation. Particles were selected at random for measurement, so long as they were obviously intact, and both ends were clearly discernible. All measured particles were initially assigned to frequency classes for graphic examination, but upper and lower "tails" containing particles of widely scattered length classes, with few particles per class, were removed for convenient presentation (Fig. 1). Fewer than 80% of the measured particles were depicted in only one frequency distribution (Fig. 1-F), particles of which ranged in length from 185 nm to 1,485 nm. We departed from the usual practice of averaging particle length for only the "major mode" (4, 5), because of the particle-length variability inherent in this study. Instead, avg particle lengths (AL) were derived as the mean for each whole population depicted graphically. Values for AL (Fig. 1) were provided only as a conventional reference statistic. Modal length, represented as a distinct frequency peak for each population, was considered to be the more meaningful parameter of virus particle populations in this study, and was used as the quantitative reference point for treatment-induced shifts in virus particle lengths.

Specific protocols used for partial purification are outlined in Table 1. Histograms of PSbMV particle lengths from unfixed leaf-dip preparations were not presented, because virus particles were rarely observable unless fixed before exposure to PTA. Formalin was the fixative used in this study, before we were aware that PSbMV particles were longer when fixed with glutaraldehyde than with formalin. Wherever not specified, virus particles were fixed with formalin. All preparations were examined in a Philips EM 300 electron microscope operated at 60 KV. Microscope magnification was calibrated by use of a Polaron diffraction-grating replica, with 2,157 lines/mm (54,800

TABLE 1. Effect of preparatory procedures on the properties of pea seedborne mosaic virus isolate W-1

Procedure		Sved. coef.[c] (S)	Relative infectivity[d]	Fixative	Modal length (s) >100 particles (nm)	Representative histogram (Fig. 1)
Clarification[a]	Purification[b]					
Chloroform-Butanol	2DC,DG(P_1)	148 S	0.1	1% Formalin; PTA	320, 520	D
				2% Formalin; PTA	600	B
				10% Formalin; PTA	290, 470	C
Chloroform-Igepon	2DC,DG(P_2)	148 S	0.5	2% Formalin; PTA	Random	G
				2% Formalin; Shadow[e]	500 to 640	F
Chloroform-Igepon	DC,PEG,DG(P_3)	148 S	0.5	2% Formalin; PTA	675	H
				3.5% Glutaraldehyde; PTA	750	I
Chloroform	DC,PEG,DG(P_4)	148 S	1.0	3.5% Glutaraldehyde; PTA	770	[g]
Chloroform (leaf tissue)[f]	DC,PEG,DG(P_4)	---	1.0	3.5% Glutaraldehyde; PTA	770	L

[a]Eighteen grams of pea-root tissue were homogenized in 180 ml fresh 0.01 M sodium diethyldithiocarbamate (NaDIECA) containing 0.01 M cysteine-HCl. The juice was expressed through cheesecloth and emulsified 30 min at room temp with one volume chloroform:*n*-butanol (1:1), one-half volume chloroform containing 0.1% Igepon T73, or one-half volume chloroform (12, 13).

[b]Abbreviations: DC = one cycle differential ultracentrifugation; DG = rate-zonal sucrose density-gradient centrifugation (2); PEG = selective sedimentation of PSbMV by centrifugation in a solution of sucrose (0.3 g/ml), polyethylene glycol (0.04 g/ml), and sodium chloride (0.007 g/ml) (12, 13); P = purification methods 1 through 4, respectively.

[c]Sedimentation coefficient ($S_{20,w}$) determined in ref. 12 by comparison with tobacco mosaic virus in rate-zonal density-gradient centrifugation in sucrose (3), uncorrected for infinite dilution.

[d]Relative lesion numbers per leaf produced on *Chenopodium amaranticolor* Coste & Reyn. by solutions containing approximately 0.1 mg/ml purified virus.

[e]Shadowed with platinum-palladium.

[f]Leaf tissue homogenized in NaDIECA + cysteine containing 0.01 M ethylenediaminetetraacetic acid (EDTA).

[g]This histogram not included; presented in ref. no. 10.

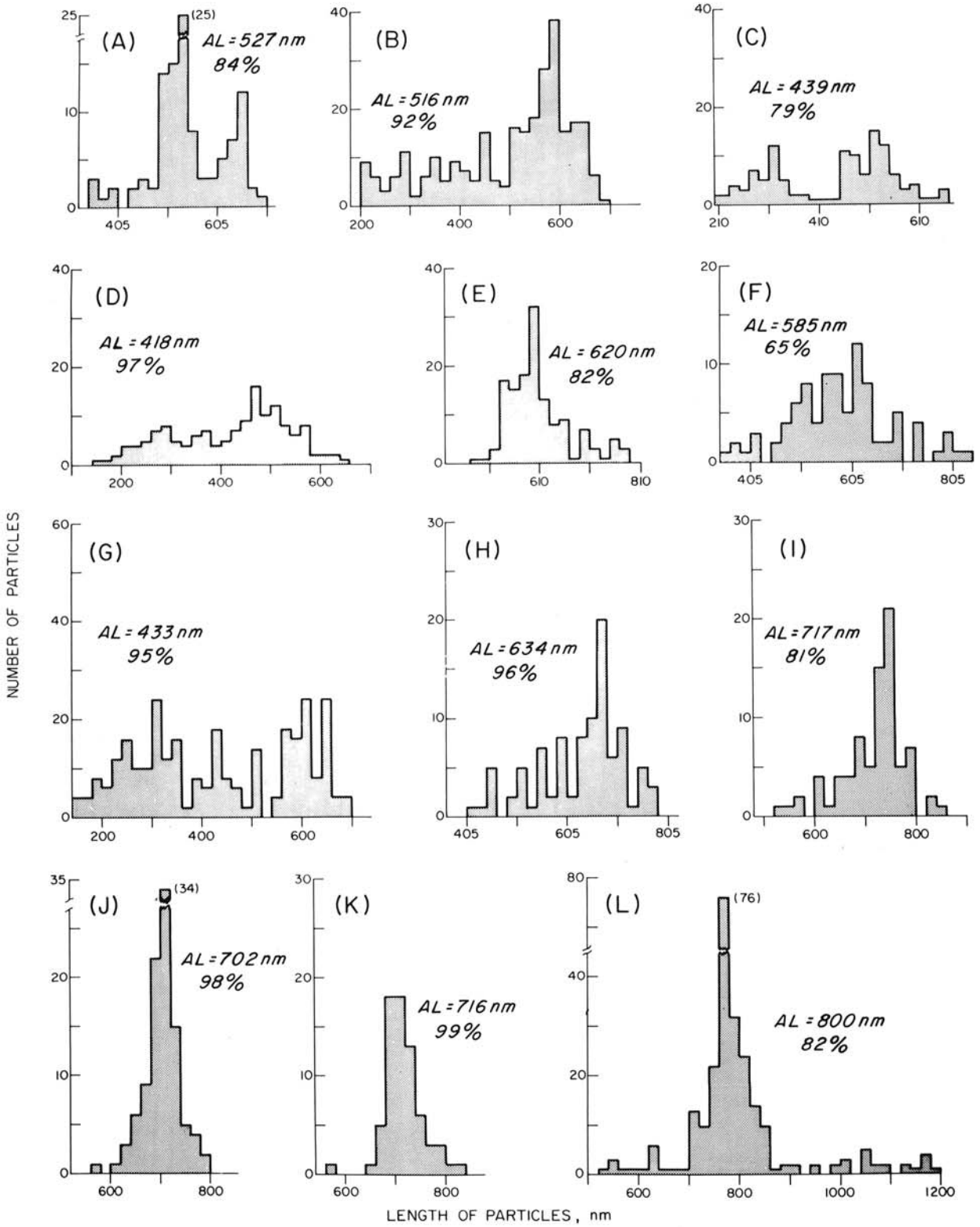

Fig. 1-(A to L). Histograms representing pea seedborne mosaic virus particle-length distributions. *Isolates.* Histogram A, field isolate PS-2. B through I and L, seed-derived isolate W-1 (ATCC No. PV 184). J and K, seed-derived isolate S-1. *Preparation Methods.* Histograms A, E, J, and K, derived from particles prepared from infected pea leaves by the leaf-dip method. B, C, and D, particles purified by method P_1 (see Table 1). F and G, particles purified by method P_2. H and I, particles purified by method P_3. L, particles purified by method P_4. *Fixatives.* Histograms A, B, E, F, G, and H, particles fixed with 2% formalin. C and D, particles fixed with 10% and 1% formalin, respectively. I through L, particles fixed with 3.5% glutaraldehyde. AL, average length of each histogrammed population (not major mode only). Percentages indicate the proportion of particles retained per histogram after removal of upper and lower tails of the distribution.

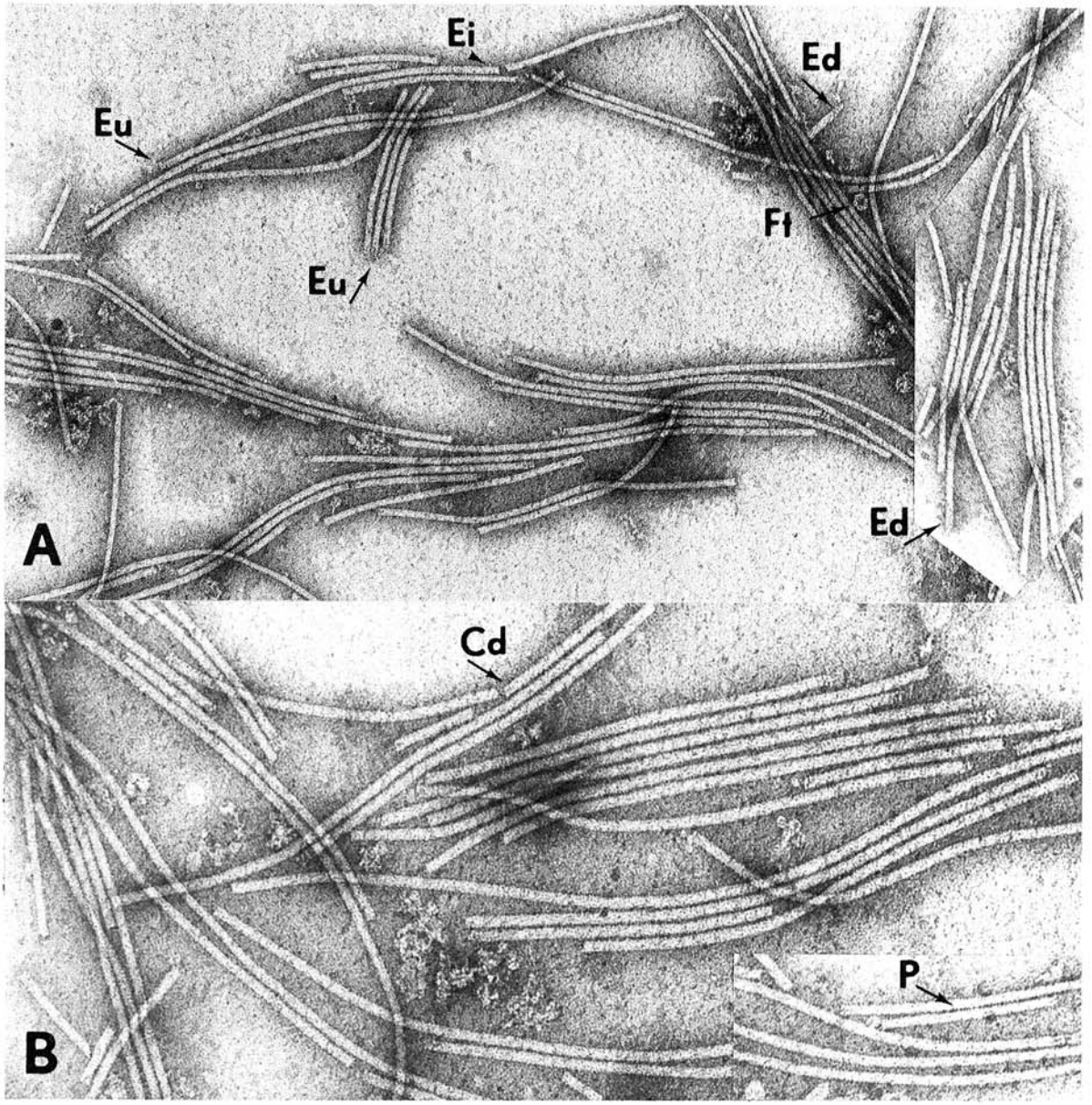

Fig. 2-(A, B). Pea seedborne mosaic virus particles partially purified by chloroform-butanol (method P_1). **A)** Most particles showed one intact, square terminal (E_1) and one unraveled (E_u) or degraded (E_d) terminal. Transverse views of well-preserved fragments (F_t) (ring of fragments), revealed the hollow particle structure (× 53,800). **B)** Partial degradation of the capsid (C_d) revealed a basic axial repeat of 34 ± 1Å (19) (× 108,200). Although protein subunits were not discernible at high magnification, peripheral periodicity (P) was apparent in some cases. Magnifications given include the inserts.

lines per inch). Tobacco mosaic virus (TMV) particles were repeatedly measured during the course of this study, with the length mode serving as a calibration reference. Photographic prints were made with a mercury lamp point light source enlarger.

RESULTS.—*Particle-length distributions.*—All field isolates initially tested (9) for particle length, except one (PS-2), were characterized in leaf-dip preparations by unimodal length distributions ranging between 500 nm and 600 nm. The bimodal particle length distribution shown by isolate PS-2 (Fig. 1-A) corresponded to the respective 517 nm and 650 nm particle lengths reported by workers in Oregon and Wisconsin (9, 17). The modal lengths of partially purified (Fig. 1-B) and leaf-dip (Fig. 1-E) preparations of isolate W-1 were mutually corroborative and verified previous (9) size estimates for this virus. Numerous separate, short (200- to 400-nm) particles in the former, however, suggested fragmentation during purification. Fragmentation in subsequent preparations was not reduced by varying the concn of

formalin fixative (Fig. 1-C, 1-D), by eliminating butanol from the program (Fig. 1-G), or by shadow-casting particles in place of PTA (Fig. 1-F); but was reduced by substituting sucrose-polyethylene glycol centrifugation (12, 13) for a cycle of differential centrifugation (Fig. 1-H), and by substituting glutaraldehyde for formalin as the virus-particle fixative (Fig. 1-I). Particle-length modes exceeding 700 nm were subsequently established for repeated leaf-dip preparations of isolate S-1 (Fig. 1-J, 1-K) and for isolate W-1 purified from leaf tissue (Fig. 1-L) and fixed with glutaraldehyde. Fixation by formalin substantially reduced PSbMV particle degradation in comparison with particles exposed to PTA without prior fixation, and it was therefore concluded that it provided insufficient fixation, rather than being deleterious in itself. Particles of PSbMV examined by Inouye (11) and of pea leafroll mosaic virus (assumed to be a strain of PSbMV) by Bos (1) comprised respective length distributions with single, dominant modes of 750 nm. The relative instability of U.S. isolates encountered in this and a previous study (9), therefore, suggests a distinction between our isolates and those reported elsewhere.

The modal length of TMV particles (at least 100 particles per trial), measured in four trials during the course of this study by means of our calibration standard, was 285 nm, 305 nm, 295 nm, and 295 nm (Fig. 1).

Ultrastructure of PSbMV particle fragments.—PSbMV particles comprising Fig. 1-C were examined electron-microscopically for possible visual evidence of induced fragmentation. Particles of the two distinct length modes showed degrees of terminal degradation varying from intact, square ends to unravelled ends lacking secondary structural integrity (Fig. 2). Particles of both the short (200-360 nm) and long (420-380 nm) modes were arranged by groups, respectively, with (i) two intact terminals, (ii) one intact and one degraded terminal, and (iii) two degraded terminals. Two-thirds of the particles in each mode showed one intact and one unravelled end, with the averages of their combined (short plus long) lengths approximating 790 nm. Only 1% of the particles showed two unravelled ends, the remainder having two intact ends. Based on the lengths of 87 particles having one degraded end, and assuming a relationship between breakage and terminal degradation, the average breaking point of particles in this preparation was 295 nm from one end and 495 nm from the other (ends not designated or known).

The longer mode of Fig. 1-C, indeed, approximated the particle length of 517 nm initially reported (9) for this virus.

A different type of breakage or structural anomaly may have been encountered with particles derived by purification Method P_3 and fixed with formalin (Fig. 1-H). In this case, the well-defined modal length of purified particles was 100 nm longer than that from dip preparations (Fig. 1-B or 1-E) and 100 nm shorter than that from preparations purified by Method P_4 (Fig. 1-L). Moreover, the length mode of particles purified by Method P_3 were 80 nm longer when fixed with glutaraldehyde (Fig. 1-H) before exposure to PTA than with formalin (Fig. 1-I). External structural periodicity of the viral capsid (Fig. 2-B) was observable in electron micrographs of intact particles, and was directly measurable on certain particles with partially degraded capsids (Fig. 2-B, see Cd). The basic repeating structure exposed on such particles comprised 34 ± 1Å intervals, which agrees with other members of the potato virus Y group measured by optical diffraction patterns and discussed by Varma et al. (19) (Fig. 2).

Preparatory methods.—Clarification procedures had a pronounced effect on both PSbMV particle length and infectivity (Table 1). The chloroform-butanol procedure (16) (method P_1) had the most deleterious effect on both characteristics. Preparations clarified by chloroform containing 0.1% Igepon T-73 (method P_2) still showed particles of various sizes if subsequently concd by two cycles of differential ultracentrifugation, but in contrast were five times more infectious than those from method P_1. When one cycle of sucrose-polyethylene glycol (PEG) centrifugation (13) was substituted (method P_3) for one differential centrifugation, fewer of the short particles were observed. Preparations clarified by one-half volume chloroform and concd by one cycle each of differential and of PEG-sucrose centrifugation (method P_4) were ten times more infectious than those from method P_1, and showed a marked particle-length mode of 770 nm when fixed with glutaraldehyde. Thus, infectivity of purified PSbMV preparations was correlated directly with increases in particle length and with enhanced unimodality. Sedimentation coefficients ($S_{20,w}$) (13) were unaltered by clarification or purification methods, being derived for four preparations (Table 1) by rate-zonal density-gradient centrifugation (3) as 147.9 ± 1.4 S (uncorrected for infinite dilution) (Table 1).

DISCUSSION.—Random points of breakage of virus particles would be expected to result in a random distribution of particle lengths. Distribution around two distinct modes, on the other hand, connotes breakage near a specific point. Specific-point breakage of PSbMV particles is suggested in Fig. 1-C and 1-D, with length modes approximating 300 nm and 500 nm. Independently of such breakage, presumably, we observed atypically short particles [590-nm modes for dip preparations (Fig. 1-A, 1-E); (675)-nm mode for P_3-purified particles (Fig. 1-H)], which were not accompanied by complementary fragments. The deviation of such particles from 750 nm or 770 nm (Fig. 1-I, 1-L) approximates the magnitude of pepper veinal mottle virus particle elongation induced by magnesium ions (8). In our studies (13), the presence of magnesium (0.02 M or 0.2 M $MgCl_2$) induced precipitation of purified PSbMV particles, which was reversible by addition of 0.01 M ethylenediaminetetraacetic acid (EDTA). We did not compare the lengths of particles before and after exposure to magnesium. Aside from a possible interaction between fixative agent and magnesium ions, however, the glutaraldehyde-affected "particle elongation" (Fig. 1-I vs. 1-H) observed in this study was probably unrelated to the effect of magnesium. There was no observable decrease in flexousness of glutaraldehyde-treated PSbMV particles, as opposed to magnesium-treated PVMV particles (8), but PSbMV particles may have been fixed in the flexuous state. The precedent that particle length of viruses of the potato virus Y group was reversibly increased by addition of magnesium ions (8) suggests, apart from particle

breakage, that fixative-related differences (d) in PSbMV particle length (d = 75 nm to 100 nm) may have reflected degrees of contraction of the virus-particle helix.

In the present study, the modal particle length derived for PSbMV from glutaraldehyde-fixed leaf-dip preparations (700 nm) agrees with that reported in Czechoslovakia for the pea leaf rolling virus (14), while those derived from glutaraldehyde-fixed purified preparations (750 nm, 770 nm) agree with particle lengths reported for PSbMV (11) and pea leafroll mosaic virus (1) in Japan and The Netherlands, respectively. Irrespective of the exact nature of particle-length anomalies associated with PSbMV preparation procedures, we believe the data imply a potential informational hazard for other members of the potato virus Y group. Viruses with particle lengths of 650 nm or 800 nm, as reviewed by Weintraub and Ragetli (20), but which induce formation of pinwheels, should perhaps be re-evaluated for particle-length artifacts and as possible members of the potato virus Y group.

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