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Physical Properties of New Jersey Serotype of Vesicular Stomatitis Virus and Its Defective Particles

(laser light scattering/defective interfering particles)

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ABSTRACT **The wild-type New Jersey serotype of vesicular stomatitis virus generated two types of defective interfering T-particles. The physical properties of these particles and the wild-type virion were determined by laser light scattering spectroscopy, sedimentation measurements, and electron microscopy.**

Defective interfering particles, generated by some animal viruses in tissue cultures, can be considered a special group of deletion mutants capable of interfering with viral replication (1). Their possible importance in certain persistent virus infection has been recognized recently (2). The length of the bullet-shaped, defective T-particles of vesicular stomatitis virus (VSV) decreases with the deletion of the virus RNA leading to a change in the sedimentation properties of the virus which allows the T-particles to be separated from each other and the virion (3). It has been shown that different temperature sensitive mutants generate T-particles of different sizes (4). Moreover, wild-type virions from various sources also differ in the size of the T-particles which they produce (5). Although the relationship between the mutation or the history of the wild type and the T-particle is not understood, the latter is quite characteristic and reproducibly generated by a given VSV virion (4, 5). We have recently applied laser light scattering spectroscopy in conjunction with sedimentation measurements in order to characterize the physical properties of some T-particles isolated from the Indiana serotype of VSV (6). Subsequent studies of the New Jersey serotype of VSV in our laboratories have shown that this virus also generates several types of T-particles. A detailed characterization of the properties of two of these particles generated by different wild-type isolates is reported in this communication.

MATERIALS AND METHODS

Purification of Virion and T-Particles. The wild-type Indiana and New Jersey serotypes of VSV, kindly supplied by Dr. C. R. Pringle, were propagated in BHK-21 clone 13 cells as described previously (4). The original stocks were clonally purified according to the method of Stampfer *et al.* (7). The final clones were enriched as described by Unger and Reichmann (8). The long T-particle was produced after the twenty-third serial undiluted passage of the virion plaque isolate. The New Jersey serotype short T-particle was produced after the third serial undiluted passage of the original crude stock.

Abbreviations: VSV, vesicular stomatitis virus; PTA, phosphotungstate.

The virus was purified as described previously (8), except that an additional sucrose density centrifugation over a 25 ml linear 15–30% sucrose gradient in 3 E buffer (0.12 M Tris·acetate, 0.06 M sodium acetate, 0.003 M EDTA, pH 7.4) in a Beckman SW 25.1 rotor at 22,500 rpm at 4°C for 90 min was carried out in order to minimize contamination of the T-particle zones with virion particles and, in addition, to free the particle preparations from aggregates. The upper halves of the final zones were removed and dialyzed overnight against 3 E buffer at 4°C. These preparations were used immediately for sedimentation, light scattering, and electron microscopy studies. Freezing was avoided in order to minimize disruption of particles.

Molecular Weight Determinations. The molecular weights of the virion and the T-particles were determined from sedimentation and diffusion measurements utilizing the standard equation (9):

$$M = \frac{SRT}{D(1 - \bar{v}\rho)} \qquad [1]$$

(R = gas constant, T = temperature in °K, D = diffusion coefficient, S = sedimentation coefficient, $\bar{v}$ = partial specific volume of the macromolecule, and ρ = solvent density). The sedimentation coefficients were determined in a Beckman model E analytical ultracentrifuge in the standard 12 mm cell at 15,220 rpm. The virion and T-particle solutions were in 3 E buffer (4) and photographs were taken with the UV optics. The partial specific volume, $\bar{v}$, was obtained by measuring the sedimentation coefficients of a given particle in two solvents of different densities. It was originally suggested by Schachman that D_2O was a suitable solvent for such measurements (10). The particles, originally in 3 E buffered H_2O were sedimented through 3 E buffered D_2O in a synthetic boundary cell at 17,250 rpm. Partial specific volumes were calculated by comparing sedimentation coefficients in H_2O and D_2O (10).

The diffusion coefficients were measured by quasi-elastic Rayleigh laser light scattering (11–13). The laser light being scattered from the solution of macromolecules is Doppler-broadened by Brownian motion. For spherical particles, the resultant spectrum has a Lorentzian line-shape with a half-width at half-height given by (14).

$$\Delta\nu_{1/2} = DK^2/2\pi \qquad [2]$$

$$K = \frac{4\pi n}{\lambda_0} \sin\left(\frac{\theta}{2}\right) \qquad [3]$$

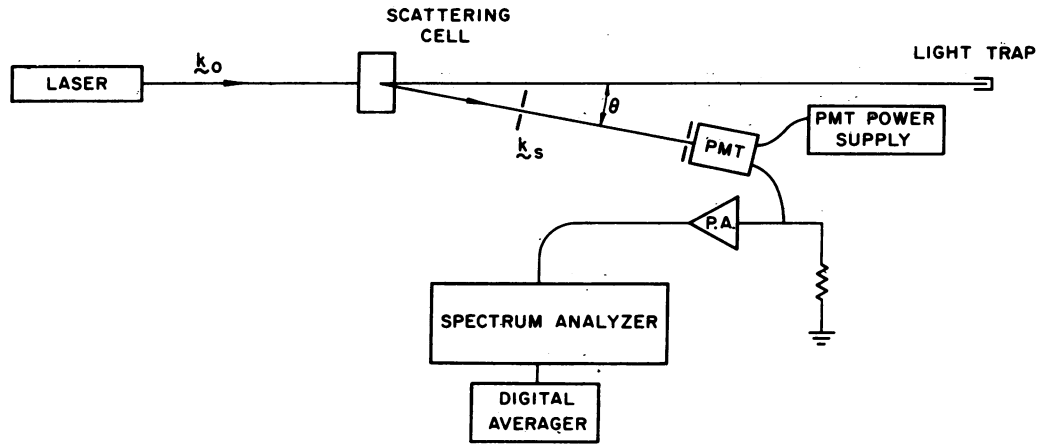

FIG. 1. Diagram of the light scattering apparatus.

where K is the magnitude of the scattering vector, n is the index of refraction of the scattering solution, λ_0 is the laser wavelength in vacuo, and θ is the scattering angle. Previous light scattering experiments on coliphages (15), the Indiana serotype of VSV (6), and on several spherical RNA viruses (16) indicated that the theory can be applied to particles with small axial ratios.

The light scattering apparatus used to measure the diffusion coefficients is shown in Fig. 1. The laser source was a Spectra-Physics model 165-03 Argon ion laser. The scattered light was collimated by pinholes and detected by an RCA 7265 phototube. The signal from the phototube was preamplified by a Princeton Applied Research model 113 preamplifier and the frequency analyzed by a Federal Scientific model UA 15A spectrum analyzer. The instrument was calibrated with 910 Å polystyrene latex spheres (PSLS) (Dow Chemical Co., Midland, Mich., run no. LS-1132-B). A $D_{20,w}$ value of $4.69 \pm 0.10 \times 10^{-7}$ cm²/sec was obtained from the data as shown in Fig. 2. This is in good agreement with the theoretical value of 4.71×10^{-7} cm²/sec calculated from the Stokes-Einstein equation (9).

Light scattering measurements were made only on freshly prepared samples which were always in 3 E buffer. Each sample was filtered into a clean light scattering cell through a 0.8 μm millipore filter treated with 0.1% bovine serum albumin solution to prevent absorption of virus particles on the filter (15). Multiple measurements were made at angles of 58.1°, 90.0°, and 121.9° for each sample.

Electron Microscopy. The size and shape of the New Jersey virion and its T-particles was examined by transmission electron microscopy, since homogeneity is prerequisite to reliance on the light scattering data. The particles were stained directly with potassium phosphotungstate (PTA) at pH 7.0 or after prefixation with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. The suspensions were deposited on carbon stabilized, parlodion coated, 300 mesh copper grids and examined in a JEM 100 C electron microscope at 80 KeV.

RESULTS AND DISCUSSION

Fig. 2 shows a plot of the spectral half-width, $\Delta\nu_{1/2}$, from the light scattering measurements as a function of the square of the scattering vector, K. Linear plots are obtained for all data and the diffusion coefficient, D, is obtained from the slopes according to Eq. [2]. Virus sample concentrations between 10 and 50 μg/ml were used and no concentration dependence was observed within this range.

Fig. 3 shows four electron micrograph plates. Plate 1 is the New Jersey virion stained with PTA. Severe disruption of the particles, with uncoiling of the ribonucleoprotein can be observed. Plate 2 is the same preparation of the New Jersey virion which has been prefixed in glutaraldehyde before staining. As shown in this micrograph, the particles have retained their integrity and were homogeneous with varying degrees of stain penetration being the only difference. The lower right hand corner insert of plate 2 shows a detailed enlargement of one of the particles. Plates 3 and 4 are the long and short T-particles, respectively, each having been prefixed with glutaraldehyde before staining. In each case the particles show good homogeneity. The lower right hand corner insert of plate 4 shows a detailed enlargement of one of the short T-particles. It is clear from the micrographs that PTA staining by itself can cause severe disruption of these particles. The micrographs

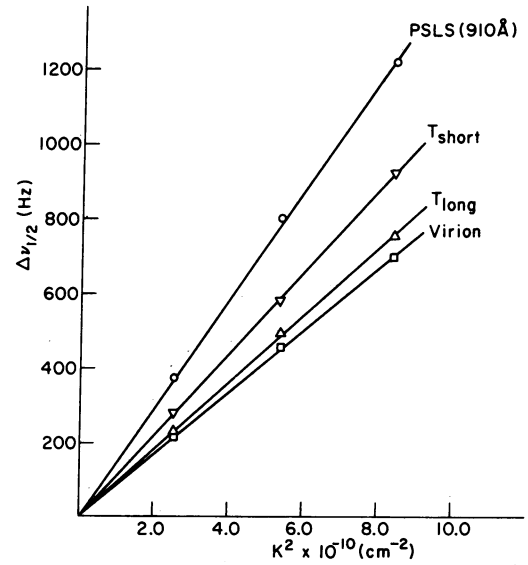

FIG. 2. A plot of the observed spectral half-widths from the light scattering data as a function of the square of the scattering vector. Data are given for polystyrene latex spheres (PSLS) and the New Jersey serotype of vesicular stomatitis virus (virion) and its defective T-particles. Each point represents the average of several points.

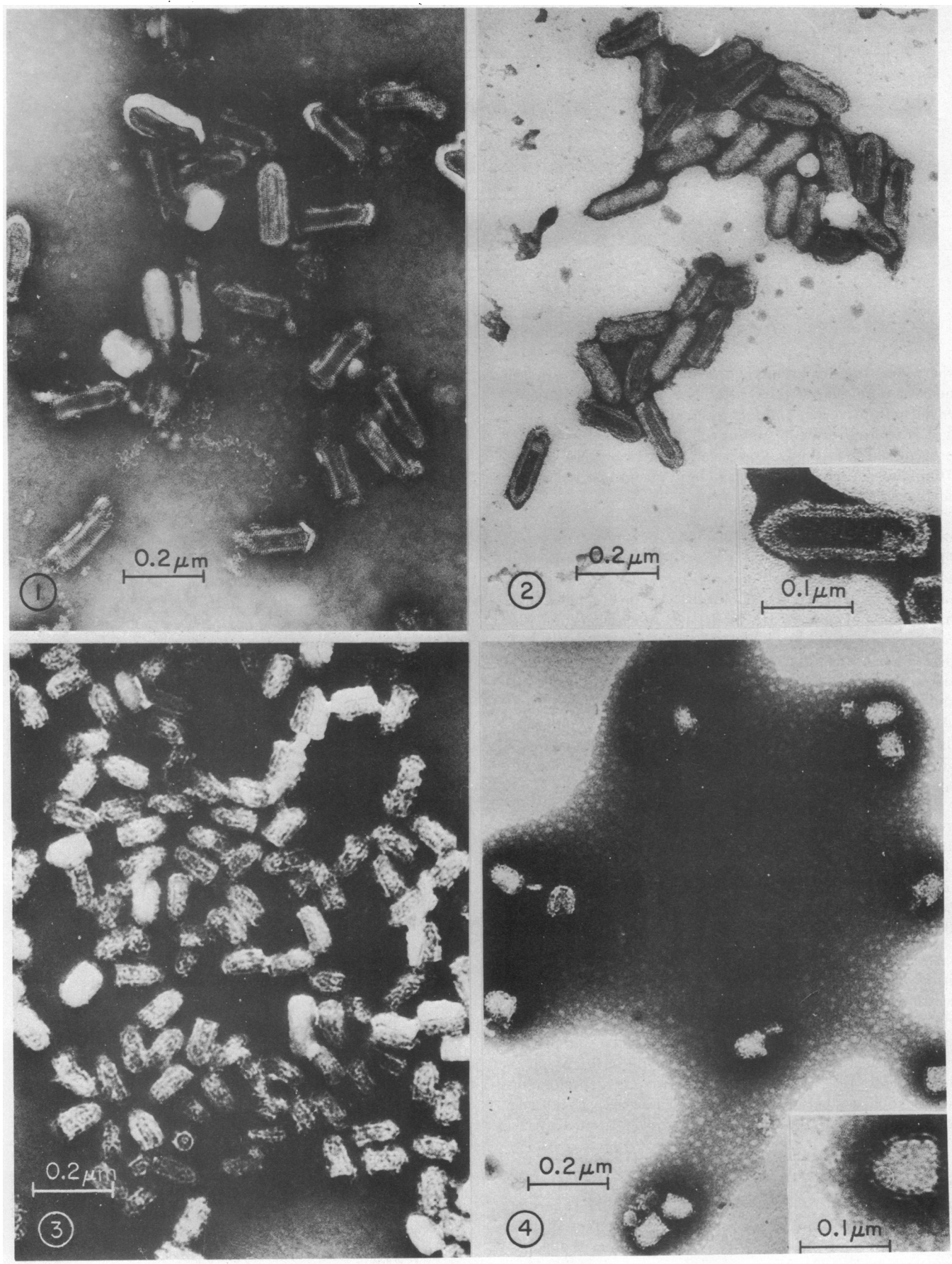

FIG. 3. *(Legend appears at top of next page.)*

FIG. 3 (*on preceding page*). Electron micrographs of the New Jersey virion and its defective T-particles. (1) Virion stained with potassium phosphotungstate (PTA). (2) Virion prefixed with 2% glutaraldehyde before staining with PTA. (3) Long T-particle prefixed with 2% glutaraldehyde before staining with PTA. (4) Short T-particle prefixed with 2% glutaraldehyde before staining with PTA.

TABLE 1. *Some physical properties of the Indiana virion and the New Jersey virion and its defective T-particles*

Particle	$D_{20,w} \times 10^8$ (cm²/sec)	$s_{20,w}$ (svedbergs)	$\bar{v}$ (cm³/g)	Molecular weight $\times 10^{-6}$	Size from electron micrographs (Å)	
					Length	Width
Indiana virion	2.69 ± 0.10	667 ± 20	0.83 ± 0.02	355 ± 45	1830 ± 80	760 ± 60
New Jersey virion	2.68 ± 0.10	657 ± 20	0.83 ± 0.02	351 ± 45	1820 ± 80	700 ± 60
Long T-particle	2.97 ± 0.18	523 ± 20	0.83 ± 0.02	252 ± 35	1350 ± 70	715 ± 60
Short T-particle	3.46 ± 0.14	393 ± 15	0.83 ± 0.02	163 ± 25	980 ± 60	740 ± 60

All error limits are double standard deviations except the electron microscopy data which are single standard deviations.

also clearly indicate that the individual preparations were homogeneous and should have yielded reliable values of the diffusion coefficient by light scattering measurements.

Table 1 is a summary of the results obtained by analyzing the light scattering, sedimentation, and electron microscopy data. The electron microscopy data were obtained by direct measurement of at least 50 particles. All of the data correlate well. Measurements that were made on the Indiana virion are also included in the table for the sake of comparison. The data show that the two virions are virtually identical in their gross physical properties. Previous determinations of the molecular weight of the Indiana virion resulted in a value of 2.9×10^8 (6). The difference between this result and the present result is largely due to small differences in the $\bar{v}$ values. Because of the low values of $1 - \bar{v}\rho$, a small error in $\bar{v}$ becomes highly significant in the molecular weight calculations. Both sets of data, however, are well within the overlapping experimental errors.

Previous investigations in the Indiana serotype have shown that the extent of the RNA deletion giving rise to a defective T-particle is reflected in its relative molecular weight (6). Studies of similar correlations between the RNAs of the New Jersey T-particles are currently in progress.

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