THE LENGTH OF THE INFLUENZA VIRUS SPIKES MEASURED BY PHOTON CORRELATION SPECTROSCOPY

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1. Introduction

The study of the spectra of intensity fluctuation of laser light scattered from the suspension of viruses provided a new tool for the investigation of their diffusion coefficients and, with some assumptions, their dimensions and shape in solution [1–8]. Recently, the technique of self-beating spectroscopy has been applied to the determination of the translation diffusion coefficients and average diameters of the myxoviruses (Sendai and influenza viruses) [9]. It was shown that samples of these viruses isolated and purified by usual methods (differential centrifugation and column chromatography) constitute a suspension of virus particles which are rather uniform in their dimensions and hydrodynamic properties. This fact allowed the measurement of the average diffusion coefficient and average diameter of virions in solution with good accuracy.

In this paper the diffusion coefficients and diameters of influenza virions and subviral particles after removing surface projections (spikes) by one of the proteolytic enzymes, bromelain, are measured. A combination of these values enables to calculate the length of the projections formed by two viral glycoproteins, hemagglutinin and neuraminidase.

2. Materials and methods

A/PR8/34 (HON1) and A/MRC-11 (H3N2) (the biological recombinant of A/Port Chalmers/1/73 and A/PR8/34) strains of influenza virus were propagated in the allantoic cavity of day 9 embryonated eggs. The allantoic fluid was harvested after 72 h of incubation at 37°C and cellular debris was removed by low speed centrifugation (2000 × g, 30 min). The influenza virus was pelleted from the allantoic fluid by centrifugation at 100 000 × g (rotor 35, centrifuge L5-65, Beckman, 4°C, 45 min), resuspended in phosphate-buffered saline (pH 7.2) and purified by one cycle of centrifugation in a sucrose gradient (20–60%) and by chromatography on a Sepharose-4B column (2 × 30 cm). The virus concentrate was then exhaustively dialyzed against a solution of 0.1 M NaCl + 0.02% NaN_3.

Bromelain-treated virus particles (subviral particles) were obtained essentially as in [10] employing the bromelain (Sigma) treatment of influenza virions and consequent purification by linear 25–60% sucrose gradient centrifugation. Smooth-surfaced particles (fig.1B) were collected as a fraction banding at 35% (w/w) sucrose concentration and were dialyzed against 0.1 M NaCl + 0.02% NaN_3.

The protein content in preparations of viruses and subviral particles was estimated by the method in [11] using BSA as a standard.

The SDS–10% polyacrylamide slab-gel electrophoresis was performed essentially as in [12]. Samples were electrophoresed for 16 h at 10 mA. The slabs were photographed, cut vertically into sections and analyzed by a recording reflection photometer EPI-65 'Carl Zeiss' (Jena, DDR).

For electron microscopy the suspensions of viruses and subviral particles were applied to carbon-coated

electron microscopic grids. The samples were stained with 1% uranylacetate in 20% methanol and examined in a JEM-100B 'Jeol' electron microscope with an instrument magnification of 50 000. Lengths (diameters) of viral and subviral particles were measured directly on magnified negatives. The digitizer was interfaced with a Hewlett Packard 9825A calculator and a 9862A plotter. Length distribution is presented in the form of histograms.

The light scattering measurements were performed by the Malvern type 4300 digital photon correlation spectrometer equipped with the Spectra-Physics He–Ne laser, model 124A, max power 18 mW (λ_o = 6328 Å) and the electronic temperature control unit, model RR 56.

The buffer viscosity with respect to that of water at 20°C ($\eta/\eta_{20,w}$ = 1.0317) was determined with a Zimm rotational viscosimeter as in [13].

In the case of monodisperse particles undergoing only translational diffusion during Brownian movement, the correlation function of the intensity fluctuation of the scattered light at homodyne detection has the form [14]:

$$R(rt) = B + A \exp - 2Grt)$$

where r is a number correlator channel, t is sample time, B defines the base line (zero correlation), A is a constant which depends on experimental conditions for a given run:

$$G = 1/\tau_c = Dq^2$$

where τ_c is correlation time, D is coefficient of translational diffusion and q is the magnitude of the scattering vector given by:

$$q = \frac{4\pi n}{\lambda_o} \sin \theta/2$$

In the last equation, n is the solution refractive index, λ_o is the wave-length in vacuum of the incident laser light and θ is the scattering angle.

In our experiments the correlation spectrometer data were analyzed by computer. The programme allowed to approximate the experimental data by one exponent and determine the correlation time τ_c, the diffusion coefficient of particles in water at 20°C – $D_{20,w}$ and an average diameter of particles using the Stokes-Einstein equation:

$$d = \frac{kT}{3\pi\eta D_T}$$

where k is the Bolzmann constant, T is temperature and η is the viscosity of the solution. The relative accidental error of the measurement of these parameters was ~1%. The calibration of the spectrometer and checking of the homodyne type of detection were made with Dow Chemicals latex (0.091 and 0.234) suspensions.

All parameters were determined for viral and subviral particles in 0.1 M NaCl + 0.02% NaN_3. Suspensions were subjected to low-speed centrifugation prior to spectroscopy measurements to remove possible aggregates.

3. Results and discussion

The influenza virions are roughly spherical particles (diam. 110–120 nm) with closely-spaced projections on the outer surface (spikes), formed by virus-specific glycoproteins, hemagglutinin and neuraminidase [15].

The hemagglutinin spikes are the rod-like projections 40 Å diam. and 140 Å length attached to the virion surface by interaction of the hydrophobic part of hemagglutinin with the lipid bilayer. These spikes are thought to consist of trimers of the HA-glycoprotein with mol. wt 80 000 [16].

The neuraminidase spikes are tetramers of glycosylated Na-polypeptide with mol. wt 55 000. The total length of neuraminidase spikes is ~140 Å [17].

Thus the neuraminidase and hemagglutinin spikes isolated from influenza virions by detergents have a similar molecular mass (210 000–240 000 daltons) and a similar length (140–160 Å). The resolution of modern electron microscopes does not allow to identify individual spikes on the virion surface, although the spikes themselves are distinguishable as a layer of surface projections (fig.1A).

The spikes can be removed from the influenza virions by treatment of virus suspension by proteolytic enzymes like bromelain, trypsin, chymotrypsin and

Fig.1A

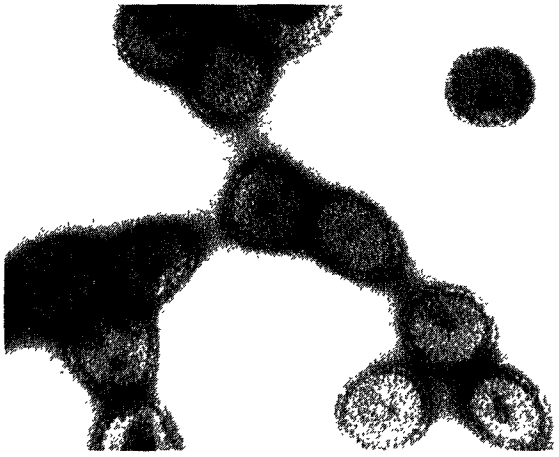

Fig.1B

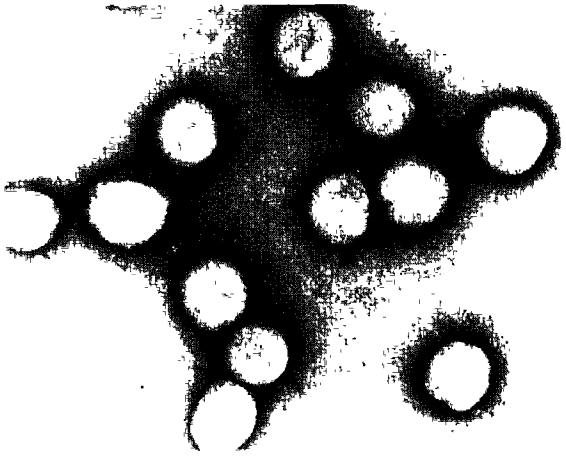

Fig.1. Virions (A) and bromelain-treated particles (B) of MRC-11 influenza virus negatively stained with 1% uranyl acetate. × 150 000.

pronase [14,16]. As a result of the interaction of these enzymes with virus particles, the surface glycoproteins can be either degraded or solubilized from the virions as relatively intact molecules.

In this paper we measured the average diameter of virus particles before and after removal of the hemagglutinin and neuraminidase spikes by bromelain to determine their length.

Special precautions were used to check the completeness of the removal of the spikes under our experimental conditions. For this purpose we used electron microscopy (fig.1) and electrophoresis in polyacrylamide gel. Figure 2 represents the results of virus and subviral particle electrophoresis. The preparation of subviral particles did not contain HA_1, HA_2 and NA polypeptides but contained the internal proteins of influenza virion – the matrix protein (M), the RNP protein (NP) and polymerases (P).

Figure 3 represents the correlation curves of intensity fluctuation of laser light scattered by influenza virions and subviral particles (MRC-11). The experimental data are rather close to the single exponential curve. This reflects the relatively high degree of monodispersity of our samples.

Table 1 shows the results of the determination of diffusion coefficients and average diameters of virus and subviral particles of both virus strains used. The length of the spikes (L) was calculated by the formula:

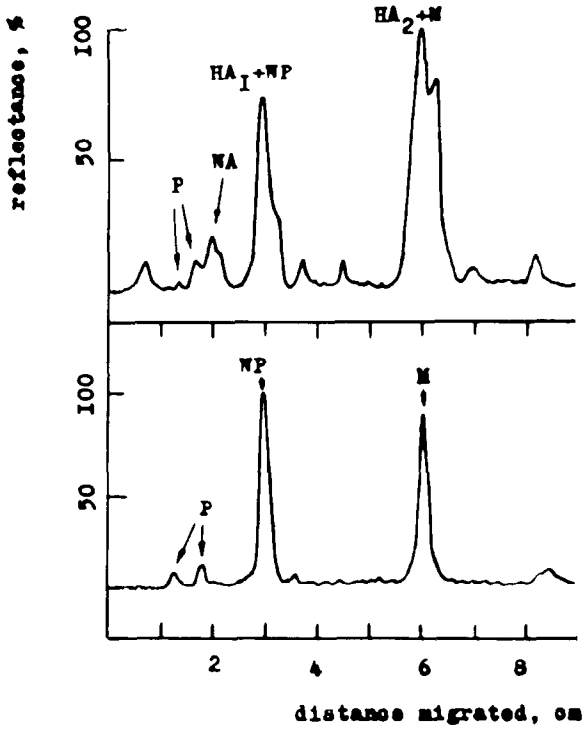

Fig.2. SDS–polyacrylamide gel electrophoresis analysis of polypeptide components of virions (top) and bromelain-treated particles (bottom) of MRC-11 influenza virus. *Abbreviations*: HA_1, HA_2, heavy and light chain of hemagglutinin polypeptides, respectively; NA, neuraminidase polypeptide; NP, nucleoprotein; M, matrix protein; P, polymerases.

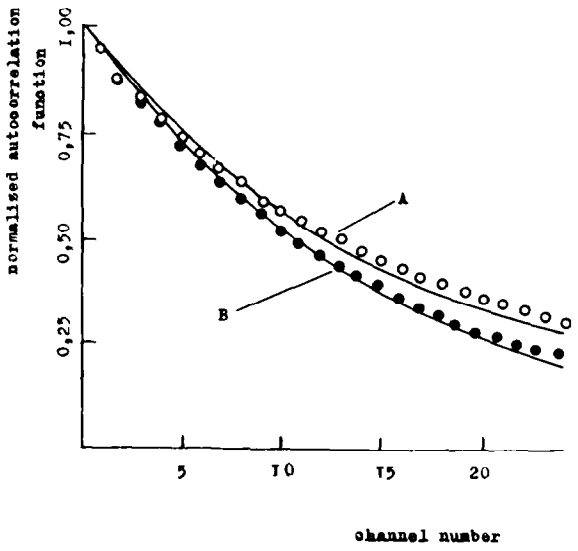

Fig.3. The autocorrelation function for laser light scattered from virus (A) and subviral particles (B) of MRC-11. The curves represent data calculated by a computer for monodisperse particles. The scattering angle $\theta = 90°$, the wavelength of incident laser light $\lambda_0 = 6328$ Å.

$$L = (d_V - d_{SV})/2$$

where d_V and d_{SV} are the diameters of virus and subviral particles, respectively.

The data of length measurements of 'complete' (isolated by detergents) and 'incomplete' (isolated by enzyme solubilization) hemagglutinin of influenza virus by electron microscopy have been reported in [18]. These values were 160 ± 15 Å and 125 ± 10 Å, respectively. Our values, 120 ± 11 Å (MRC-11) and 110 ± 13 Å (PR-8), are very close to the length of 'incomplete' hemagglutinin measured [18].

It is reasonable to try to calculate the depth of penetration of influenza spikes into the lipid bilayer by using the length of 'complete' hemagglutinin from [18] and the value of average length of spikes (115 ± 13 Å), obtained in this work. However, the large error of this calculation (we obtained 45 ± 28 Å) did not allow the estimation of exactly how deep the spikes penetrate into the lipid shell of influenza virus.

It was interesting to compare the diameters of influenza virions and the bromelain-treated particles measured by photon correlation spectroscopy and by electron microscopy. For this purpose we analyzed the electron microscopic photographs of our preparations by the Hewlett-Packard calculator. The results (histograms) are presented in fig.4. The average diameters of the influenza viruses and those of the subviral particles are somewhat smaller than the corresponding values measured by photon correlation spectroscopy and there is some noticable scatter of the particle diameters. These facts probably can be explained by the different level of the dehydration of the different virions and subviral particles due to the negative staining technique employed. Nevertheless, as expected, spike length determined by electron microscopy (110 ± 25 Å)* is rather close to the corresponding value measured by photon correlation spectroscopy.

* The given value is the difference between the mean lengths of the viral and subviral particles respectively within the corresponding peak fractions of each histogram

Table 1
The translation diffusion coefficients and average diameters of influenza virions and subviral particles and the length of influenza virus (MRC-11 and PR-8) spikes

Particles	Suspension (mg/ml)	$D_{20,w} \times 10^7$ (cm^2/s)	d (Å)	L (Å)
Virions MRC-11	0.3	0.346	1197	120 ± 11
Subviral particles MRC-11	0.3	0.433	957	–
Virions PR-8	1.0	0.293	1415	110 ± 13
Subviral particles PR-8	1.0	0.347	1194	–

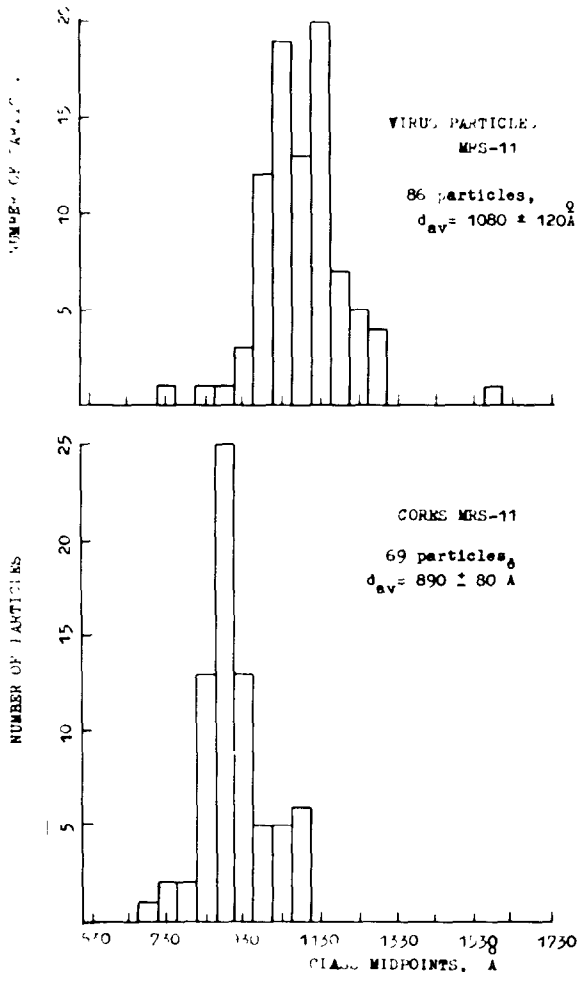

Fig.4. Length (diameter) distribution of influenza MRC-11 virions and corresponding subviral particles obtained during analysis of electron microphotographs by an H-P 9825A (Hewlett Packard) calculator.

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