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Some Physicochemical Properties of Erysimum Latent Virus

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Abstract

Sedimentation velocity, diffusion coefficient and sedimentation equilibrium measurements gave a molecular weight of $5 \cdot 90 \times 10^6$ for the intact Erysimum latent virus. The molecular weight of the empty shell was estimated to be $3 \cdot 92 \times 10^6$ and the protein subunit to be 21 600. The RNA content calculated from the molecular weights of the full and empty particles is 33%, in agreement with that estimated from the buoyant density in caesium chloride. However, a direct phosphorus determination gave an RNA content of only 28%.

Introduction

Erysimum latent virus (ELV) is a definitive member of the tymovirus group (Shukla and Gough 1980) and many of its properties have already been investigated (Shukla and Schmelzer 1972; Shukla *et al.* 1973, 1975, 1980). However, unlike other tymoviruses, ELV is not serologically interrelated (Shukla *et al.* 1980). To assist in the elucidation of the three-dimensional structure of the virus (cf. Colman *et al.* 1980) and its serological affinities, the molecular weight of ELV and its components have been determined by sedimentation velocity, diffusion coefficient, and sedimentation equilibrium methods. The results of these determinations are reported here and also other physicochemical properties of the virus.

Materials and Methods

ELV was propagated in chinese cabbage and purified by the butanol–chloroform method described previously (Shukla *et al.* 1973). The coat protein subunit was isolated using 60% (v/v) acetic acid (Shukla *et al.* 1980). Analytical ultracentrifugation experiments were carried out at 20°C with a Beckman model E ultracentrifuge equipped with schlieren and Rayleigh interference optics. Sedimentation velocity experiments were performed at 22 000 rpm and sedimentation coefficients were corrected to the density and viscosity of water at 20°C. Diffusion coefficients were measured in the ultracentrifuge at 2 800 rpm by applying the conventional reduced height–area method to the spreading of a preformed boundary in a synthetic boundary cell.

Molecular weights of the virus were measured in 0·05 M sodium phosphate buffer, pH 7·0, by the meniscus-depletion method (Yphantis 1964) using solution column heights of 3 mm in regular double sector cells and the fluorocarbon FC43 (Beckman) as a base fluid. Speeds of 2 400–4 000 rpm were employed and for increased stability at these low speeds, the heavy An-J rotor was used, and 10% (w/v) sucrose was incorporated in all solutions: the equilibration time was 72 h. The molecular weight of the protein subunit was determined in 8 M urea, 0·05 M sodium phosphate buffer, pH 7·0,

at 44 000 rpm. The photographic plates were measured with the aid of a Nikon measuring microscope. The computer program of Roark and Yphantis (1969) was used to calculate the number-, weight-, and *Z*-average molecular weights for points along the solution column in the meniscus-depletion experiments.

Apparent partial specific volumes were determined from the densities of dialysed purified bottom component (infectious nucleoprotein) and its diffusate. The densities were measured at $20 \pm 0{\cdot}02$°C with a precision density meter DMA-02C (Anton Parr, Graz). Protein concentrations were determined from the dry weights of the solution and its diffusate as described by Woods (1979). The absorbance and specific refractive index increment were determined on the same solutions as used for the partial specific volume measurements. Insufficient top component (empty protein shell) was available for partial specific volume measurements, so the value was calculated from the amino acid composition of the purified protein subunit (Shukla *et al.* 1980). This value was also used for calculating the molecular weight of the protein subunit in 8 M urea.

Buoyant densities of the top and bottom components were determined by equilibrium centrifugation in caesium chloride carried out at 25°C in the analytical ultracentrifuge with schlieren optics. A single sector, 12 mm thick, aluminium centerpiece was employed at a speed of 52 000 rpm. One experiment was carried out using a carbon-filled epoxy double sector centerpiece and a Beckman photoelectric scanning absorption optical system. Identical results were obtained with both optical systems. The solution was buffered with 0·01 M sodium phosphate, pH 6·5, and the pH was measured also at the conclusion of the run. The mean pH was $6{\cdot}50 \pm 0{\cdot}05$. The initial densities of the caesium chloride solutions were measured with a precision density meter. The preparation of the solutions, conduct of the experiment, and the calculations were carried out as described by Ifft (1969). The buoyant densities were corrected to atmospheric pressure.

The molecular weight of the protein subunit was also determined by electrophoresis in polyacrylamide gel containing 0·1% (w/v) SDS (SDS-PAGE) (King 1970) using polyacrylamide concentrations of 11, 12, 12·5, 13, 13·5 and 14% (w/v). Protein kinase, bovine serum albumin, ovalbumin, carbonate dehydratase, trypsin inhibitor and α-lactalbumin (Pharmacia) were used as standards. The gels were run until the bromophenol blue was close to the end of the gel. The position of the bromophenol blue was marked with nichrome wire and the gels stained overnight with 0·02% (w/v) Coomassie Blue G (ICI, Australia) in 50% (v/v) methanol, 5% (v/v) glacial acetic acid. Destaining was carried out in 7·5% (v/v) glacial acetic acid, 7·5% (v/v) methanol using dye-absorbent pads (Gradipore). The mobilities of the proteins, relative to the bromophenol blue, were calculated and the data analysed using the Ferguson plot (Ferguson 1964; Frank and Rodbard 1975).

The isoelectric point of the bottom component was determined by iso-electric focussing in 4% (w/v) polyacrylamide gels. The gels were prepared and run by the method of Rice and Horst (1972). Staining and pH measurements were done according to Marshall and Blagrove (1979).

Phosphorus determination (Fogg and Wilkinson 1958) was carried out on samples of the bottom component which had been freeze-dried after dialysis against several changes of 1 M NaCl and deionized water. Using the phosphorus value obtained, the RNA percentage was then calculated, utilizing the base composition of ELV RNA (Shukla *et al.* 1980).

Results and Discussion

Fig. 1*a* shows the sedimentation velocity pattern of an ELV preparation. The faster moving peak corresponds to the bottom component and the slower moving peak to the top component of the virus; this is a characteristic feature of the tymoviruses (Koenig and Lesemann 1979). From the areas of the peaks corrected for radial dilution and assuming equal refractive index increments for the two components, the top component corresponds to 18·5% of the purified viral preparation. The sedimentation coefficients as a function of protein concentration are shown in Fig. 2 for the two components. The value of $S_{20,w}$ at zero concentration for the bottom component (113·1 S) is in good agreement with that (113·4 S) previously obtained (Shukla *et al.* 1973).

The top and bottom components were purified by centrifugation in a sucrose density gradient (Shukla *et al.* 1973). The density-gradient centrifugation was repeated

until the purified components gave single peaks when examined by sedimentation velocity at concentrations of $>1\%$ (w/v). Electron microscopy of the purified components showed them to be free of each other. The purified bottom component was quite stable and showed no evidence of top component after storage at 4°C in phosphate buffer containing 0·02% (w/v) sodium azide for up to one year. The $S_{20,w}$ values at zero concentration of the purified components were identical to those determined in the mixture (Fig. 1*a*), indicating that no changes had occurred during the fractionation procedure.

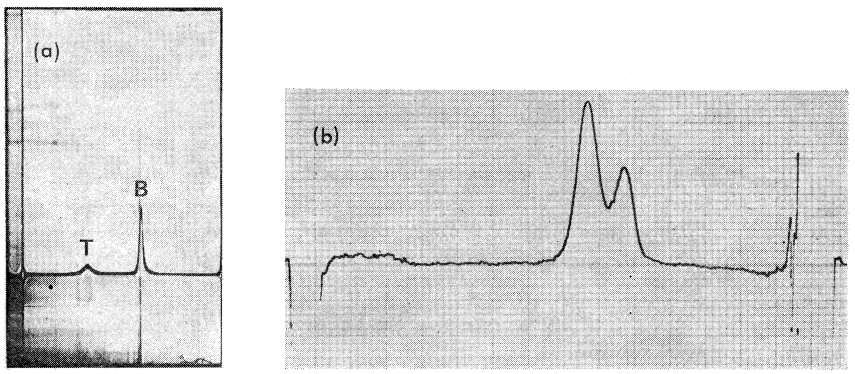

Fig. 1. (*a*) Sedimentation velocity pattern of an unfractionated ELV preparation showing the top (T) and bottom (B) components. Sedimentation is from left to right. (*b*) Photoelectric scanning trace (265 nm) of ELV bottom component at equilibrium in caesium chloride of initial density 1·4288 g/ml, 0·01 M sodium phosphate, pH 6·5, at 52 000 rpm and 25°C. Sedimentation from left to right, buoyant densities being 1·412 and 1·426 g/ml, respectively.

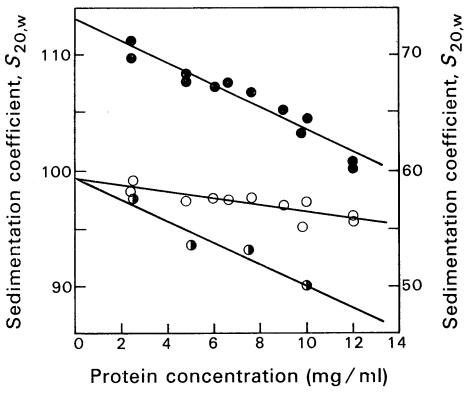

Fig. 2. Sedimentation coefficient as a function of initial protein concentration. ● Bottom component (left ordinate). ○ Top component (right ordinate) in an unfractionated ELV preparation where the concentration is the total of top and bottom components. ◑ Purified top component (right ordinate).

Isopycnic centrifugation of the bottom component in caesium chloride showed two closely spaced bands, the less dense being present in greater amount (Fig. 1*b*) and a minor band of higher buoyant density which was only detected at high initial virus concentrations. The top component also gave two closely spaced bands and several minor bands of higher buoyant density when subjected to centrifugation in caesium chloride. Double-banding in caesium chloride density gradients has been observed with turnip yellow mosaic virus, cowpea mosaic virus, and radish mosaic

virus and is discussed in more detail by Matthews (1974). The presence of double bands in the top component suggests that they are due to some property of the protein shell rather than the RNA and this could be due to species with different binding capacities for caesium ions. However, in the case of radish mosaic virus, double-banding occurred in caesium chloride gradients but not in rubidium bromide gradients (Matthews 1974). It is important to know the effect of any density heterogeneity on the physicochemical characterization of the virus. If two species of the same molecular weight existed in dilute phosphate buffer with a difference in densities the same as that found in caesium chloride then estimations of the buoyancy factor show that their sedimentation coefficients would differ by about 5% and their apparent molecular weights by the same amount. Such heterogeneity would not be evident in a conventional sedimentation-velocity experiment and would be barely detectable in a meniscus-depletion, sedimentation-equilibrium experiment.

Table 1. Some physiochemical properties of Erysimum latent virus

All measurements were done in 0·05 M sodium phosphate, pH 7·0, except the protein subunit which was studied in 8 M urea in 0·05 M sodium phosphate, pH 7·0, and the buoyant-density experiments which contained 0·01 M sodium phosphate, pH 6·5

Property	Bottom component	Top component	Protein subunit
Sedimentation coefficient, $S_{20,w}$, at infinite dilution (S)	113·1	59·2	
Diffusion coefficient, $D_{20,w}$ (cm^2/s)	$1·35 \times 10^{-7}$	$1·40 \times 10^{-7}$	
Isoelectric point	$5·40 \pm 0·05$		
Absorbance(A)[A]	9·7 (260 nm)		0·48 (276 nm)
$A_{260\ nm}/A_{280\ nm}$	1·76		
$A_{260\ nm}/A_{276\ nm}$			0·74
Refractive index increment (ml/g)	0·170		
Partial specific volume (ml/g)	0·684		0·732
Buoyant density in CsCl (g/ml)[B]	1·412, 1·426	1·256, 1·262	
Molecular weight:			
Sedimentation equilibrium	$5·85 \times 10^6$	$3·77 \times 10^6$	21 600
Svedberg equation[C]	$5·96 \times 10^6$	$3·83 \times 10^6$	
No. of subunits in the particle (180) × molecular weight of subunit		$3·92 \times 10^6$	
SDS-PAGE (Ferguson plot)			19 400

[A]Concentration 1 mg/ml, 1 cm light path.
[B]Values for the two major bands are reported.
[C]The value of $S_{20,w}$ at 7 mg/ml was used since $D_{20,w}$ was measured at this concentration.

The molecular parameters of the bottom and top components are given in Table 1. The meniscus-depletion experiments showed constancy of the molecular weight along the solution column indicating a high degree of homogeneity of both components. The diffusion coefficients of the bottom and top components are similar, as expected, since electron micrographs of full and empty particles show that the diameters are identical, and no difference can be detected in the particle shape. Molecular weights from the Svedberg equation are given in Table 1 and these are in good agreement with those from the sedimentation-equilibrium data. Other methods of molecular weight calculation from the sedimentation coefficient alone do not give such good agreement. Thus using the average virus particle diameter of 26·5 nm (Colman *et al.* 1980) to calculate the frictional coefficient from Stoke's law for spherical particles

gives values which are 10–15% lower. This suggests that the hydrodynamic radius is larger than the value determined from shadowed electron microscope preparations. Empirical relationships between molecular weights and sedimentation coefficient are often used to calculate molecular weights. The application of equation (9) of Squire and Himmel (1979) to our data gives a molecular weight for the bottom component in agreement with the sedimentation-equilibrium and Svedberg values, but the value for the top component was 25% less. The frictional ratios, f/f_0, calculated by means of the usual equations, are 1·23 for the bottom component and 1·47 for the top component. We may consider f/f_0 to be the product of two terms (Oncley 1941), f/f_e, a term due to hydration, and f_e/f_0, a term due to shape. The virus particles are polyhedral and close to spherical so we may assume f_e/f_0 to be 1, then the hydration factor may be calculated from the equation (Oncley 1941):

$$w = [(f/f_e)^3 - 1]\,\bar{v}\,\rho,$$

where w is the hydration expressed as grams of water bound by 1 g protein, $\bar{v}$ is the partial specific volume of the protein, and ρ is the density of water. The value of w for the bottom component is 0·59 g water per gram of protein which is very close to the average value for proteins (Squire and Himmel 1979). The value for the top component is 1·59 g water per gram of protein. This is greater than values found for most proteins and also the increase in hydration over that for the virus is greater than can be accounted for by the space formerly occupied by the RNA. It is not surprising that a hollow protein shell would have unusual properties. A procedure for the determination of RNA content of spherical viruses from the sedimentation coefficients of full and empty particles (Reichmann 1965) is therefore of doubtful validity since one of the assumptions is that the full and empty particles have the same shape and hydration.

The molecular weight of the ELV protein subunit (Table 1) obtained by sedimentation equilibrium (21 600) is in excellent agreement with values obtained from SDS-PAGE in 7·5% (w/v) polyacrylamide (21 700) and in amino acid analysis (21 770) (Shukla *et al.* 1980). However, a more extensive investigation has shown that the molecular weight determined in SDS gels is dependent on the acrylamide concentration. Application of the Ferguson plot gave 19 400 for the molecular weight which is about 10% lower than the other values. Combination of the molecular weight calculated from the amino acid analysis with our value of $3{\cdot}8 \times 10^6$ for the top component gives 175 subunits. The virus particles are polyhedral and if we assume icosahedral symmetry (Colman *et al.* 1980) then crystal symmetry dictates that there should be a multiple of 60 subunits (Caspar and Klug 1962). Hence we can assume 180 subunits and a top component molecular weight of $3{\cdot}92 \times 10^6$.

If the molecular weights of the bottom and top components are taken as $5{\cdot}90 \times 10^6$ (average) and $3{\cdot}92 \times 10^6$, respectively, then the molecular weight of the ELV RNA is $1{\cdot}98 \times 10^6$ and comprises 33·6% of the virus. The RNA content can also be calculated from the empirical equation (Sehgal *et al.* 1970) which relates buoyant densities in caesium chloride to RNA contents. Thus, ELV with a buoyant density of 1·42 g/ml would be expected to have an RNA content of 32%. The direct determination of phosphorus on the bottom component gave values of 2·7 and 2·8% from two different virus preparations. This gives an RNA content of about 28% which is significantly lower than the values calculated by indirect methods. Using indirect methods for calculating the percentage of RNA, values for 12 different

tymoviruses have been found to range from 32 to 38% (refer Koenig and Lesemann 1979). In view of the differences in values of percentage RNA between the direct phosphorus determination and indirect methods obtained in this paper, the indirect methods should be viewed with some reservations.

The physicochemical properties of ELV (Table 1) are very similar to those of other tymoviruses except for the $S_{20,w}$ value of the top component (59·2 S) which is higher than for any other tymovirus (Koenig and Lesemann 1979).

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