Determination of the sedimentation coefficient of vaccinia virus DNA by analytical ultracentrifugation

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ABSTRACT

By releasing intact molecules of vaccinia virus DNA, it has been possible to determine the sedimentation coefficient by analytical ultracentrifugation. The value found was 92S, which corresponds to a molecular weight of 161×10^6 .

The purpose of this paper is to report a determination of the sedimentation coefficient of intact DNA molecules from vaccinia virus by analytical ultracentrifugation.

The release of pox virus DNA molecules with a high molecular weight has been achieved only for the Fowl Pox virus.¹ The facility with which this DNA is released by SDS can be accounted for by the high-content in lipids in the coat of this virus. For mammalian pox virus, the best purified DNA preparation present sedimentation coefficients equivalent to half the estimated molecular weight.²

In order to study intact DNA molecules, it was necessary to avoid shearing by suppressing all manipulation after releasing the DNA. Therefore the release was done either on the top of sucrose gradients according to Sarov & Becker³, in the pit of band forming center pieces for analytical ultracentrifugation or in dialysis bags (for removal of SDS) for subsequent observation by electron microscopy. Equal volumes of vaccinia virus suspension and DNA releasing solution (1 mg/ml pronase B, 0.6 % SDS, 0.002 M EDTA 0.2X SSC at pH 7.4) were carefully brought into contact. Mixing was achieved by diffusion over night at 37°C.

The efficiency of the isolation procedure was tested on a sucrose gradient (Fig. 1) using virus labelled in the DNA and proteins. The sedimenting peak of DNA is free from protein contamination. And at equilibrium, in CsCl (40,000 rpm for 48 hr at

25°C, in a rotor AND of Spinco Analytical Ultracentrifuge model E the buoyant density was found to be for vaccinia virus DNA released in the centrifugation cell, the same as the one found for DNA extracted and purified as described previously.⁴

The sedimentation experiments on the viral DNA released in the sample pit of a band forming center piece were done according to Bruner & Vinograd⁵ using a CsCl bulk solution. For experiments the sample pit received from 10 to 20 μ l of a virus suspension corresponding to 54 μ g DNA/ml and the same volume of releasing solution, before the cell is completely assembled. After an over night incubation at 37°C, the sector was filled with the CsCl bulk solution (0.01 M Tris, pH 7.4, 0.001 M EDTA and CsCl), and the cell was centrifuged at 32,000 rpm, 20°C. The linearity of the graph $\lg r$ versus time is a good evidence for the homogeneity of the sedimenting DNA in the bulk solution. The results of experiments are summarized in Fig. 2. The values ($S^\circ \eta r \times \rho$) submitted to a linear regression gave a regression coefficient of -0.997, corresponding to a value of the standard sedimentation coefficient ($S^\circ 20_w$)w: 92.02 $\pm$ 0.48 (*) S for CsDNA. The calculated values of the graphic parameters for vaccinia virus and E. coli DNA used to control the technique are summarized in table I.

Table I. Graphic parameters of S

DNA origin	($S^\circ 20_w$)w	$\bar{v}_s$	$\bar{v}_s/Nf_s$	$\bar{M}_s/Nf_s$	$\rho^\circ = 1/\bar{v}_s$	Buoyant density
E. coli	36.33	0.586	51.517	87.846	1.705	1.7035
Vaccinia virus	92.03	0.618	149.132	241.161	1.617	1.6948

$\bar{v}_s = \bar{v}_s M_s$ = partial molecular volume of solvated molecules

Two points should be mentioned for vaccinia virus DNA : First, the difference between ρ° and the buoyant density, and second, the relation between the sedimentation coefficient and the molecular weight.

With regard to the first point, it has been previously reported that a difference exists between the observed buoyant density and the extrapolated value for various DNAs. That is, the graph of $S^\circ \eta r \times \rho$ is linear over a wide density range but

Fig. 1. Sucrose gradient (15-30 % w/w, 0.005 M Tris, pH 7.3, 0.1 M NaCl, 0.5 % SDS) centrifugation for 5 hr at 23,000 Rev/min. in a SW 50, 1 rotor at 20°C, of DNA released from purified ^3H tdR, ^{14}C A.A. labeled vaccinia virus, incubated over night with the releasing solution. The 43 fractions were precipitated onto filters with cold 5 % Trichloroacetic acid.

Fig. 2. Graph values $S^\circ \eta r \times \rho$:

$S^\circ \eta r$ versus ρ is a linear function of the density over a wide range of densities

$$S^\circ \eta r = \frac{M_s}{N f_s} (1 - \bar{v}_s \rho)$$

When S° , M_s , $\bar{v}_s$, f_s are the observed sedimentation coefficient, the molecular weight, partial specific volume and frictional coefficient of the solvated species, and ρ , ηr and N are the density and relative viscosity of the solvent and Avogadro's number.

The extrapolated value at $\rho = 1$ is $(S^\circ 20_w)_w$ Standard sedimentation coefficient of the Cs DNA salt and at $S^\circ \eta r = 0$, $\rho = \rho^\circ = 1/\bar{v}_s$ buoyant density.

it does not extrapolate to the correct buoyant density.⁵ The trivial hypothesis of protein contamination can be excluded because such a difference of density would correspond to 15 % w/w proteins. This should have been detected during sucrose gradient sedimentation (Fig. 1) and it would have given a non linear time-distance relation, due to salting out of proteins linked to DNA. An other hypothesis should be an interaction of SDS, CsCl, and DNA. But Smart and Bonner⁶ show that ADN dehis-tonized either by salt concentration or by detergent has the same sedimentation coefficient.

Other hypotheses could be formulated. One, the most probable, is that $\bar{v}_s$ and f_s deviate at salt concentrations approaching the buoyant density. An other is that moving and non-moving (eg. at equilibrium) molecules are solvated differently. Still a third is that the anomalous behaviour derives from the fact that the DNA is present in "State III", as suggested by Peters & Müller.⁷ This state is too fragile to be seen by the Kleinschmidt method or during equilibrium ultracentrifugation.

Concerning the second point, the relation between S and M is standardized for the size of the studied molecules by two formulas :

$$S = 0.00244 M^{0.543} \quad 8 \quad (\text{T2 DNA (sodium salt) ranging from 30 to } 130 \cdot 10^6 \text{ daltons})$$

$$\lg_{10}(S^{\circ}20W - 3) + 1.859 = 0.449 \lg_{10} M \quad 9 \quad (\text{various DNAs (sodium salt) ranging from 0.3 to } 130 \cdot 10^6 \text{ daltons})$$

The ratio of the standard sedimentation coefficient for the Cs and Na forms should be the ratio of the molecular weight of the respective Cs and Na salt of nucleotides : 1.33: Bruner & Vinograd⁵ found a value of 1.31, and they considered it in good agreement. Using 1.33 and 1.31 the values for Na-DNA were found to be 69.2 and 70.6, respectively, which correspond to $158\text{--}164$ and $157\text{--}165 \times 10^6$ daltons. The average value is 161×10^6 daltons. This value corresponds to a molecule of $87 \mu\text{m}$, using a M/L value of $1.85 \text{ dalton}/\mu\text{m}$ according to Freifelder

formula (**)¹⁰. This length is in good accord with the length given by Sarov & Becker.³

This provides evidence for a linear molecule and reveals the weakness of the third hypothesis mentioned above.

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(*) $\pm (\sigma/\sqrt{n})$: Standard deviation on the average of parallel projection to the regression line of every point, it is the incertitude of the graphic determination of the (S²Ow)_w value.

(**) M/L (dalton/μm) = 1.54×10^6 (A + T) % + 2.42×10^6 (G + C) %

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