ON THE DETERMINATION OF THE SEDIMENTATION COEFFICIENT OF DNA IN A PREPARATIVE ULTRACENTRIFUGE

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Sedimentation constants of DNA were calculated from the path of macromolecules, travelled during the centrifugation in a density gradient in a preparative ultracentrifuge. The possibility was verified, whether the dependence of viscosity and density on the distance from the rotation axis can be expressed in terms of an equation derived from the measurement of these values in model solutions, corresponding, as to their composition, to different points of the gradient. Results of the experiment, in which a DNA of a known $S_{20,w}$ was centrifuged, have shown that this procedure is applicable to both sucrose and H_2O–D_2O mixture gradients. Tables are presented for a fast calculation of $S_{20,w}$ in both types of gradient, when a centrifuge VAC 60 is employed (Janetzki-Leipzig).

The determination of the sedimentation coefficient of DNA by centrifugation in a sucrose density gradient is a method used more and more frequently. The sedimentation coefficient $S_{20,w}$ is calculated from the path macromolecule has travelled during the centrifugation.

$$S_{20,w} = \frac{\mathrm{d}r/\mathrm{d}t}{\omega^2 r} = S_{t,m} \frac{1 - \bar{v}\varrho_{20,w}}{1 - \bar{v}\varrho_{t,m}} \frac{\eta_{t,m}}{\eta_{20,w}} . \qquad (1,2)$$

The velocity at a given point of the gradient is then

$$v = \frac{\mathrm{d}r}{\mathrm{d}t} = S_{t,m}\omega^2 r = S_{20,w}\omega^2 r \frac{1 - \bar{v}\varrho_{t,m}\eta_{20,w}}{1 - \bar{v}\varrho_{20,w}\eta_{t,m}} , \qquad (3)$$

where $\eta_{t,m}$ is the viscosity and $\varrho_{t,m}$ the density of the medium, $\bar{v}$ is the partial specific volume (for DNA 0·556), $\eta_{20,w}$ is the viscosity and $\varrho_{20,w}$ the density of water at 20°C, v is the sedimentation velocity, r is the radial distance from the rotation axis and ω is the angular velocity. By integration of equation (*3*) one obtains a relationship between the time and the path travelled by the particle. It is often the case that no equation relating ϱ and η on r exists, especially if the gradient contains other compounds beside sucrose or if another gradient but sucrose is used. The integration is made possible by the assumption that the velocity of particle movement is constant throughout the gradient. This procedure is used for the sucrose gradient which is most frequently applied[1] to DNA centrifugation. The values ϱ and η for the correction factor determining the relation between $S_{t,m}$ and $S_{20,w}$ may be found in the literature[1].

For isokinetic movement one may further determine $S_{20,w}$ or the molecular weight of an unknown sample of DNA by comparing the paths travelled by DNA with a known sedimentation coefficient and that by the unknown sample[2]. However, calibration of the gradient with DNA

of known molecular weight is rather laborious and, moreover, the constant-velocity condition need not be always fulfilled.

We attempted then to find the simplest possible procedure that would be based on the physical parameters of the solutions used and that would be widely applicable to any type of gradient, regardless of whether we are dealing with isokinetic centrifugation or not.

EXPERIMENTAL

Gradients. The experiments were done on a gradient of 5–20% sucrose, and a gradient of 50 to 100% D_2O. In addition to components ensuring the increase in density, both gradients contained 1M-NaCl, 0·001M-EDTA and 0·01M Tris. The gradients were prepared in mixing chambers[3]. The linearity, reproducibility and stability of the gradient were controlled refractometrically in the case of sucrose and by adding ^3H_2O in the case of heavy water gradients. A VAC-60 centrifuge (Janetzki, Leipzig) was used with a 3 × 5 ml swing-out rotor. After the run, the polyethylene tube was pierced and the tube content was gradually displaced by adding constant volumes of 40% sucrose. The individual fractions were withdrawn with a capillary connected with the cap of the tube.

Dependence of ϱ and η on r. Several model solutions were prepared, corresponding to different heights of the gradient. The density of these solutions was measured with a hydrometer, viscosity with Ostwald's viscometer.

Deoxyribonucleic acid was isolated from the T 7 phage by the phenol method of Messie and Zimm[4]. DNA was labelled with thymidine-[^{3}H]. 0·1 ml of a solution containing 0·5 μg DNA was placed on the top of the gradient. Detection was carried out by radioactivity measurement in a Mark I (Nuclear Chicago) scintillation spectrometer.

RESULTS AND DISCUSSION

The measurement of density of solutions corresponding by their composition to different levels of the gradient showed that with sucrose and D_2O gradients, ϱ may be expressed easily as a linear function of r. However, linearity between r and η exists only for gradients of 50–100% D_2O. When using sucrose solutions we obtained a relationship expressed by $\eta = [(3{\cdot}39r - 10{\cdot}75)^{1{\cdot}49} + 109{\cdot}0] \,.\, 10^{-2}$.

If ϱ and η are known for any point of the gradient one may substitute into equation (3) to obtain the velocity with which a particle of a given $S_{20,w}$ is moving and, by integration, obtain the path travelled by the particle or the time required for reaching any given point of the gradient. For practical purposes it is advantageous to use the second possibility. The time required for sedimentation down to a certain depth is inversely proportional to $S_{20,w}$. It is thus enough to compute the integral for one value of $S_{20,w}$ and the table obtained is then applicable to any length of centrifugation at constant ω. When changing the speed of the centrifuge one has to divide the data by the ratio of the squares of revolutions per min. Table I shows the results of numerical integration whence it follows that below 2·3 cm the rate of particle

sedimentation in the sucrose gradient is practically constant. In a H_2O–D_2O gradient the rate increases downward.

To verify the applicability of the Table, DNA from phage T 7 was centrifuged in several runs in a neutral and an alkaline sucrose gradient and in D_2O gradients. Fig. 1 shows typical sedimentation profiles thus obtained.

The path *D* travelled by the particle in the course of centrifugation was determined from the position of the peak before centrifugation and after it. Table I then permits to determine the corresponding value of *t* for the path interval obtained. The $S_{20,w}$ of an unknown DNA is then calculated as

$$S_{20,w} = 100t/t_x , \qquad (4)$$

where t_x is the time of centrifugation of the unknown sample. In this way, the following values of $S_{20,w}$ were obtained. Neutral gradients, sucrose: 32·16 and 32·55; H_2O–D_2O: 32·11 and 33·33; alkaline gradients, sucrose: 33·28 and 34·43; H_2O–D_2O: 35·83 and 34·54 S. Davison and Freifelder[5] report for T 7 phage DNA a value of 32·2 ± 0·5 S, Crothers and Zimm[6] 33·7 S, Leighton and Rubenstein[7] 32·6 S. In an alkaline gradient, Studier[8] obtained 37·2 ± 0·5 S. There exists satisfactory agreement between the various values for neutral gradients. With alkaline gradients the discrepancy amounts to almost 10%. One of the reasons may be the dependence of $S_{20,w}$ of denatured DNA in an alkaline medium on the ionic strength of the solutions[8]. A change in the ionic strength from 0·1 to 1·0 results in a 33% increase of the sedimentation coefficient. In comparison with the neutral gradients where the sedimentation coefficient does not depend on ionic strength over a wide range, the situation with alkaline gradients is more complex and one may expect the results not to be reproducible.

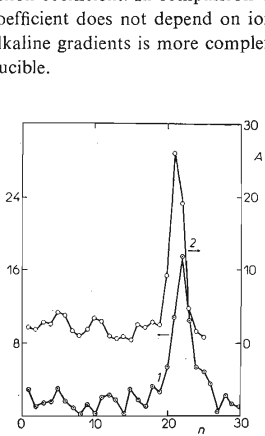

FIG. 1

Sedimentation Profile of T 7 Bacteriophage DNA in a Neutral Sucrose Gradient and a H_2O–D_2O gradient

Ultracentrifuge Janetzki VAC 60, 40,000 r.p.m., 3 × 5 ml swing-out head; *A* total activity (%) recovered in the gradient, *n* the number of fractions. ● 176 min, 5—20% sucrose gradient; ○ 172 min, gradient of 50—100% H_2O–D_2O.

TABLE I

Sedimentation Time of a 100 S DNA from the Top of Gradient to a Distance D

Velocity and sedimentation time were calculated for a VAC 60 centrifuge (Janetzki - Leipzig), 3 × 5 ml swing-out head, 30000 r.p.m. D distance from the top of gradient, v velocity at given point, t total time of sedimentation, r_{top} 4·65 cm.

Sucrose			H_2O–D_2O		Sucrose			H_2O–D_2O	
D cm	$v \cdot 10^{-6}$ cm s^{-1}	t s	$v \cdot 10^{-6}$ cm s^{-1}	t s	D cm	$v \cdot 10^{-6}$ cm s^{-1}	t s	$v \cdot 10^{-6}$ cm s^{-1}	t s
0·0	354	0	349	0	2·4	396	6 293	477	5 828
0·2	361	559	360	564	2·6	396	6 798	486	6 244
0·4	367	1 108	372	1 110	2·8	396	7 302	495	6 651
0·6	373	1 648	383	1 639	3·0	396	7 807	504	7 052
0·8	377	2 181	395	2 152	3·2	396	8 312	513	7 445
1·0	382	2 708	406	2 653	3·4	395	8 818	522	7 830
1·2	385	3 230	416	3 140	3·6	394	9 325	530	8 212
1·4	388	3 747	427	3 614	3·8	393	9 833	538	8 586
1·6	391	4 261	437	4 077	4·0	392	10 343	547	8 955
1·8	392	4 772	447	4 529	4·2	390	10 854	555	9 319
2·0	394	5 280	457	4 972	4·4	389	11 368	562	9 676
2·2	395	5 787	467	5 404					

To determine the sedimentation coefficient of DNA during centrifugation in a density gradient one must know the dependence of ϱ and η on r. We tested whether one may use direct measurement of these values in model solution corresponding by their composition to different levels of the gradient and to deduce the required dependence. The results of experiments where good agreement was obtained for the $S_{20,w}$ of T 7 DNA phage as obtained here and as reported in the literature indicate that the procedure is applicable and sufficiently reliable. Moreover, it is applicable to any type of gradient even if the ϱ and η of their components cannot be found tabulated.

Determination of the sedimentation coefficient of DNA found after centrifugation at a certain level of the gradient is prerequisite for calculating the distribution of molecular weights in a given DNA sample. The results reported here indicate that the operation may be carried out without much difficulty for any type of gradient, simply on the basis of changes of ϱ and η and of the centrifuge parameters.

So far we have considered only a relative accuracy of the procedure, *i.e.* accuracy related to other methods of gradient calibration. In model solutions the measurement of ϱ and η and the relationships derived therefore may be in error of less than 2%. In a practical experiment the accuracy of a result will depend on whether the actual gradient shape agrees with the theory. However, this condition underlies the accuracy of all other indirect methods of determining $S_{20,w}$ in a preparative centrifuge, such

as calibration of the gradient with the aid of a standard[2,9] of known $S_{20,w}$. It is hence believed that in comparison with those methods the procedure described here is equally accurate.

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