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Physical Properties of Chick Interferon

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ABSTRACT

KREUZ, LEO E. (The Johns Hopkins University School of Medicine, Baltimore, Md.), AND ALLAN H. LEVY. Physical properties of chick interferon. J. Bacteriol. **89**:462–469. 1965.—The sedimentation coefficient, diffusion coefficient, and molecular weight of chick-embryo interferon were determined by zone centrifugation and equilibrium sedimentation in cesium chloride density gradients, and by chromatography on Sephadex G-100 columns. Purified interferon is not available in quantities sufficient to permit direct analysis by chemical or physical means; its relative concentrations were determined, therefore, by bioassay. I^{125}-human serum albumin was used as an internal reference in all experiments. The sedimentation coefficient of chick-embryo interferon is 2.2 to 2.3*S*; the diffusion coefficient is 9.5×10^{-7} cm^2 sec^{-1}. A molecular weight of 26,000 was calculated from the sedimentation and diffusion coefficients, and a range of 25,000 to 34,000 daltons was obtained from equilibrium-sedimentation analyses.

Interferon is a protein synthesized by animal cells in response to viral infection. When transferred to cells of the same or related species, it protects them against infection with a wide variety of viruses (Isaacs and Lindenmann, 1957; Wagner, 1963). Despite widespread interest in the biological activity of interferon, relatively few attempts have been made to characterize the physical properties of the molecule. In early crude experiments, interferon produced in chick embryos was subjected to ultracentrifugation in a separation cell, and the sedimentation coefficient was estimated to be in the range of 2*S* (Wagner and Levy, 1960). Sedimentation velocity studies of a highly purified preparation of chick interferon have indicated a molecular-weight range of 20,000 to 30,000 (Lampson et al., 1963). It has also been shown that interferons derived from either mouse or chick tissues sediment in sucrose gradients at a velocity approximately that of lysozyme, 1.9*S* (Rotem and Charlwood, 1963). Recently, Merigan (1964), using chromatography on Sephadex, reported a molecular-weight value of 38,000 for a purified chick interferon. A similar value was reported by Phillips and Wood (1964) with a crude preparation.

The present studies were undertaken to obtain additional knowledge of the physical properties of chick-embryo interferon. I^{125}-human serum albumin (I^{125}-HSA) was used as an internal reference in all experiments. The relative concentrations of interferon were measured by the biological-assay method of viral plaque inhibition. The sedimentation coefficient of interferon was determined by zone centrifugation in cesium chloride density gradients. The diffusion coefficient of interferon was determined by molecular-sieve chromatography on a calibrated Sephadex column, according to a method recently described by Ackers (1964). The molecular weight of interferon was calculated from values obtained for the sedimentation coefficient and the diffusion coefficient. The molecular weight of interferon has also been calculated independently from calibrated equilibrium-sedimentation analyses; the results confirm earlier observations (Kreuz and Levy, 1963).

The experiments reported in this communication provide evidence for the homogeneity of interferon with respect to both molecular size and buoyant density.

MATERIALS AND METHODS

Interferon. Interferon was obtained from pooled allantoic fluids harvested from 13-day-old chick embryos 3 days after infection with the WS strain of influenza A virus (Wagner, 1961). Allantoic fluid was freed of all detectable hemagglutinin and more than 99.9% of infectious virus by centrifugation at 65,000 × *g* for 1 hr and recentrifugation of the supernatant fluid at 104,000 × *g* for an additional hr. The supernatant fluid was then concentrated fourfold by pervaporation in dialysis bags at 4 C, followed by dialysis against 0.1 M

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phosphate-buffered saline (PBS), *p*H 7.2. The material so prepared is termed chick interferon.

Human serum albumin (HSA). HSA was obtained from a commercial source (Behringwerke Aktiengesellschaft, Marburg-Lahn, Germany), and the radioactive iodine derivative (I^{125}-HSA, 42 μg of nitrogen per ml) was prepared in the Department of Physiological Chemistry, Johns Hopkins University School of Medicine.

Bioassay. Interferon was assayed by a modification of the plaque-inhibition method described by Wagner (1961). Monolayer cultures of chick-embryo cells were exposed for 1 hr to dilutions of the test material. The cell layers were then infected with an estimated 100 plaque-forming units of vesicular stomatitis virus (VSV) and overlayered with nutrient agar containing neutral red. Interferon activity was quantitated by calculating the per cent reduction in the number of plaques appearing in 44 to 48 hr on interferon-treated cell monolayers compared with the number of plaques on untreated control monolayers. The interferon concentration of each fraction was assayed by reference to a dose-response curve for the original interferon preparation determined concurrently. Since the interferon assay is most reliable in the range of 50% plaque inhibition (Lindenmann and Gifford, 1963), samples with interferon activity were diluted to obtain plaque counts between 30 and 70% of controls. Each dilution was tested in quadruplicate and, if necessary, reassayed at more closely spaced dilutions. Interferon concentrations of each sample are expressed as 1,000 times the reciprocal of that dilution of the original interferon preparation which corresponds to the same per cent plaque reduction as the sample tested.

Zone centrifugation. Centrifuge tubes were filled with 4.8 ml of a solution of CsCl dissolved in PBS, specific gravity 1.13. Density gradients were formed by centrifugation at 100,000 $\times$ *g* in the SW-39 swinging-bucket rotor of a Spinco model L preparative ultracentrifuge for 48 hr at 4 C. Interferon samples of 0.1 ml, to which had been added I^{125}-HSA (approximately 12,000 count/min), were then layered above the preformed density gradients, and the tubes were centrifuged for an additional 10.5 or 12.66 hr. Three-drop samples were then removed from the bottom of each tube through a hole made with a hot 22-gauge needle; drop formation was stabilized by the pressure of 18 cm of water transmitted by an air column to the meniscus of the sample. The radioactivity of each sample was measured in a scintillation counter. Specific gravities of samples were determined by weighing in a 50-μliter pipette; the CsCl was then removed by dialysis for 48 hr against PBS. Samples were then brought to equal volumes by the addition of PBS and assayed for interferon on chick-embryo cell monolayers. Viscosities of CsCl solutions over the density range of the zone centrifugation were determined with a 2-ml Ostwald viscometer immersed in a 4 C water bath. Distilled water was used as the reference fluid.

Molecular-sieve chromatography. Powdered Sephadex G-100 was equilibrated with PBS; the slurry was used to fill a glass column, 2.25 cm in diameter, to a bed volume of 112 ml. Interferon 0.1 ml supplemented with I^{125}-HSA (approximately 20,000 count/min) was layered above the column, and PBS was allowed to flow through. Seventy 50-drop samples were collected during a 4-hr period (17.5 ml/hr) by an automatic fraction collector fitted with a drop counter. After the radioactivity was counted, the samples were assayed for interferon.

Equilibrium sedimentation. Samples containing 4.8 ml of interferon, to which had been added I^{125}-HSA (approximately 100,000 count/min), were brought to a starting specific gravity of 1.32 by the addition of CsCl powder. After centrifugation at 100,000 $\times$ *g* for 72 hr at 4 C in the SW-39 rotor, two-drop samples were collected from the bottom of each tube. Specific gravity, radioactivity, and interferon content were measured as described above.

RESULTS

Zone centrifugation. Figure 1 shows the results of two zone sedimentation analyses of mixtures of interferon and HSA, for 10.5 hr (A) and 12.66 hr (B). It can be observed that interferon bands as a homogeneous molecular species. The skewness of the sedimentation curve for albumin in Fig. 1A may be attributed to the presence of dimers and higher order polymers that are known to be present in small amounts in HSA preparations (Pederson, 1962). In addition to polymerization, the skewness of the sedimentation curve for human serum albumin in Fig. 1B may possibly be attributed to the presence of several different albumin monomers of approximately equivalent molecular weights, as suggested by Pederson (1962).

Martin and Ames (1961) have suggested that the sedimentation coefficient of an unknown molecule centrifuged in a linear sucrose density gradient with a molecule of known sedimentation coefficient can be estimated from the simple ratio of distances moved by the molecules, provided that the molecules move with constant velocity within the gradient. Based on this assumption, we estimated the sedimentation coefficient of chick interferon in zone centrifugation in a CsCl density gradient from the ratio of distances moved in centrifugation by chick interferon and a reference molecule (HSA) by use of the approximation:

$$S^{\circ}_{20,w}(u) = \frac{\Delta X_u}{\Delta X_v} \cdot S^{\circ}_{20,w}(r) \qquad (1)$$

where X_u and X_v are the distances moved by the unknown and reference macromolecules, re-

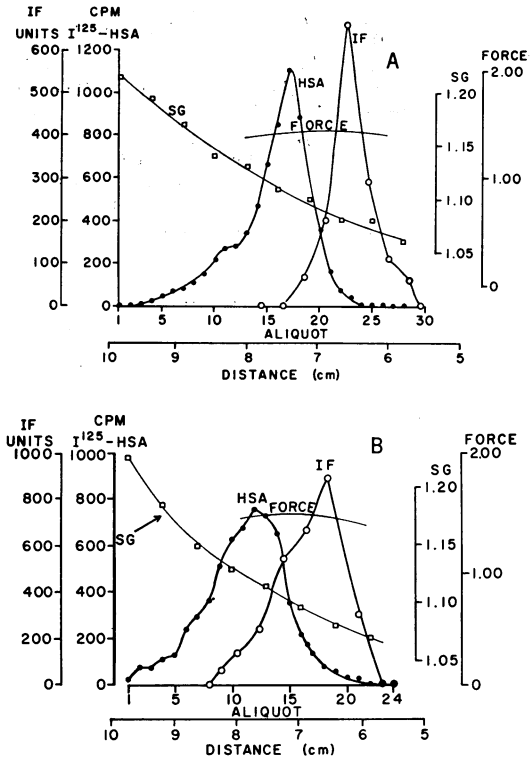

FIG. 1. *Distribution of chick-embryo interferon (IF) and I^{125}-HSA (HSA) in preformed CsCl density gradients after sedimentation for 10.5 hr (A) and 12.66 hr (B). Sample number and corresponding distance from the center of the axis of rotation are indicated on the abscissa.* ○ = *the average of the concentration of interferon in two adjacent fractions; each fraction was assayed independently on four bioassay plates. Specific gravity (SG) is expressed as grams per milliliter, and the calculation of relative force (FORCE) is described in the text.* ● = *count/min of I^{125}.*

spectively, and $S^{\circ}_{20,w}$ is the standard sedimentation coefficient (water as the solvent at 20 C) of the unknown molecule (u), and the reference molecule (r).

In density-gradient centrifugation, molecules are centrifuged through solvent zones varying in centrifugal force, buoyancy, and viscosity. The approximation expressed in equation 1 will be valid only under the following restrictions. (i) Both molecules are centrifuged under conditions such that the velocity of each molecular species is approximately constant over the distance moved. (ii) The distances moved by the molecules are small in comparison with the distance from the rotation center. (iii) The partial specific volumes of the unknown and reference molecules are approximately equal.

These conditions were satisfied by the experimental conditions employed in the present studies (Table 1). The interferon and the reference protein were of similar effective density, and were centrifuged for short distances in comparison with the distance from the center of rotation. The condition of constant velocity was approximated by choosing a density gradient such that buoyancy and viscosity nearly counter-balanced centrifugal force. This is illustrated graphically by the approximate constancy of the lines labeled "force" in Fig. 1A and B. These lines, representing the calculated values for the relative force at each point in the centrifugations, were calculated as the product of the distance from the rotation center times the difference between the effective density of the molecule and the solution density at that locus. The measured change in viscosity with distance was slight. This constancy of the lines of relative force indicated an approximately constant velocity of each molecule over the path of centrifugation, thus satisfying condition (i). It was determined that CsCl solutions of initial specific gravities in the range of 1.10 to 1.15 g/cc and spun at 100,000 × g resulted in density gradients within which interferon and HSA moved at nearly constant velocity.

The sedimentation coefficient of interferon was directly calculated by substituting into equation 1 the experimental values from the 10.5-hr run for the distances moved by interferon and by albumin from meniscus to peak (Fig. 1A; Table 1, lines 1a to 1c), and the known sedimentation coefficient for HSA (Table 1, line 9b). In this manner, $S^{\circ}_{20,w}$ for interferon was calculated to be 2.3.

Similar calculations from the data for the 12.66-hr zone centrifugation (Fig. 1B) gave a value of 2.2S for the sedimentation coefficient. The close agreement between the values, obtained from zone centrifugations of differing durations, was empirical evidence for the reliability of the method.

Molecular-sieve chromatography. Figure 2 shows the results of chromatography of a mixture of chick interferon and I^{125}-HSA passed through a Sephadex G-100 column. The skewness of the albumin curve is again attributable to partial polymerization (Pedersen, 1962). Interferon does not exhibit such skewness, suggesting that the interferon preparation studied was homogeneous with respect to its molecular size, with no suggestion of polymerization.

It has been demonstrated empirically that a calibrated molecular-sieve column can be used for estimating the diffusion coefficient of a macromolecule present in impure form and in unknown

TABLE 1. *Experimentally determined and calculated values for sedimentation velocity analysis*

Item no.	Measurement	Symbol	Value	Comment
	Distance from rotation center to:			
1a	Center of meniscus	X_1	5.5 cm	Directly measured
1b	I^{125}-HSA peak	$X_{2_{HSA}}$	7.41 cm	Directly measured
1c	Interferon peak	$X_{2_{IF}}$	6.59 cm	Directly measured
1d	Point of mean distance traveled by I^{125}-HSA	$\bar{X}_{HSA}$	6.45 cm	Directly measured
1e	Point of mean distance traveled by interferon	$\bar{X}_{IF}$	6.05 cm	Directly measured
2	Slope of density gradient	a	8×10^{-3} g/cc-cm	Directly measured
3	Slope of viscosity gradient	b	-2.5×10^{-4} poise/cm	Directly measured
4	Viscosity of meniscus sample		1.36×10^{-2} poise	Directly measured
5	Specific gravity of meniscus sample		1.055 g/cc	Directly measured
6a	Speed of centrifugation	f	39,000 rev/min	Directly measured
6b	Square of angular velocity	ω^2	1.764×10^7 radians/sec	Calculated
	Partial specific volume			
7a	I^{125}-HSA	$\bar{v}_{HSA}$	7.34×10^{-1} cc/g	Shulman, 1953
7b	Interferon	$\bar{v}_{IF}$	7.7×10^{-1} cc/g	Approximated as the reciprocal of the buoyant density of interferon
8	Time of sedimentation		3.96×10^4 sec	Directly measured
	Sedimentation coefficient			
9a	Interferon	$S^{\circ}_{20,w_{IF}}$	$2.2\text{–}2.3 \times 10^{-13}$ sec^{-1}	Calculated
9b	I^{125}-HSA	$S^{\circ}_{20,w_{HSA}}$	4.31×10^{-13} sec^{-1}	Shulman, 1953

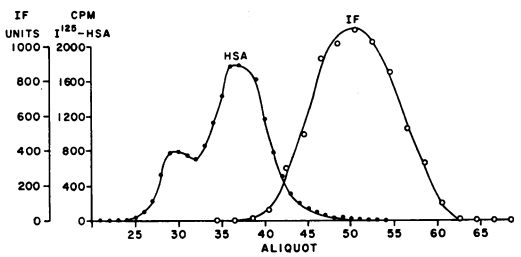

FIG. 2. *Elution of interferon (IF) and I^{125}-HSA (HSA) from a Sephadex G-100 column. Samples are 1 ml each. ○ = the average of the interferon concentration of two adjacent fractions; each fraction was measured independently on eight bioassay plates.*

absolute concentration, provided some suitable assay for determination of relative concentration is available (Ackers, 1964). The molecular weight can then be calculated from the diffusion coefficient determined by gel filtration and the sedimentation coefficient.

The determination of the diffusion coefficient of a molecule, by the method of Ackers (1964), is based on an estimation of the Stokes radius of the molecule by use of a column of calibrated gel-pore radius. The diffusion coefficient can then be calculated from the Stokes-Einstein equation.

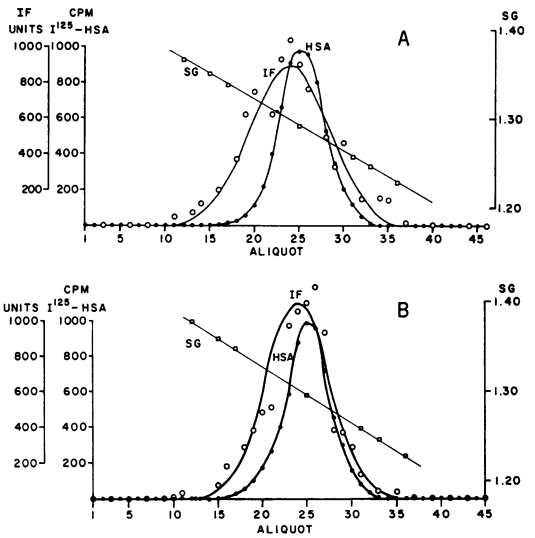

FIG. 3. *Distributions of interferon (IF) and I^{125}-HSA (HSA) in CsCl density gradient equilibrium sedimentations. The concentration of interferon in each fraction is represented by ○; each fraction was assayed on four bioassay plates. SG = specific gravity. ● = count/min of I^{125}. A and B represent the results of different runs.*

TABLE 2. *Molecular sieve chromatography of interferon on Sephadex G-100 column*

Measurement	Symbol	Value	Comment
Volume of column	V_c	112 ml	Directly measured
Volume of unbound internal solvent	V_i	64 ml	Calculated from stated water regain of Sephadex
Effluent volumes I^{125}-HSA polymer.	V_0*	28 ml	Directly measured
I^{125}-HSA monomer	$V_{e_{HSA}}$	37 ml	Directly measured
Interferon	$V_{e_{IF}}$	50 ml	Directly measured
Stokes radius I^{125}-HSA	a_{HSA}	3.62 mμ	Reference value (Ackers, 1964)
Interferon	a_{IF}	2.24 mμ	Calculated from $V_{e_{IF}}$, r
Gel-pore radius of Sephadex column	r	10.2 mμ	Calculated from a_{HSA}, V_i, V_0, $V_{e_{HSA}}$

* The molecular size of the albumin polymer is such that its effluent volume represents the total volume of external water.

The total volume of external water, V_0, was determined by measuring the effluent volume from the column from time of sample application to the time of efflux of the HSA polymer peak (Table 2). The volume of unbound solvent internal to the gel phase, V_i, was calculated from the stated water regain of the Sephadex. The effluent volumes of both the I^{125}-HSA monomer peak and the interferon peak were measured directly.

Ackers (1964) prepared tables relating the volume of external water, the volume of unbound solvent, the effluent volume of a known macromolecule, and the Stokes radius of that macromolecule to the effective gel-pore radius of the particular column. By use of Ackers' tables and the data in Table 2, the column was calibrated as having an effective pore radius of 10.2 mμ. The Stokes radius for interferon was then determined from the same tables by use of the calculated gel-pore radius and the measured effluent volume of interferon; its value was 2.24 mμ.

The free-diffusion coefficient, D°, for interferon was calculated from the Stokes-Einstein equation:

$$D^\circ = \frac{kT}{6\pi\eta a} \qquad (2)$$

where k is the Boltzman constant, T is temperature in Kelvin degrees, η is the viscosity of the medium, and a is the Stokes radius. $D^\circ_{20,w}$ for interferon was calculated to be 9.5×10^{-7} cm^2 sec^{-1}, with an error of approximately 5%.

The diffusion coefficient of a molecule is related to its sedimentation constant and molecular weight, M, at infinite diiution by the Svedberg equation:

$$M = \frac{S^\circ_{20,w} RT}{D^\circ_{20,w}(1 - \bar{V}\rho_0)} \qquad (3)$$

where R is the gas constant, $\bar{V}$ is the solute partial specific volume, and ρ_0 is the density of water at 20 C. Substituting the experimentally determined values for $S^\circ_{20,w}$ (zone centrifugation, Table 1, line 9a) and $D^\circ_{20,w}$ (molecular-sieve chromatography), the molecular weight of interferon was calculated to be 26,000. This value is consistent with the molecular-weight range of 25,000 to 34,000 as determined by equilibrium sedimentation (see below).

Equilibrium sedimentation. The results of the combined equilibrium sedimentation of interferon and I^{125}-HSA are shown in Fig. 3A and B. The best gaussian fits of the interferon bioassay points for the two sets of data have been determined by linear regression lines constructed through plots of the natural logarithm of the interferon concentration against the square of the distance of each fraction from the region of maximal interferon concentration (Kreuz and Levy, 1963). The gaussian nature of the equilibrium distribution of interferon in a linear density gradient is evidence for its density homogeneity. The effective buoyant density of interferon for both runs was 1.3.

Meselson, Stahl, and Vinograd (1957) showed that the standard deviation of the distribution curve for a given macromolecular species in a linear density gradient is inversely proportional to the square root of the molecular weight of the molecule. The molecular weight can be calculated from the following equation:

$$M = \frac{\rho RT}{\frac{d\rho}{dr_0}\omega^2 r_0 \gamma^2} \qquad (4)$$

where ρ is the effective density of the macromolecule, $d\rho/dr_0$ is the density gradient, ω is the angular velocity, r_0 is the distance from the center of rotation to the locus of effective density, and γ is the standard deviation of the distribution of

TABLE 3. *Equilibrium sedimentation analyses*

Symbol*	Run 1		Run 2	
	Interferon	HSA	Interferon	HSA
ρ	1.296†	1.290	1.302	1.295
$d\rho/dr_0$	0.0752	0.0752	0.0825	0.0825
ω^2	1.764×10^7	1.764×10^7	1.764×10^7	1.764×10^7
r_0	7.50	7.39	7.35	7.42
γ^2	0.122	0.0458	0.082	0.0483

* Symbols are defined in text.
† All values are in centimeters, grams, and seconds.

the macromolecule. The reciprocal of the effective density is used as an approximation of the partial specific volume.

Substituting the experimentally determined values (Table 3) in the above formula, molecular weights of 25,000 and 34,000 were calculated for interferon; the molecular weight of HSA was calculated to be 57,000 and 64,000, respectively, for the two sedimentation runs. The present values for the molecular weight of interferon are in close agreement with those previously reported by us in earlier experiments in which the I^{125}-HSA was not included as a reference protein (Kreuz and Levy, 1963). Additional evidence for the reliability of the method may be adduced from the simultaneously determined molecular-weight values of 57,000 and 64,000 for I^{125}-HSA; these are only slightly lower than the molecular-weight range of 65,000 to 70,000 determined for HSA by analytical ultracentrifugation (Baldwin et al., 1955; Edsall and Foster, 1948; Klainer and Kegeles, 1955).

DISCUSSION

Interferon is present in extremely low absolute concentration in all biological materials investigated to date. It has been estimated that the interferon content of allantoic fluid obtained from virus-infected chick embryos, a source of interferon with high biological activity, is 0.0017 to 0.0062 mg/ml (Lampson et al., 1963). The low concentration of interferon precludes analysis of physical properties by optical, analytical, ultracentrifugal, chemical, or other conventional methods that require either purified or concentrated material. However, with a sensitive bioassay, physical measurements can be made at concentrations near the theoretically ideal condition of zero concentration.

The precision attained in the plaque-inhibition bioassay for interferon is indicated by the substantial agreement of values calculated for both molecular weight and sedimentation coefficient in different runs. Also, the molecular weight reported in this communication agrees well with the range reported previously (Kreuz and Levy, 1963). That the bioassay itself is a valid estimate of relative interferon concentration can be adduced by the similar values for molecular weight calculated from equilibrium-sedimentation data and from diffusion and sedimentation coefficients, because these two independent methods of calculating molecular weight are based on different properties of the molecule.

It was of some interest to compare the value for the sedimentation coefficient of interferon calculated by the simple approximation of ratio of distances (equation 1) with a value corrected more precisely for zone variations in density and viscosity. Nomura, Hall, and Spiegelman (1960) developed an integral relationship which permits the calculation of the corrected sedimentation coefficient of an unknown molecule centrifuged in a linear sucrose density gradient simultaneously with a molecule of known sedimentation coefficient. Using a different, although related, means of calculation, we have determined that the values based on equation 1 differ by only 5% from the corrected values (see Appendix).

The method described for determining the sedimentation coefficient can be applied to many other proteins, the presence of which can be detected by an appropriate biological assay. By the application of appendix equation 6, it is possible to calculate the error introduced by the use of the simple approximation of relative distances (text equation 1).

APPENDIX

The error in the sedimentation coefficient resulting from the use of text equation 1 may be estimated as follows.

The sedimentation coefficient of a spherical molecule determined at position x in centrifugation may be written as:

$$S = \frac{V_x}{\omega^2 x} = \frac{M(1 - \bar{V}\rho_x)}{6\pi N r \eta_x} \qquad (1)$$

where V_x is the velocity of the molecule at x·

$\omega^2 x$ is the centrifugal force; M, $\bar{V}$, and r are, respectively, the molecular weight, partial specific volume, and Stokes radius of the molecule; ρ_x is the solvent density at x; η_x is the solvent viscosity at x; and N is Avogadro's number. In our centrifugation experiments, density and viscosity are, to a good approximation, linear functions of distance from the rotation center (Fig. 1A and B):

$$\rho_x = \rho_1 + ax$$
$$\eta_x = \eta_1 + bx$$

where a and b are constants, ρ_1 and η_1 are the density and viscosity, respectively, at the meniscus. Substituting into appendix equation 1 and multiplying through by $\omega^2 x$ gives:

$$V_x = \frac{M(1 - \bar{V}\rho_1)\omega^2 x - M\bar{V}a\omega^2 x^2}{6\pi Nr(\eta_1 + bx)} \quad (2)$$

But $M\ (1 - \bar{V}\rho_1) = S_1 6\pi Nr\eta_1$ from appendix equation 1. Substituting into appendix equation 2 gives:

$$V_x = \frac{S_1 6\pi Nr\eta_1\omega^2 x - M\bar{V}a\omega^2 x^2}{6\pi Nr(\eta_1 + bx)} \quad (3)$$

Since $V_x = dx/dt$, the time required for the molecule to move from x_1 to x_2 is given by:

$$\int_{x_1}^{x_2} \frac{dx}{V_x} = \Delta t \quad (3a)$$

Substitution for V_x (appendix equation 3) and integration from x_1 to x_2 give:

$$\Delta t = \frac{1}{S_1\omega^2} ln\left(\frac{x_2}{x_1}\right) - K \quad (4)$$

where $K =$

$$\frac{6\pi Nr}{M\omega^2}\left(\frac{\nu_1}{(1 - \bar{V}\rho_1)} + \frac{b}{\bar{V}a}\right) \cdot ln\left(\frac{1 - \bar{V}\rho_1 - \bar{V}ax_2}{1 - \bar{V}\rho_1 - \bar{V}ax_1}\right) \quad (4a)$$

Since $ln(x_2/x_1) \simeq \Delta x/\bar{x}$ (this approximation introduces less than 1% error in our data), where Δx is the distance moved and $\bar{x}$ is the mean distance moved from the rotation center, appendix equation 4 reduces to:

$$\Delta t \simeq \frac{1}{S_1\omega^2} \cdot \frac{\Delta x}{\bar{x}} - K \quad (5)$$

In zone centrifugation experiments, albumin and interferon were centrifuged simultaneously. Thus, Δt for interferon (Δt_i) and Δt for albumin (Δt_a) were equal. Combining equations (appendix equation 5) for interferon and albumin gives:

$$\frac{S_i}{S_a} = \frac{\Delta x_i}{\Delta x_a}\left(\frac{\Delta t + K_a}{\Delta t + K_i} \cdot \frac{\bar{x}_a}{\bar{x}_i}\right) \quad (6)$$

This formula is similar in form to text equation 1, except for the addition of the bracketed term. This term, therefore, can be considered as a correction factor for the simpler approximation given in text equation 1.

Δt, K, and $\bar{x}$ values for interferon and HSA were calculated from reference values and experimental values from the first sedimentation-velocity experiment and Table 1. With these data, appendix equation 6 becomes:

$$S_i = \frac{\Delta x_i}{\Delta x_a} \cdot S_a \cdot 1.05 \quad (7)$$

(In this calculation the partial specific volume of interferon was taken as equivalent to the partial specific volume of albumin.) Thus, the use of text equation 1 would underestimate the Svedberg constant of interferon by 5%. The error utilizing the approximation is comparable for the second zone centrifugation experiment.

ACKNOWLEDGMENTS

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