BBA 45270

CONCERNING THE MOLECULAR WEIGHT, SHAPE, AND SIZE OF POLYGLUCOSE ISOLATED FROM HeLa CELLS

F. SOKOL*, I. L. GRAVES AND W. W. ACKERMANN

Department of Epidemiology and Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor, Mich. (U.S.A.)

(Received April 12th, 1965)

SUMMARY

Light-scattering measurements were made on a preparation of polyglucose isolated from HeLa cells. The molecular weight was determined, from a Zimm plot of the data, to be $4.6 \cdot 10^6$. The character of the angular dependence of the particle-scattering factor suggests the shape of the particle in solution with 779 Å gyration radius approximates that of a rigid rod, 2700 Å in length with a thickness of 50 Å. By quantitative electron microscopy, using reference latex spheres, the molecular weight was estimated to be $1.35 \cdot 10^6$. Electron micrographs show the preparation when air-dried on a collodion membrane to be composed of relatively uniform spheroid particles, approx. 300 Å in diameter.

INTRODUCTION

The extraction of HeLa cells from tissue cultures with phenol and deoxycholate yields three high-molecular-weight species, RNA, DNA and a polyglucose. The latter precipitates partially with the RNA from 20% ethanol and the remainder with the DNA from 50% ethanol[1]. The buoyant densities of DNA and polyglucose are identical in CsCl and they are not separated by density-gradient centrifugation. The RNA and polyglucose are separated by this method to yield a sharp band of polyglucose in the form of predominantly homogeneous particles, visible in electron micrographs (spheroid, 300 Å in diameter). These micrographs have been previously published[1]. It has been suggested that the structures are subunits of glycogen granules and that they interact *in vitro* with the nucleic acids. The present study is concerned with the physical properties of the polyglucose as deduced from light-scattering measurements and quantitative electron microscopy. The molecular weight, shape and size have been estimated by two independent methods.

MATERIALS AND METHODS

Preparation of polyglucose

The preparation of polyglucose used in these experiments has been published previously[1]. Essentially, HeLa cells grown in tissue cultures were extracted with

* Permanent address: Institute of Virology, Czechoslovak Academy of Sciences, Bratislava, Czechoslovakia.

phenol, in the presence of deoxycholate and 1 M NaCl, as described by COLTER *et al.*[2]. RNA and polyglucose were precipitated from the extract with 20 % ethanol, separated by density-gradient centrifugation in CsCl. The sharply banded polyglucose taken from the CsCl gradient was diluted and reprecipitated with ethanol (50 %).

The concentration of polyglucose was determined from the weight of a dried sample and also by glucose determination (diphenylamine–HCl reagent[3]).

Latex spheres

The reference latex spheres were obtained from Dow Chemical Co., Midland, Mich. The number of spheres per unit volume of a standard was determined from the weight of a dried sample and the weight of a single sphere (given by Dow Chemical Co. as $0.382 \cdot 10^{-15}$ g).

Light-scattering measurements

A sample of polyglucose was dispersed in 0.13 M NaCl and 7 mM phosphate at pH 7.2 and the solution freed of dust by centrifugation at 10000 rev./min (SW 25 swinging head of a Spinco centrifuge) for 20 min directly in cylindrical light-scattering cells of 10 ml volume. The intensity of scattered light was measured at 30°, 37.5°, 45°, 60°, 75°, 90°, 105°, 120°, 135°, 142.5° and 150°, respectively, with a Sophica photoelectric apparatus. Unpolarized light of 546 mμ wavelength was used.

The measured reduced intensity of light scattered at angle θ (R_θ, see ref. 4) was corrected for the difference in refractive indices of the solvent (n_0) and the environment of the detector (benzene, n_b) by the multiple n_0^2/n_b^2 and for the horizontal component of the scattered light, as well as for the differences in the scattering volumes at various angles by multiplying with $\sin\theta/(1 + \cos^2\theta)$.

Pure benzene with reduced intensity of scattered light at 90° of $16.3 \cdot 10^{-6}$ was used as a scattering standard[5]. For the refractive index increment (dn/dc) of the polyglucose at 546 mμ, 0.152 was taken. The latter value was found for dextran[6].

Enumeration of polyglucose particles

A standard suspension with a known number of latex spheres was diluted in bovine serum albumin (0.05 % final concentration) and treated with sonic waves (10 kcycles) for 3 min. Appropriate dilutions of the latex and glycogen were combined and a drop of this mixture was placed on a collodion membrane which was floating on water. Copper grids were placed on a sintered glass block which was submerged in the water. The grids were brought up under the drop of the latex–glycogen mixture and the drop then was lifted out of the water. The liquid in the drop was rapidly pulled through the sintered glass by capillary action as the sintered glass was placed on a moist towel. This technique was developed to minimize aggregation caused by drying. The grids with the collodion membrane on top were removed from the sintered glass and shadow-cast with palladium at an angle of 7:1.

RESULTS

Molecular weight by Zimm plot

The reduced intensity of light scattered at angle θ, obtained with two concentrations of polyglucose are plotted in Fig. 1 according to the method of ZIMM[7,8].

The constant K is equal to $2\pi^2 n_0^2 (dn/dc)^2 / N_0 \lambda_0^4$ (see ref. 4) where N_0 is Avogadro's number and λ_0 the wavelength (in cm) of the monochromatic light *in vacuo*, and c is the concentration in g/ml. In Fig. 1 some of the lines connecting the points for the same angle, but different concentrations, are omitted from the lower part of the curve for the sake of clarity. The extrapolated intercept on the ordinate represents the reciprocal of the weight-average molecular weight which is estimated from this value to be $4.6 \cdot 10^6$.

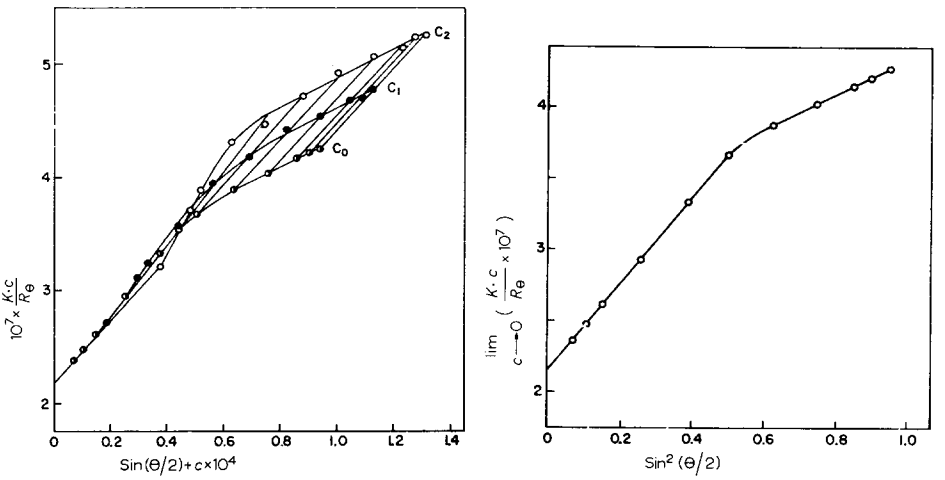

Fig. 1. ZIMM plot of light-scattering data obtained with polyglucose from HeLa cells. Angles measured: 150°, 142.5°, 135°, 120°, 105°, 90°, 75°, 60°, 45°, 37.5° and 30° (0° by extrapolation). Concentrations of polyglucose used: c_2, $3.72 \cdot 10^{-5}$ g/ml; c_1, $1.86 \cdot 10^{-5}$ g/ml; c_0, extrapolated to zero concentration.

Fig. 2. The scattering envelope of polyglucose isolated from HeLa cells, plotted from the data in Fig. 1.

Shape and size of the molecule

The gyration radius (r_n) of the molecule was calculated using the expression of ZIMM AND STOCKMAYER[9]:

$$r_n = \frac{\lambda_0 \sqrt{3}}{4 \pi n_0} \sqrt{m \times \text{mol.wt.}}$$

where

$$m = \lim_{\substack{\theta \to 0 \\ c \to 0}} \frac{d\,(Kc/R_\theta)}{d\,[\sin^2 (\theta/2)]}$$

The dependence of $\lim_{c \to 0} (Kc/R_\theta)$ on $\sin^2 (\theta/2)$ is shown in Fig. 2. For the polyglucose the calculated gyration radius was 779 Å. From a consideration of the estimated molecular weight and the diameter of a spherical particle calculated from the gyration radius ($d = r_n \sqrt{20/3}$), the molecule cannot be spherical.

The character of the dependence of the particle-scattering factor

$$P(\theta) = \lim_{c \to 0} R_\theta / R_0$$

upon $\sin^2 (\theta/2)$ excludes also the shape of a monodisperse or polydisperse coil. The

closest resemblance, although far from identity, was found between the data and the angular dependence of the particle-scattering factor of rigid, monodispersed rods. This is shown in Fig. 3 where the particle-scattering factor of monodispersed rods of 779 Å gyration is plotted against $\sin^2(\theta/2)$ and compared with the observed data.

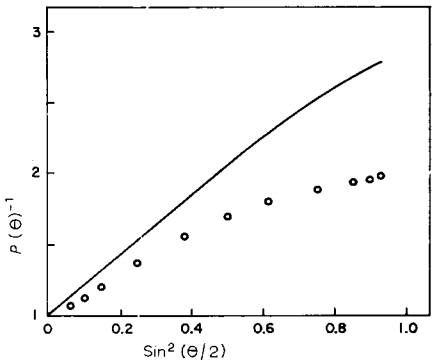

Fig. 3. The dependence of the reciprocal of the particle-scattering factor of monodispersed rigid rods of 779 Å gyration radius upon $\sin^2(\theta/2)$ is given by the solid curve; that obtained experimentally for polyglucose given by the open circles.

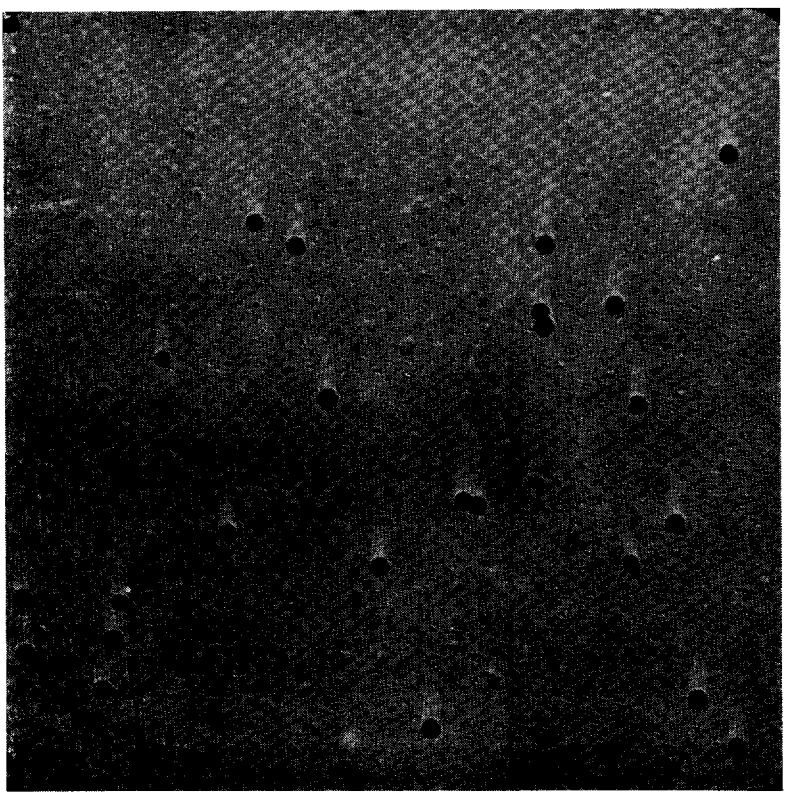

Fig. 4. An electron micrograph of a field containing a mixture of latex spheres (880 Å) and polyglucose particles, shadow-cast with palladium at angle of 7:1.

TABLE I

DETERMINATION OF MOLECULAR WEIGHT OF POLYGLUCOSE BY ELECTRON MICROSCOPE PARTICLE COUNTING

Expt.	*Sample*			*Observed*				*Calculated*	
	*Latex–polyglucose proportion**	*Latex concn.*** *(g/ml × 10¹¹)*	*Polyglucose dilution***	*Counts**** *Latex*	*Polyglucose*	*No. of fields*	*Average ratio polyglucose/latex*	*Polyglucose concn.*§ *(g/ml × 10¹⁴)*	*Mol. wt. × 10⁶*
1	2:1	9.17	300	555	1623	17	3.61	9.90	1.16
2	1:1	6.88	200	318	1498	10	5.49	7.55	1.53
3	2:1	9.17	300	1154	3123	23	2.98	8.18	1.39
4	1:1	6.88	200	611	2002	14	3.71	5.12	2.24
5	4:1	11.0	500	515	970	15	2.14	11.8	0.968
6	3:1	10.3	400	684	1674	14	3.36	13.4	0.823
							Average 1.35 ± 0.37		

* Sample counted prepared from various proportions of latex (1:200 dilution of standard containing $2.74 \cdot 10^{14}$ particles per ml) and polyglucose (1:100 dilution of standard containing 1898 μg polyglucose per ml).

** Concentration of latex in sample counted expressed as particles per ml; the fold dilution of polyglucose in sample based upon original standard (1898 μg/ml).

*** Particle counts from electron micrographs represent the totals from all fields counted.

§ Concentration of polyglucose expressed as particles $\times 10^{11}$ per ml of the original standard (1898 μg/ml).

If the polyglucose molecules were nearly rod-shaped their average length would be 2700 Å and their thickness 50 Å based upon the relationship: l (length of rod) = $r_n\sqrt{12}$. It is to be emphasized that these relationships have been deduced for monodisperse rigid rods and that the polyglucose sample measured is probably polydisperse, as indicated by the marked difference between the weight- and number-average molecular weights.

Molecular weight by particle count

Appropriate dilutions (Table I) of latex spheres containing known numbers of spheres were combined with a 1:100 dilution of polyglucose (which contained originally 1898 μg/ml) in proportions which varied from 1:1 to 1:4. From this mixture electron microscope grids were prepared (*cf.* METHODS) and electron micrographs of random fields were made (Fig. 4). The ratio of small polyglucose particles (300 Å) to larger latex spheres (880 Å) was determined by counts of 10–20 fields for each sample. From these ratios (Table I) and the known concentration of spheres, the concentration of polyglucose particles was determined in the mixture. From the dilution factor, the number of polyglucose particles in the original sample was determined. This allowed the average weight per particle to be calculated from the weight concentration. The molecular weight was then determined (from the product of Avogadro's number and the weight per particle in grams) to be $1.35 \cdot 10^6$ (Table I). The principle involved is essentially that employed by others for the study of virus particles[10–12].

DISCUSSION

Quantitative enumeration of particles by electron microscopy has been applied primarily to virus particles and reference latex spheres[10–13]. The method should, however, be generally applicable to true molecular entities which are sufficiently resolved in electron micrographs. Polyglucose, obtained by phenol extraction, is in the proper size range for both light-scattering measurements and quantitative electron microscopy. The degree of homogeneity of the particles (as revealed in previous electron micrographs[1]) suggested a direct comparison of the methods for determination of shape, size and molecular weight should be made. Considering that light-scattering data yield a weight-average molecular weight while the particle-counting method gives a number average, the agreement of $4.6 \cdot 10^6$ to $1.34 \cdot 10^6$ is sufficient to be meaningful. Any aggregation in solution would greatly weight the light-scattering data in the direction of values in excess of the true molecular weight and may account for the larger value. Indeed this is suggested by the electron micrographs prepared for quantitation (Fig. 4).

It is also interesting that the plot (Fig. 3) of the particle-scattering factor upon $\sin^2(\theta/2)$ does not precisely fit any of the common models for large molecules. Nor are the data in clear agreement with the evidence of the electron micrographs reported previously[1]. But as yet there is no considerable accumulation of experience with varied methods of preparation of grids for the electron microscope. It may be recalled that a molecule like DNA assumes forms in electron micrographs ranging from long threads to spherical particles.

However, if one accepts, from the light-scattering data, that in solution the shape of the particle approximates a rigid rod, then the additional evidence of the

electron microscope (indicating a flattened spheroid) suggests that polyglucose is capable of intermolecular interaction and can assume some secondary structure. This increases the possibilities for specific interactions with other large molecules which might account for the difficulty in separating polyglucose from high-molecular-weight RNA and DNA[1].

Possibilities of similar intracellular interactions have prompted studies, now in progress, of the synthesis and accumulation of polyglucose in HeLa cells, where the normal nucleic acid metabolism has been deranged by the action of vaccinia or poliovirus.

ACKNOWLEDGEMENTS

This investigation was supported in part by U.S. Public Health Service Grant No. AI 05876-01 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

F. S. was supported in part by a stipend from the U.S. Public Health Service, Trainee Grant No. 5T7 AI 60-05.

REFERENCES

1 Z. M. MARTINEZ SEGOVIA, F. SOKOL, I. L. GRAVES AND W. W. ACKERMANN, *Biochim. Biophys. Acta*, 95 (1965) 329.
2 J. S. COLTER, R. A. BRAN AND K. A. O. ELLEN, *Biochim. Biophys. Acta*, 55 (1962) 31.
3 F. E. SNELL AND C. T. SNELL, *Colorometric Methods of Analysis*, Vol. 3, Van Nostrand, Princeton, 3rd Ed., 1953, p. 312.
4 P. DOTY AND Y. T. EDSALL, *Advan. Protein Chem.*, 6 (1951) 35.
5 C. I. CARR AND B. H. ZIMM, *J. Chem. Phys.*, 18 (1950) 1616.
6 F. A. BOVEY, *J. Polymer Sci.*, 35 (1959) 167.
7 B. H. ZIMM, *J. Chem. Phys.*, 16 (1948) 1093.
8 B. H. ZIMM, *J. Chem. Phys.*, 16 (1948) 1099.
9 B. H. ZIMM AND W. H. STOCKMAYER, *J. Chem. Phys.*, 17 (1949) 1301.
10 R. C. WILLIAMS AND R. C. BACKUS, *J. Am. Chem. Soc.*, 71 (1949) 4052.
11 E. KELLENBERGER AND W. ARBER, *Virology*, 3 (1957) 245.
12 D. H. WATSON, *Biochim. Biophys. Acta*, 61 (1952) 321.
13 D. G. SHARP, *Proc. Soc. Exptl. Biol. Med.*, 70 (1949) 54.