SEDIMENTATION AND VISCOSITY STUDIES ON THE CAPSULAR AND SOMATIC POLYSACCHARIDES OF PNEUMOCOCCUS TYPE III*

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INTRODUCTION

For a thorough review of the work that has been done on the polysaccharides of pneumococcus Type III, one can refer to Burger (1). The major part of this review is given to the pioneering investigations of the immunological properties of these polysaccharides. The somatic and capsular polysaccharides investigated in this present work were obtained as by-products during the preparation of sodium desoxyribonucleate from pneumococcus Type III (2). The polysaccharides were obtained during the preparation procedure in much the same manner as was employed by McCarty and Avery (3).

Burger (1) in his review observes that very little physicochemical work has been done on the polysaccharides from pneumococcus Type III. In view of this fact, it was decided that an investigation into the sedimentation and viscosity behavior of these substances should be undertaken.

Methods

The sample of somatic polysaccharide was that described in a previous work (2), and was obtained in the form of a dry white powder.

The preparation of capsular polysaccharide was obtained from a crude preparation of the desoxyribonucleate at the point of deproteinization by the Sevag (4) procedure. When the deproteinization mixture was saturated with NaCl, the capsular polysaccharide precipitated out. It was removed by centrifugation, washed with absolute alcohol then with ether, and dried at 30°C. in a vacuum oven. This material after drying was a white powder.

Sedimentation was at 59,733 R.P.M. at 20°C. using the Spinco ultracentrifuge. The interpretation of the results has been previously described (5). The solvent used was 0.2 M NaCl. The equations for the curves of regression of S_{20} (sedimentation constant corrected to water as solvent at 20°C.) on c (concentration in grams/100 ml.) were calculated by determining the coefficients for

$$S_{20} = \frac{A + Bc}{1 + Dc}$$

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by solving the following simultaneous equations:

$$\begin{aligned} nA + B\Sigma c - D\Sigma cS &= \Sigma S \\ A\Sigma c + B\Sigma c^2 - D\Sigma c^2 S &= \Sigma cS \\ A\Sigma cS + B\Sigma c^2 S - D\Sigma c^2 S^2 &= \Sigma cS^2 \end{aligned}$$

It was found that by using this set of simultaneous equations, coefficients for the final equations of regression could be calculated which fit the data for the polysaccharides better than those obtained by using the set of simultaneous equations (5) which proved best for desoxyribonucleic acids. The equations of regression were analyzed statistically by the same method employed formerly (5). The value of A is the value of S_{20} at zero concentration.

The viscosity studies were carried out at 20 ± 0.1°C. using the ordinary Ostwald laboratory viscometer with a flow time of 80 to 100 seconds for water. Density determinations were made using the Ostwald pycnometer (6). The polysaccharides were dissolved in 0.2 M NaCl, placed in a Visking casing, and dialyzed against 0.2 M NaCl for 24 hours. Before viscosity determination, the solutions were filtered through a coarse sintered glass filter to remove large particles. The concentrations were determined as was described previously (5). The relative viscosities, η_r, were calculated. For the somatic polysaccharide, the regression line for ln η_r on c was calculated by the method of least squares. This curve was analyzed statistically and interpreted. The slope of this line gave the weight intrinsic viscosity. For the capsular polysaccharide, the reduced viscosity, $(\eta_r - 1)/c$, was calculated in which c is the concentration in grams per 100 ml. It was found for this polysaccharide that the regression line for η_{sp}/c on c, in which $\eta_{sp} = \eta_r - 1$, gave a better straight line than when the regression of ln η_r on c was used. The regression line for η_{sp}/c on c was therefore calculated by the method of least squares. The intercept of this curve gives the weight intrinsic viscosity. This curve was analyzed statistically and interpreted.

The volume intrinsic viscosities could be calculated by multiplying the weight intrinsic viscosity by 100 and dividing by the respective partial specific volumes.

The partial specific volumes of the polysaccharides were determined as previously described (7).

RESULTS

In Fig. 1 is given an ultracentrifuge diagram of capsular polysaccharide. It would appear to be a single component indicating no great amount of contaminating material. An ultracentrifuge diagram of the somatic polysaccharide is given in Fig. 2. This material has one main component representing the somatic polysaccharide and a faster smaller component which would appear to be capsular polysaccharide as contaminant. This contaminant exists to the extent of about 10 to 15 per cent at most. Owing to the low concentration and diffusion, the peak of the contaminant has flattened.

A summary of the sedimentation data is given in Table I for both the somatic and capsular polysaccharides. The equations for the curves of regression of S_{20} on c are listed together with the standard error of estimate of the curve, S_y, and the correlation coefficient, r, in Table I.

In Table II is given a summary of the viscosity data for the somatic and the capsular polysaccharides. The equation for the curve of ln η_r *vs.* c, standard error of slope, σ_s, and the correlation coefficient, r, in the case of the

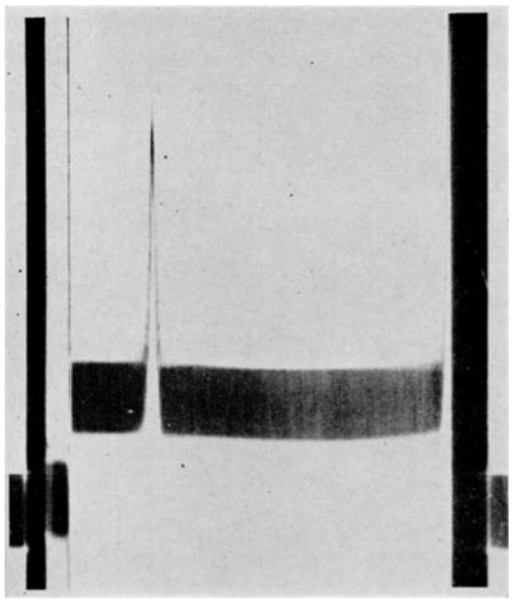

FIG. 1. Capsular polysaccharide, 0.6 per cent solution in 0.2 M NaCl, at end of 96 minutes, and schlieren angle of 55°. Migration toward the right.

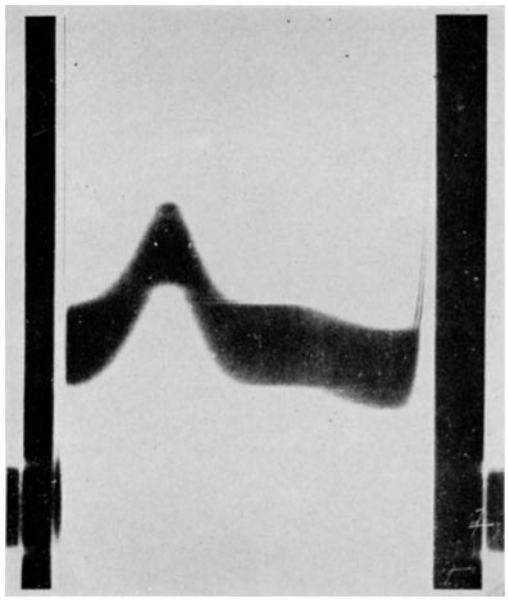

FIG. 2. Somatic polysaccharide, 1.0 per cent solution in 0.2 M NaCl, at end of 128 minutes, and schlieren angle of 45°. Migration toward the right.

TABLE I

Summary of Sedimentation Data

Somatic polysaccharide		Capsular polysaccharide	
c	S_{20}	c	S_{20}
gm./100 ml.	*Svedberg units*	*gm./100 ml.*	*Svedberg units*
5.00	0.67	1.00	1.22
4.00	0.79	0.80	1.43
3.00	0.99	0.60	1.58
2.00	1.18	0.40	1.92
1.00	1.53	0.20	2.85
0.80	1.54	0.10	3.20
0.60	1.63		
$S_{20} = \frac{1.89 - 0.12c}{1 + 0.19c}$		$S_{20} = \frac{4.06 + 0.45c}{1 + 2.73c}$	
$S_y = 0.03$		$S_y = 0.13$	
$r = 0.997$		$r = 0.985$	

TABLE II

Summary of Viscosity Data

Somatic polysaccharide		Capsular polysaccharide	
c	$\ln \eta_r$	c	η_{sp}/c
gm./ 100ml.		*gm./100 ml.*	
0.578	0.378	0.247	16.61
0.385	0.247	0.164	13.52
0.247	0.166	0.105	11.67
0.193	0.131	0.082	10.88
0.144	0.095	0.061	10.43
0.082	0.058	0.035	9.62
$\ln \eta_r = 0.66\ c$		$\eta_{sp}/c = 8.32 + 32.91\ c$	
$\sigma_s = 0.01$		$\sigma_I = 0.12$	
$r = 0.99992$		$r = 0.998$	
Volume intrinsic viscosity = $\frac{0.66}{0.608} \times 100 = 108.55$		Volume intrinsic viscosity = $\frac{8.32}{0.529} \times 100 = 1572.78$	

somatic polysaccharide are also given in Table II. The equation for η_{sp}/c *vs.* c, the standard error of the intercept, σ_I, and the correlation coefficient, r, in the case of capsular polysaccharide are likewise given in Table II.

The partial specific volume for the capsular polysaccharide was found to be 0.529 and represents the average of determinations made at four different

concentrations with a standard deviation of 0.008. The partial specific volume for the somatic polysaccharide was found to be 0.608 and represents the average of determinations made at three different concentrations with a standard deviation of 0.001.

When the molecules of the polysaccharides were considered to be prolate ellipsoids of revolution and the equations of Simha, Perrin, and Svedberg (8) were used, dimensions and molecular weights were calculated for the polysaccharides. Using a volume intrinsic viscosity of 108.55, a sedimentation constant of 1.89, and a partial specific volume of 0.608, the following values were calculated for the somatic polysaccharide: axial ratio, 37.35; molecular weight, 26,400; diameter of molecule, 0.97 mμ; and length of molecule, 36.18 mμ. Using a volume intrinsic viscosity of 1572.78, a sedimentation constant of 4.06, and a partial specific volume of 0.529, the following values were calculated for the capsular polysaccharide: axial ratio, 171.16; molecular weight, 171,800; diameter of molecule, 1.04 mμ; and length of molecule, 177.87 mμ.

Considering the polysaccharides to be flexible chain molecules one obtains by making the indicated substitutions in the Mandelkern and Flory (8, 9) equation the following values of molecular weight; for the somatic polysaccharide, 31,500 and for the capsular polysaccharide, 267,500.

DISCUSSION

Notwithstanding the fact that both the somatic and capsular polysaccharides are similar to the desoxyribonucleate with regard to alcoholic precipitating properties, the polysaccharides are as a whole much smaller in size than is the desoxyribonucleate. The sedimentation constants for the polysaccharides are much smaller than the sedimentation constant of the desoxyribonucleate. Of the two, the sedimentation constant of the capsular polysaccharide is larger than that of the somatic polysaccharide. The plots of S_{20} *vs.* c for both polysaccharides are not straight lines as in the case of proteins but are curves as in the case of desoxyribonucleates. The curves are flatter for the polysaccharides than in the case of desoxyribonucleates, but nevertheless they are curved. The sedimentation constant shows a great deal of dependence on concentration. All this sedimentation behavior points to a high degree of asymmetry on the part of both polysaccharides.

The viscosity work showed the capsular polysaccharide to have a much higher viscosity than the somatic polysaccharide. The ln η_r *vs.* c gave the best relation for the somatic polysaccharide viscosity data, while η_{sp}/c *vs.* c gave the best relation for the capsular polysaccharide data. In both cases the intrinsic viscosities indicate as did the sedimentation data high degrees of asymmetry on the part of both polysaccharides. Much could probably be learned by studying the viscosity behavior of these polysaccharides in various

solvents. The viscosity of the somatic polysaccharide is in error somewhat by the presence of a small amount of capsular polysaccharide. It is felt that a correction would be superfluous for this contaminant in view of the uncertainty in determining the absolute concentration of the contaminant.

The partial specific volume of the somatic polysaccharide is significantly higher than that of the capsular polysaccharide.

When the polysaccharides were considered to be flexible chain molecules, much larger molecular weights were obtained than when the molecules were assumed to be prolate ellipsoids of revolution.

SUMMARY

Sedimentation constants at infinite dilution have been found to be 1.89 and 4.06 for the somatic and capsular polysaccharides, respectively, from pneumococcus Type III.

Intrinsic viscosities have been determined for the somatic and capsular polysaccharides of pneumococcus Type III using the Ostwald viscometer.

Molecular weights and dimensions have been calculated for the somatic and capsular polysaccharides of pneumococcus Type III assuming the molecules to be prolate ellipsoids of revolution. Values for the somatic polysaccharide are: molecular weight, 26,400; diameter, 0.97 mμ; and length, 36.18 mμ. Values for the capsular polysaccharide are: molecular weight, 171,800; diameter, 1.04 mμ; and length, 177.87 mμ.

The molecular weights were calculated for the somatic and capsular polysaccharides of pneumococcus Type III assuming the molecules to be flexible chains. The value of the molecular weight of the somatic polysaccharide is 31,500 and the value for the molecular weight of the capsular polysaccharide is 267,500.

The molecules of both the somatic and capsular polysaccharides exhibit high degrees of asymmetry.

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