Acknowledgments.—The authors wish to thank A. Van Hook and C. A. Winkler for reading the manuscript.

Summary

The linear rate of crystallization of *p,p*-DDT from some supercooled binary melts has been determined, as a function of temperature and melt concentration. The second component of the melt may increase or decrease the rate. The apparent activation energy for the crystallization of pure *p,p'*-DDT is 33.4 kcal.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN, AND THE DEPARTMENT OF MEDICINE, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, THE PRESBYTERIAN HOSPITAL]

Fractionation and Physical–Chemical Studies of a Commercial Preparation of the Specific Polysaccharide of Type I Pneumococcus[1]

BY ROBERT A. ALBERTY AND MICHAEL HEIDELBERGER

The specific polysaccharide of Type I pneumococcus (SI)[2] is similar to the proteins in its electrochemical and solubility characteristics because it is also an amphoteric colloidal electrolyte. For this reason an attempt was made to apply the alcohol fractionation techniques which have been developed in the purification and separation of plasma proteins[3,4] to the purification of this polysaccharide. As with proteins, electrophoresis has proved a useful method for analyzing crude products[5] and those obtained by fractionation and in determining the isoelectric points of the components.

Experimental

The electrophoresis experiments were carried out in the usual Tiselius apparatus equipped with a cylindrical lens schlieren optical system. The mobilities were calculated by using the conductivity of the buffer in Donnan equilibrium with the electrophoresis sample determined at the temperature of the thermostat, 1°. Because of the high viscosity of the polysaccharide solutions the concentrations of the electrophoresis samples were made 0.5% or less.[6] Throughout the paper, mobilities on the descending side are given, as well as analyses obtained by averaging the results from the ascending and descending patterns.

The SI polysaccharide used as starting material was prepared by E. R. Squibb and Sons and supplied by the Commission on Pneumonia of the U. S. Army Epidemiological Board. The electrophoresis pattern for this preparation at *p*H 3.9, 0.10 ionic strength acetate buffer, is shown in Fig. 1a. The fast component which makes up 19% of the area of the moving peaks in the pattern contains nucleic acid, and the largest peak represents the SI polysaccharide. These components were identified after electrophoresis by withdrawal of small samples from various levels in the electrophoresis cell by means of a syringe with a long needle. The sample taken just below the fastest ascending peak showed the typical ultraviolet absorption of nucleic acid, with a

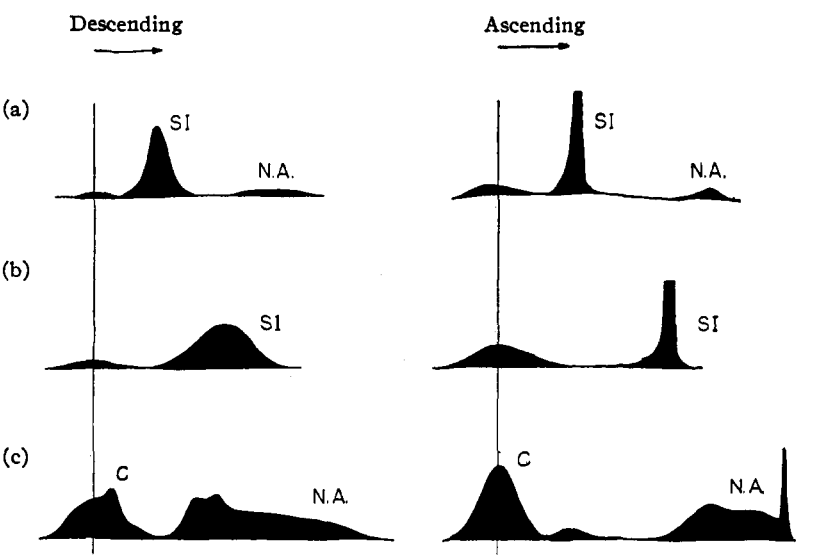

Fig. 1.—Electrophoresis diagrams: (a) commercial preparation, *p*H 3.90, $\Gamma/2 = 0.10$, after 144 minutes at 3.60 volts/cm.; (b) fraction A, *p*H 6.90, $\Gamma/2 = 0.10$, after 79 minutes at 3.28 volts/cm.; (c) fraction C, *p*H 6.90, $\Gamma/2 = 0.10$, after 80 minutes at 3.26 volts/cm.

(1) Presented before the Symposium on Sugars and Amino Sugars in Biochemistry, American Chemical Society Meeting, Atlantic City, April, 1947.

(2) (a) Avery and Heidelberger, *J. Exptl. Med.*, **42**, 367 (1925); (b) Heidelberger, Goebel and Avery, *ibid.*, **42**, 727 (1925); (c) Heidelberger, Kendall and Scherp, *ibid.*, **64**, 559 (1936).

(3) Cohn, Strong, Hughes, Mulford, Ashworth, Melin and Taylor, THIS JOURNAL, **68**, 459 (1946).

(4) Deutsch, Gosting, Alberty and Williams, *J. Biol. Chem.*, **164**, 109 (1946).

(5) As indicated in preliminary tests carried out several years ago with Dr. Dan H. Moore at Columbia University.

(6) The relative viscosity of a 0.1% solution of SI in 0.9% sodium chloride is 1.69; ref. 2c.

maximum at 255 mμ. Samples tested serologically showed that the slower moving component was SI and that the C polysaccharide (the somatic polysaccharide common to all types of pneumococci) did not move in the electrical field or moved so slowly it was not resolved from the salt boundary. The C substance had previously been observed[7] to move with a low mobility (-2.2×10^{-5} at *p*H 7.85 and -1.2×10^{-5} at *p*H 6.20) at 0.05 ionic strength. Since there was no resolution at 0.10 ionic strength it was difficult to estimate the amount of C substance present, but it could not exceed 10% because the area of the stationary boundary was 10–15% of the total area of the electrophoretic pattern in experiments at various *p*H and ionic strengths (see Fractionation). The areas in the electrophoresis pattern were not weighted for differences in refractive index increment for the polysaccharide and nucleic acid since the increments are presumably about the same.[8] Although a trace of nucleoprotein was believed present, it did not appear in the electrophoresis patterns because of the small amount or because its mobility was close to that of one of the other components.

In spite of the high viscosity of the polysaccharide solutions the velocity of the nucleic acid boundary on the ascending side where it moves up into buffer was only slightly higher (about 15%) than on the descending side where it moved into the viscous polysaccharide solution. This difference is caused by the presence of the conductivity gradient at the stationary boundary, the nature of which is understood[9] so that the mobility of the nucleic acid is relatively independent of the viscosity due to the polysaccharide.

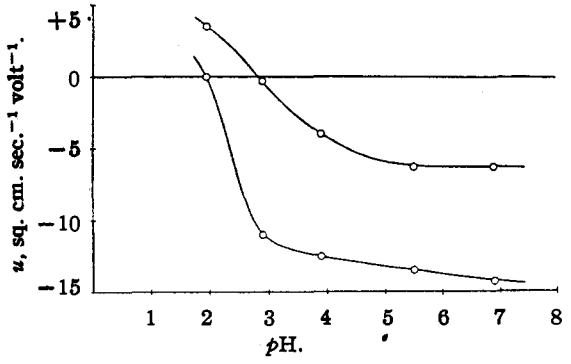

Fig. 2.—Determination of isoelectric points at $\Gamma/2 = 0.10$; upper curve, SI polysaccharide; lower curve, nucleic acid.

Results and Discussion

1. Determination of Isoelectric Point.—The isoelectric point of the SI and nucleic acid in the crude mixture were determined at 0.10 ionic strength from mobility determinations at several *p*H with sodium glycinate, acetate and cacodylate buffers. Figure 2 is a plot of mobility *vs.* *p*H for the two moving components in this material. The isoelectric point for the SI at this ionic strength is *p*H 2.8, and that for the nucleic acid is *p*H 2.0. Since the experiments were performed on mixtures, there is the possibility of some interaction between the components, although this was not evidenced by the electrophoretic patterns.[10] The isoelectric point for the nucleic acid is in agreement with earlier values.[11] The presence of basic groups in the SI polysaccharide is confirmed by the positive mobility at *p*H 2.0.

A sample of SI prepared by earlier methods[2] had a mobility of -5.5×10^{-5} at *p*H 6.9, $\Gamma/2 = 0.10$, in agreement with the mobility of the commercial preparation. Another sample prepared from cultures concentrated by heat and purified in part by precipitation from alkaline solution showed a mobility of -6.5×10^{-5} at *p*H 5.5 in agreement with that obtained for the undegraded SI in spite of the known removal, under these conditions, of an O-acetyl group.[12]

2. Fractionations.—Several fractionations with alcohol were performed on the commercial preparation in the cold in attempts to obtain SI free from nucleic acid and C substance. Addition of ethyl alcohol to dilute solutions (0.5%) at the low ionic strengths ($\Gamma/2 = 0.01$ to 0.1) most suitable for the fractionation of proteins at 0° produced voluminous gels which were not suitable for fractionation. With isopropyl and *n*-propyl alcohols and the much higher ionic strengths customarily used in the precipitation of carbohydrates (3% sodium acetate), there was less tendency to form gels. Figure 3 shows the flow sheet for a fractionation with the electrophoretic analysis (electro.) and serological assay (serol.) of the various fractions. The analytical electrophoresis experiments were carried out at *p*H 6.9 in a cacodylate buffer of 0.10 ionic strength (0.02 *N* sodium cacodylate, 0.08 *N* sodium chloride). The percentage given for the "fast" component includes material with a more negative mobility than -10×10^{-5}. Fraction C contained a large amount of such material (Fig. 1c), although only a portion was nucleic acid as determined with the Beckman spectrophotometer at a wave length of 255 mμ and the extinction values given by Kunitz.[13] Fraction A showed a single symmetrical gradient in electrophoresis and precipitated 90% as much antibody[14] as the same amount of the most active sample of SI available. The electrophoresis pattern is given in Fig. 1b.

Fraction C showed an abnormally large stationary boundary in electrophoresis because of the

(7) Goebel, Shedlovsky, Lavin and Adams, *J. Biol. Chem.*, **143**, 1 (1943).

(8) Seibert and Watson, *ibid.*, **146**, 55 (1941), obtained 0.0013 for the specific refractive increment for nucleic acid.

(9) Longsworth and MacInnes, THIS JOURNAL, **62**, 705 (1940).

(10) Longsworth and MacInnes, *J. Gen. Physiol.*, **25**, 507 (1942).

(11) Stenhagen and Teorell, *Trans. Faraday Soc.*, **35**, 743 (1939).

(12) Enders and Pappenheimer, *Proc. Soc. Exptl. Biol. Med.*, **31**, 37 (1933); Avery and Goebel, *J. Exptl. Med.*, **58**, 731 (1933).

(13) Kunitz, *J. Biol. Chem.*, **164**, 563 (1946).

(14) For the method, *cf.* Heidelberger and Kendall, *J. Exptl. Med.*, **55**, 555 (1932).

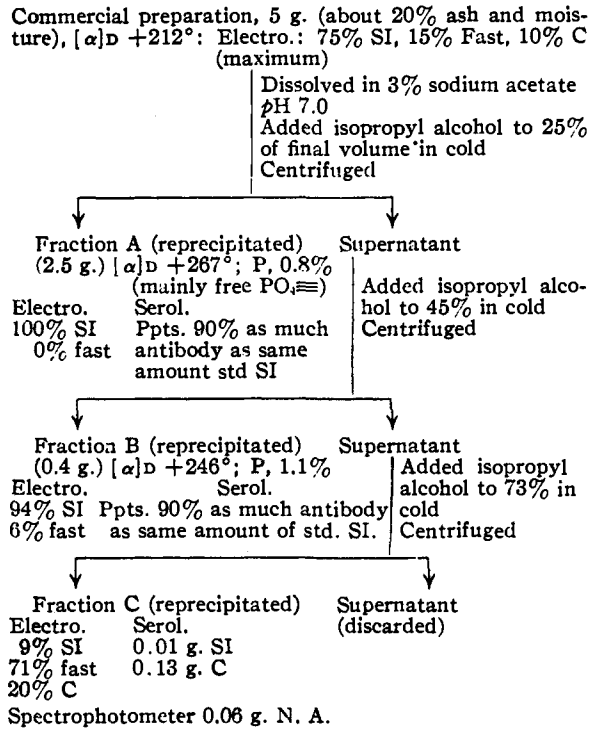

Fig. 3.—Fractionation flow sheet

presence of C substance (see Fig. 1c). The buffer dilution factor at the stationary boundary was calculated from the area of the rising and descending moving peaks. The portion of the area of the ϵ boundary due to salt was calculated by assuming the buffer dilution factor to be equal to the dilution factor for the fast peak. The values indicate that Fraction C contains approximately 20% C.[15] Electrophoresis indicated more SI in Fraction C than did serological assay, but the serological activity of SI may possibly be partially inhibited by excess C substance. Isopropyl alcohol has been found to be a useful reagent in the fractionation and purification of other specific bacterial polysaccharides.

3. Sedimentation Velocity.—Several sedimentation experiments with the purified SI were carried out at 50,400 r. p. m. in the standard Svedberg oil turbine velocity ultracentrifuge with a cell having a 12-mm. optical path. Both the sedimentation constant and the rate of boundary spreading are quite dependent on concentration as shown in Fig. 4. However, as shown by Gralén,[16] the calculated value of s taken from the apex of the peak does not correspond to the same molecules in experiments at different concentrations. Information as to homogeneity and molecular weight of this material may be obtained only by extrapolation of sedimentation constants and rate of boundary spreading to infinite dilution.

Acknowledgment.—The authors wish to express their appreciation for the interest and helpful suggestions of Dr. J. W. Williams.

Summary

Fractionation procedures with isopropyl alcohol have been applied to the purification of the specific polysaccharide of Type I pneumococcus (SI). Electrophoretic and serological assays were used for the analysis of products. The isoelectric points of the SI and nucleic acid in the crude preparation have been determined by electrophoresis.

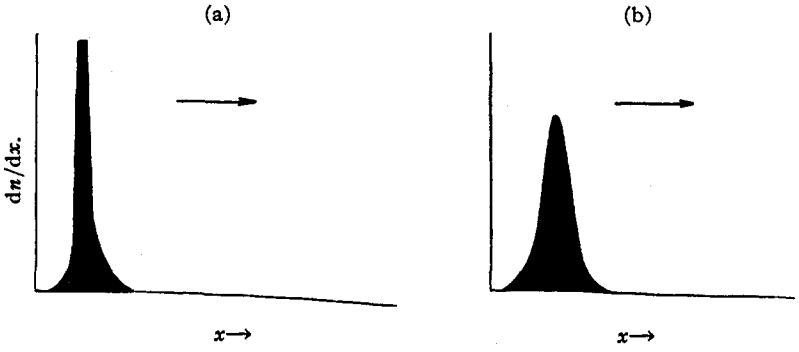

Fig. 4.—Sedimentation diagrams for purified SI (Fraction A) at 50,400 r. p.m. (pH 5.5, acetate buffer, Γ/2 = 0.10): (a) 0.5% solution after 119 minutes, s_{20w} = 1.2S; (b) 0.15% solution after 119 minutes, s_{20w} = 2.3S.

(15) The calculation of the protein dilution factor across the stationary boundaries in electrophoresis has been described by Longsworth and MacInnes, ref. 9, and by Longsworth, *Chem. Rev.*, **30**, 323 (1940). The application of these calculations to the determination of immobile components in electrophoresis will be described in a future publication.

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NEW YORK, NEW YORK

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(16) Gralén, Sedimentation and Diffusion Measurements on Cellulose and Cellulose Derivatives, Inaugural Dissertation, Upsala, 1944.