ADDENDUM

ELECTROPHORETIC AND DIFFUSION MEASUREMENTS ON CELL WALL OLIGOSACCHARIDES FROM *CORYNEBACTERIUM DIPHTHERIAE*

by

T. J. BOWEN

Department of Biochemistry, The University, Leeds (England)

INTRODUCTION

The oligosaccharide samples obtained from the wall of *Corynebacterium Diphtheriae* were prepared by Dr HOLDSWORTH (previous paper). Material prepared by the picric acid and also by the barium hydroxide route was studied but the quantity available was always small, particularly of the former.

Electrophoretic and diffusion experiments were carried out in a Michaelis buffer at pH 8 and ionic strength of 0.2 in which sodium chloride contributed 0.13 of the ionic strength. The preparation and advantages of this buffer have been described by WIEDEMANN[1]. The apparatus used was a modified TISELIUS[2] apparatus with PHILPOT-SVENSSON[3] optical system supplied by Adam Hilger, Ltd. (London).

ELECTROPHORESIS

Dialysis before electrophoresis was impossible because of the low molecular weight of the material. Donnan effects were, however, negligible since the picric acid material was uncharged and also the main component of the barium hydroxide preparation. Furthermore, the material was dissolved directly in the buffer (ionic strength 0.2) to give solutions never greater than 1%. All electrophoretic runs were performed at 0.5° C at 2.92 volts/cm and a heat dissipation below 0.15 watts/cm^3.

The oligosaccharide prepared by the picric acid procedure gave a single boundary immobile over periods up to 5½ hours. No other component could be detected under these conditions. Zero electrophoretic mobility was expected from the chemical evidence (previous paper).

One sample of the oligosaccharide prepared by the barium hydroxide route was examined under similar conditions. Two peaks were observed in the electrophoretic pattern, one stationary component accounting for most of the material, but, in addition, a component with a mobility of $-5.20 \cdot 10^{-5}$ cm^2 volt^{-1} sec^{-1} accounted for 10% of the total refractive area.

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DIFFUSION

The use of the TISELIUS apparatus for the determination of diffusion coefficients has been described by LONGSWORTH[4,5]. The water bath was maintained at 20° ± 0.01° C and stirred by a pump remotely situated to eliminate vibration. Boundaries were displaced into the cell more slowly than the 0.1 cm/min limit advocated by BYWATER AND JOHNSON[6]. A preliminary run on 1% sucrose solution gave results for the diffusion coefficient, corrected to pure water as solvent at 20° C, of $D_{20}^{H_2O} = 4.53$ and $4.61 \cdot 10^{-6}$ cm^2 sec^{-1}. This is in reasonable agreement with accepted values. Furthermore, the sucrose Schlieren peaks coincided with theoretical gaussian distributions and justified the use of the optical system for these measurements in the absence of the more usual Lamm scale or interferometric technique. The inclined wire method was used in these runs (SVENSSON[7]).

Only one diffusion run was possible on the picric acid oligosaccharide due to lack of material, but a duplicate result was obtained from the two limbs of the Tiselius cell on a 0.8% solution. The result was calculated by the area-maximum height method and, after applying a viscosity correction for the buffer, gave values of $D_{20}^{H_2O} = 2.84$ and $2.80 \cdot 10^{-6}$cm^{2}sec^{-1}. Figure 3 shows a typical Schlieren peak compared with the theoretical gaussian distribution. Close agreement between the two curves, particularly important at the maximum ordinate, indicates that the sample was monodisperse. Any inorganic matter which may have been present (see below) was not detected.

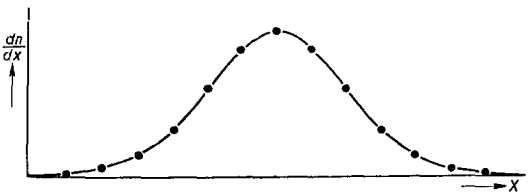

Fig. 3. Diffusion of Oligosaccharide (prepared via picric acid) after 14,970 seconds. Circles are points on the theoretical Gaussian curve.

Diffusion studies on impure materials are not generally satisfactory, but the barium hydroxide oligosaccharide was studied in an attempt to obtain additional information. Since the electrophoretic evidence indicated two components in a sample of this oligosaccharide the curves obtained of concentration gradient against cell co-ordinate were solved by the methods described by NEURATH[8]. Two diffusion runs were carried out on two different batches of the oligosaccharide. One preparation gave a major component with $D \approx 2.5 \cdot 10^{-6}$ cm^{2}sec^{-1} containing about 20% of a component with $D \approx 1.4 \cdot 10^{-6}$ cm^{2}sec^{-1}. The other sample was apparently purer and mainly contained a component with $D \approx 3.3 \cdot 10^{-6}$cm^{2}sec^{-1} and a small amount of another component which was just sufficient to prevent curves being precisely Gaussian. Of necessity, such solutions are very approximate even if the material is strictly a two-component system. However, the results are suggestive and indicate that the major component of the barium hydroxide oligosaccharide may possibly be identified with the picric acid oligosaccharide as claimed by Dr HOLDSWORTH.

ESTIMATION OF THE MOLECULAR WEIGHT

The low molecular weight of these materials was first indicated by the loss of a sample during dialysis prior to electrophoresis.

An attempt was made to determine the molecular weight of the picric acid oligosaccharide by cryoscopic methods using water as the solvent. An unexpectedly low value for the molecular weight was traced, by conductivity measurements, to an im-

purity which was probably hydrochloric acid used in the purification of the material. Two further recrystallisations from alcohol reduced the conducting impurity (calculated as HCl) from 2% to 0.5% thus ruling out the possibility of the conductivity arising from an ionisable group in the oligosaccharide. The material, already meagre, dwindled at each crystallisation, but the molecular weight determinations extrapolated to a value of approximately 1100.

Scarcity of material prevented the determination of the partial specific volume and the determination of a maximum possible value for the molecular weight as described by PEDERSEN AND SYNGE[9]. Instead, a semi-empirical method was used described by POLSON AND VAN DER REYDEN[10]. These workers found that for a molecule of "compact form" the molecular weight is related to the diffusion coefficient by the formula —

$$D = \frac{a}{M^{1/3}} + \frac{b}{M^{2/3}} + \frac{c}{M}$$

where $a = 2.74 \cdot 10^{-5}$, $b = 1.65 \cdot 10^{-5}$, and $c = 17.00 \cdot 10^{-5}$. The equation appears to be valid over a wide range of molecular weights from water to African horse sickness virus. If it holds in the present case a diffusion coefficient of $2.82 \cdot 10^{-6}$ $cm^2.sec^{-1}$ corresponds to a molecular weight of 1220. This figure will be in error if shape and hydration are not "normal" for this oligosaccharide.

Although these values for the molecular weight can only be approximate they fall into line with the end-group titration data and chromatographic evidence of Dr HOLDSWORTH. A multiple of the minimum molecular weight expected on the chromatographic analyses seems improbable.

SUMMARY

A sample of an oligosaccharide prepared from the cell wall of *C. diphtheriae* was electrophoretically pure and had zero mobility. Its diffusion coefficient was $2.82 \cdot 10^{-6}$ cm^2/sec corresponding to a molecular weight of approximately 1220.

Another sample prepared by a different method appeared to be similar but less pure.

RÉSUMÉ

Une préparation d'un oligosaccharide obtenu à partir de la paroi cellulaire de *C. diphtheriae* était électrophorétiquement pure et avait une mobilité nulle. Son coefficient de diffusion était de $2.82 \cdot 10^{-6}$ cm^2/sec, ce qui correspond à un poids moléculaire d'approximativement 1220.

Une autre préparation obtenue par une méthode différente s'est révélée semblable mais moins pure.

ZUSAMMENFASSUNG

Ein Oligosaccharid-Präparat aus der Zellwand von *C. diphtheriae* war elektrophoretisch rein und seine Mobilität war null. Sein Diffusionskoeffizient war $2.82 \cdot 10^{-6}$ cm^2/Sek, was einem Molekulargewicht von ungefähr 1220 entspricht.

Ein anderes Präparat, das mit Hilfe einer anderen Methode hergestellt war, erwies sich als dem ersten ähnlich, jedoch weniger rein.

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