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Preliminary communication

An improved procedure for the isolation of meningococcal, polysaccharide antigens, and the structural determination of the antigen from serogroup X*

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It has recently been demonstrated that the meningococcal, capsular polysaccharides are immunogenic in man, and play an important role in providing protection against meningococcal meningitis in humans[1,2]. Procedures previously employed for the isolation of the group-specific polysaccharide antigens[1,3,4] involved tedious and time-consuming methods for the removal of protein and nucleic acid contaminants. The method herein reported is simple and fast, and is adaptable to precipitation procedures employing either Cetavlon[1] or ammonium sulfate[3]; it has been shown to produce the pure, serogroup X polysaccharide, and other serogroup polysaccharides also, in good yields. Recent immunochemical studies on the meningococcal polysaccharide antigens have established that the one from serogroup A is[4] a homopolymer of partially *O*-acetylated 2-acetamido-2-deoxymannosyl phosphate, whereas those from serogroups B and C are[5] both homopolymers of sialic acid. We now report a related homopolymer, isolated from serogroup X, which is composed of 2-acetamido-2-deoxyglucosyl phosphate residues. This is the first reported identification of 2-amino-2-deoxyglucose as a constituent of the meningococcal polysaccharides.

N. meningitidis, strain 247 X (Canadian Communicable Disease Centre, Ottawa), was grown on a chemically defined[6] medium (NCDM), and the bacteria were inactivated by the addition of formaldehyde (0.5%), instead of merthiolate; this modification led to enhanced yields of the polysaccharide. Cells were harvested with a Sharpless continuous centrifuge, and ammonium sulfate (600 g per liter) was added to the culture medium, together with sodium hydrogen carbonate to maintain the neutrality of the solution. A solution of the crude, precipitated polysaccharide in the minimal volume of 0.2M phosphate buffer (pH 6.98) was extracted for 5 min at 65–70° with an equal volume of 90% phenol, by the procedure of Westphal and co-workers[7]. The aqueous extracts were combined, dialyzed, and digested with ribonuclease (Worthington Biochemical Corp.) for

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24 h at 37°. This solution was continuously dialyzed against water in a Diaflo ultrafiltration cell having a PM-10 membrane. Almost all of the contaminants remaining were removed from the polysaccharide (high molecular weight), which was retained by the membrane. Lyophilization of the aqueous solution remaining in the cell afforded the pure polysaccharide in yields of ~20 mg per liter of the original culture medium. This procedure has also been found applicable to isolation of the capsular-polysaccharide antigens of serogroups A, BO_6, Y, and Z.

The serogroup X polysaccharide was found to have a molecular weight slightly lower than that of the serogroup A polysaccharide reported by Gotschlich and co-workers[1,4], as it was only partially excluded on a column of Sephadex G-200. However, this difference in molecular weight is not attributable to the extraction procedure, as the crude, precipitated antigen behaved identically to the purified polysaccharide on a column of Sephadex G-200. Also, by using the methods of Plescia and co-workers[8], the polysaccharide was found to be of sufficiently large molecular weight to elicit antibody production in rabbits. The antibody to the pure polysaccharide was detected by immunodiffusion analysis.

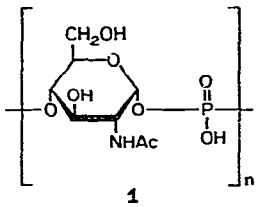
The analysis of the polysaccharide is summarized in Table I. The analysis for

TABLE I

ANALYSIS OF POLYSACCHARIDE FROM *N. meningitidis* SEROGROUP X

	Percentage, by weight	*Molar ratio*
C	29.2	
H	4.3	
N	4.3	
P	9.3	1.00
Total Ac	11.8	1.0
GlcN	51.8	1.05
Amino acids	<1	
Nucleic acid	~2–3	

amino sugar and amino sugar phosphate was performed with a Technicon auto-analyzer[9], after acid hydrolysis of the polysaccharide (4M HCl at 100°, at intervals of 4 to 24 h). 2-Amino-2-deoxyglucose was found to be the only amino sugar present, and the total content of 2-amino-2-deoxyglucose was estimated by extrapolation to zero time. This analysis was also confirmed by the Morgan–Elson method[10], after acid hydrolysis of the polysaccharide (0.1M HCl for 10 min at 100°) and subsequent incubation of the hydrolyzate with alkaline phosphatase (Sigma Chemical Co.). Phosphorus[11] and total acetyl[12] were determined by established procedures. The molar ratio of 2-amino-2-deoxyglucose to phosphorus, namely, 1.05:1.00, coupled with the lability of the polymer to mild hydrolysis with acid (0.1M HCl for 10 min at 100°) or autohydrolysis (30 min at 100°), suggest a structure similar to that depicted (1). The detection, by paper chromatography and paper electrophoresis, of a 2-acetamido-2-deoxyglucose phosphate as the sole product of mild hydrolysis with acid supports this conclusion.



1

The 2-acetamido-2-deoxyglucosyl residues are linked by phosphate diester bonds which, from the high, positive, specific rotation (+135°, *c* 1.25 in water) of the polysaccharide, are probably in the α configuration. In this respect, the polymer resembles the polymer of 2-acetamido-2-deoxymannosyl phosphate obtained[4] from *N. meningitidis* serogroup A, but, in this, the phosphate diester bonds are not (1→6)-linked. The serogroup X polysaccharide was resistant to periodate oxidation, as determined by titrimetric[13] and spectrophotometric[14,15] methods; this resistance could not be due to acetyl groups on O-3 or O-4 of the 2-amino-2-deoxyglucosyl residue as none were detected by infrared spectroscopy or *O*-acetyl analysis[16].

On the basis of the results of these chemical procedures, it was not possible to distinguish between the other structures feasible for **1**; namely, the (1→3)-, (1→4)- or mixed (1→3)- and (1→4)-linked polymers. However, recently, by use of the technique of Fourier-transform, ^{13}C nuclear magnetic resonance, it has been found that the spectrum of the polysaccharide is consistent only with the (1→4)-linked polymer depicted in structure **1**. The results of this work will be presented in detail elsewhere[17].

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