DEGRADATION STUDIES ON THE LIPOPOLYSACCHARIDE FROM *E. COLI* 071:K?:H12. SEPARATION AND INVESTIGATION OF *O*-SPECIFIC AND CORE POLYSACCHARIDES

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1. Introduction

Recently, we described the isolation and identification of 3-amino-3,6-dideoxyglucose, obtained from the lipopolysaccharide of *E. coli* 071:K?:H12 [1]. This lipopolysaccharide also contains the amin sugars glucosamine and galactosamine. Preliminary investigations led to the conclusion that 3-amino-3,6-dideoxyglucose and galactosamine are constituents of the polysaccharide portion and glucosamine is present only in the lipid component (lipid A [2]). To verify this conclusion the lipopolysaccharide was mildly hydrolyzed into lipid and 'degraded polysaccharide'. The latter was further fractionated by Sephadex filtration and on DEAE-Sephadex.

The results described here show that the 'degraded polysaccharide' can be resolved on Sephadex columns into two fractions. One of these is predominantly composed of *O*-specific sugar components [2] and is reactive in 071 antiserum. The second fraction, which is not reactive in 071 antiserum, consists almost entirely of the basal sugars [2]. Under these hydrolytic conditions the major part of KDO was split from the polymer. During Sephadex filtration the liberated KDO emerges after the second peak. Glucosamine, which constitutes part of the Salmonella core (at least in smooth forms and Ra mutants) was not detected in either polysaccharide fraction. Thus these findings not only demonstrate that *O*-reactive polysaccharide chains can be obtained rather easily but also indicate a core structure of *E. coli* lipopolysaccharide which differs from that of Salmonella [2] and Shigella [3].

2. Materials and Methods

E. coli strain P10a (serological formula 071:K?:H12) was used. Isolation and purification of the lipopolysaccharide was performed as described previously [4]. The determinations of glucose, galactose, glucosamine, heptose, and KDO were carried out as described in an earlier communication [5]. Galactosamine was assayed with the Elson-Morgan reaction after enzymatic deamination of glucosamine [6] and 3-amino-3,6-dideoxyglucose according to Ashwell et al. [7]. Phosphate was determined with the method of Lowry et al. [8]. Sedimentation and diffusion constants were determined using a Spinco Model E analytical ultracentrifuge. From these values the molecular weight was calculated. Independently, the method of Yphantis was used for the determination of molecular weight. Gel filtration was performed with Sephadex G50. For ion exchange chromatography DEAE-Sephadex A25 was used. Immune precipitation experiments were performed as described previously [9]. 071 antiserum (rabbit) was obtained from Drs. L. and F. Ørskov (Statens Seruminstitut/Copenhagen).

3. Results and Discussion

The lipopolysaccharide of *E. coli* 071:K?:H12 consists of the basal sugars [2] heptose, KDO, galactose, glucose and glucosamine. Additionally, it contains galactosamine, 3-amino-3,6-dideoxyglucose and

Table 1
Composition of the lipopolysaccharide of *E. coli* 071:K?:H12 and fractions derived therefrom. For details see text.

Components	LPS	P1	$P1_{DEAE}$	P2
3-Amino-3,6-dide-oxyglucose	9.86	21.30	24.20	0.46
Galactosamine	16.50	28.12	29.80	1.86
Rhamnose	9.98	22.80	24.80	1.45
Galactose	8.85	16.40	19.05	10.75
Glucose	4.12	2.90	1.22	17.44
Heptose	7.00	0.90	–	25.23
KDO	6.10	0.70	–	3.81
P	3.00	0.30	0.03	4.80
Lipid A	30.00	–	–	–

rhamnose. The analytical values are listed in table 1. In analyzing the values obtained it was taken into consideration that: 1) 3-amino-3,6-dideoxyglucose gives a positive reaction in the Dische assay, which was used for the determination of rhamnose (on a molar basis the color intensity of the amino sugar is 6% of that of rhamnose), and 2) galactosamine as well as galactose react with galactose oxydase, and therefore to determine galactose it was necessary to subtract the value found for galactosamine in the amino sugar assay.

The following procedure was adopted for the degradation experiments: heating of the lipopolysaccharide in 1% acetic acid at 100° for 1.5 hr, lyophilization of the reaction mixture, trituration of the material with water, centrifugation at 3000 rpm to separate into polysaccharide and lipid A. Lipid A was obtained as sediment, the 'degraded polysaccharide' was isolated from the supernatant by lyophilization. With this procedure 65% of the material was obtained as 'degraded polysaccharide' and 30% as lipid A.

The 'degraded polysaccharide' was fractionated on Sephadex G50 with water as eluant. When fractions were tested with the phenol/sulfuric acid assay [10] two peaks were found, the first being eluted immediately after the dead volume of the column. In fig. 1 the elution pattern is shown (curve A). In one case the bacterial culture used was instable in auramin and upon heatin (which is indicative of rough characteristics [11]) but agglutinated in 071 serum (characteristic for smooth forms). When the lipopolysaccharide of these bacteria was degraded the elution pattern of the resulting 'degraded polysaccharide' differed from the one described before with respect to the ratios of peak 1 and peak 2 (see fig. 1, curve B).

The material from the first peak (P1) consists predominantly of galactosamine, 3-amino-3,6-dideoxyglucose, galactose and rhamnose in a molar ratio of 1.2:1.00:0.91:1.33. These values can be reconciled with the concept of a tetrasaccharide repeating unit of the polysaccharide. On analytical ultracentrifugation this material showed one broad peak, suggesting a uniform molecular weight distribution. From its sedimentation constant ($s_{25} = 1.21 \times 10^{-13}$ sec) and diffusion constant ($D_{25} = 6.05 \times 10^{-7}$ cm sec^{-1}) a molecular weight of 13.900 was calculated. Using the method of Yphantis a value of 13.800 was found. On the assumption of an ordered structure built by

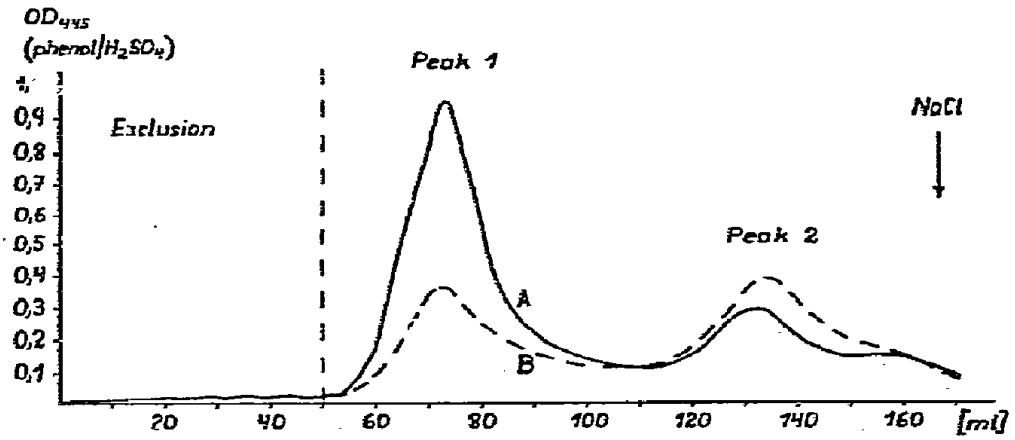

Fig. 1. Fractionation on Sephadex G50 of the 'degraded polysaccharide' derived from the lipopolysaccharide of *E. coli* 071:K?:H12. For details see text.

tetrasaccharide repeating units P1 would consist of about 17 such units.

Material from peak 2 (P2), the analyses of which are included in table 1, consists predominantly of heptose, glucose and galactose in a molar ratio of 1.28:1.00:0.62. It also contains phosphorus (ratio P:heptose is 0.77:1.00) and KDO. All these are known to be components of the basal structure of lipopolysaccharides [2]. It is important to note that this fraction does not contain glucosamine. Also there is a loss of KDO. The latter component was partially split off the lipopolysaccharide during degradation and was eluted from Sephadex columns after peak 2. The generality of this finding is currently under investigation. This lead us to test whether glucosamine would also be split off during degradation and thus escape detection in P1 and P2. However, no free glucosamine was found in the degradation mixture (aliquotes were tested after various times).

The fact that glucosamine is completely absent from the polysaccharide part of the lipopolysaccharide is of interest. It points to a core structure different from those of Salmonella [2] and Shigelle [3]. Therefore our investigations are being extended to the lipopolysaccharides of other *E. coli* strains and R-mutants. First results indicate that glucosamine indeed is not a constituent of the *E. coli* core.

As can be seen from table 1, P1 contains small amounts of phosphorus and heptose — which are components of P2. Also small amounts of glucose are present. This can be explained by either contamination of P1 with small amounts of P2, or by the fact that the lipopolysaccharide was fragmented in such a way that breakage occurred mainly in an internal structure (core) so that small basal fragments are chemically linked to long polysaccharide chains. In order to differentiate between these possibilities an aliquot of P1 was fractionated on DEAE-Sephadex. Elution with water gave a material ($P1_{DEAE}$) which was about 30% of the total and was practically free of phosphorus and heptose but still contained some glucose. Later fractions, eluted with 1% acetic acid and with 1M acetate buffer (pH 4.0), showed increasing amounts of phosphorus, heptose and glucose.

These results can be interpreted in the following way. There are obviously several weak linkages within the internal structure (core) of the lipopolysaccharide which break under the conditions of degradation. Thus

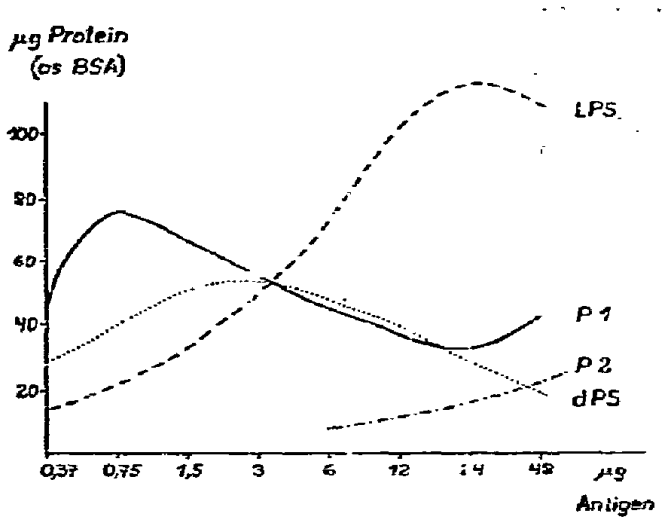

Fig. 2. Immune precipitation in 071 antiserum of the lipopolysaccharide of *E. coli* 071:K?:H12 and fractions derived therefrom. For details see text.

outer polysaccharide chains, some of which have attached core-fragments of different sizes, are left intact.

On the basis of chemical composition the conclusion can be drawn that P1 represents the *O*-specific polysaccharide chains and P2 the underlaying rough structure. This was verified by testing the reactivities of both fractions in 071 antiserum using immune precipitation. The results are given in fig. 2. As is evident from fig. 2 the crude 'degraded polysaccharide' (dPS) and to a greater extent P1 is reactive in 071 antiserum while P2 is not. For comparison the reactivity of the intact lipopolysaccharide (LPS) is included.

The results described here show that degradation of *E. coli* lipopolysaccharides — as demonstrated with *E. coli* 071:K?:H12 — brings about cleavage of the polymer at such points that separation yields: 1) *O*-reactive polysaccharide (in the case of *E. coli* 071:K?:H12 this has a molecular weight of about 14.000 and comprises about 17 tetrasaccharide repeating units), 2) core material which is not reactive in 071 antiserum (this has a glucose/galactose ratio of 2:3 and is free of glucosamine, which seems to be a general feature of the *E. coli* core region) and 3) lipid A.

References

[1] B. Jann, K. Jann and E. Müller-Seitz, Nature 215 (1967) 170.
[2] O. Lüderitz, A. M. Staub and O. Westphal, Bacteriol. Rev. 30 (1966) 192.
[3] J. H. Johnston, R. J. Johnston and D. A. R. Simmons, Biochem. J. 105 (1967) 79.
[4] O. Westphal and K. Jann, Methods in Carbohyd. Chem. (Academic Press Inc., New York, 1965) vol. 5, p. 83.
[5] B. Jann and K. Jann, Europ. J. Biochem. 2 (1967) 26.
[6] H. J. Risse and O. Lüderitz, Biochem. Z. 341 (1964) 1.
[7] G. Ashwell, N. C. Brown and W. A. Volk, Arch. Biochem. Biophys. 112 (1965) 648.
[8] O. H. Lowry, N. R. Roberts, K. Y. Leiner, M. L. Wu and A. L. Farr, J. Biol. Chem. 102 (1954) 1.
[9] K. Jann, B. Jann, F. Ørskov, I. Ørskov and O. Westphal, Biochem. Z. 342 (1965) 1.
[10] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, Anal. Chem. 28 (1955) 350.
[11] E. Kröger, Erg. Hyg. Bakteriol. Immunforsch. 29 (1955) 475.