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Chemical and serological investigations on the genus-specific lipopolysaccharide epitope of *Chlamydia*

(synthetic antigens/monoclonal antibodies/serology/GLC/MS)

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ABSTRACT **Members of the bacterial genus *Chlamydia* are responsible for widespread disease among humans and animals, including endemic trachoma in developing countries, venereal disease in developed countries, and a variety of other diseases such as infantile pneumonia and lymphogranuloma venereum. Although there is little genetic relatedness between and large antigenic diversity between and among the two chlamydial species, one antigenic determinant has been preserved among all serovars: the genus-specific lipopolysaccharide epitope. In this report, the tools of molecular genetics, monoclonal antibodies, and analytical and synthetic chemistry have been combined to determine the structure of this epitope. This epitope is attributed to the presence of a trisaccharide of 3-deoxy-D-*manno*-octulosonic acid (KDO) of the sequence KDO*p*-(2→8)-KDO*p*-(2→4)-KDO. The structure includes a unique linkage of two KDO residues through a 2.8-linkage.**

Chlamydiae are pathogenic, obligatory intracellular parasites causing a variety of diseases in animals and humans (1). In developing countries, *Chlamydia trachomatis* causes hyperendemic trachoma (≈350 million cases), which is the world's leading cause of secondary blindness. In developed nations, Chlamydiae are the major cause of nongonococcal urethritis, which is assumed to be the most frequent, sexually transmitted disease (2). Chlamydiae have a unique developmental cycle, including metabolically inactive but infectious elementary bodies that differentiate inside the host cell into metabolically active, noninfectious, multiplying reticulate bodies (3). Chlamydiae may also cause persistent chronic infections (4). Albeit the molecular mechanisms of intracellular chlamydial differentiation are not known, surface components of Chlamydiae have been assumed to participate in this process as well as in the early steps of adhesion and penetration (5, 6). They are tentatively also responsible for the inhibition of phagosome–lysosome fusion, another characteristic feature of the chlamydial infection (7).

It is known, however, that surface components of Chlamydiae represent antigens among which genus-, species-, and subspecies-specific antigens have been found (8–11). One of the major antigens is the lipopolysaccharide (LPS), which is chemically (10, 12–14) and serologically (12, 14–17) related to the LPS of enterobacterial rough mutants of the Re chemotype (18). It contains several antigenic determinants cross-reacting with LPS of enterobacterial Re mutants (12, 14–17) and *Acinetobacter calcoaceticus* (16) or with the lipid A component (14, 15). In addition, the chlamydial LPS harbors a genus-specific, not cross-reactive, periodate-sensitive epitope (10, 17) against which Caldwell and Hitchcock (17) have prepared a mouse monoclonal antibody.

Recently, Nano and Caldwell have reported on the expression of the chlamydial genus-specific epitope in an *Escherichia coli* strain harboring a recombinant plasmid, pFEN207, containing an insert of *C. trachomatis* DNA (19). Strains containing this recombinant plasmid synthesized, in addition to the parental LPS, a second, rough-type LPS population that contained the *Chlamydia*-specific epitope. The authors hypothesized that the cloned DNA fragment encoded for a glycosyltransferase that determined the *Chlamydia*-specific epitope and hindered further synthesis of the parent LPS leading to a truncated LPS molecule. The chemical nature of this epitope and, thus, the specificity of the enzyme are unknown.

Chlamydiae are hazardous and difficult to grow in large quantities, and this has hampered the preparation of large amounts of chlamydial LPS required for chemical analysis by conventional methods. In contrast, the recombinants, expressing the chlamydial LPS epitope, are simple to grow and thus serve as a convenient source for the preparation of appropriate quantities of LPS, allowing a structural analysis of the genus-specific epitope of *Chlamydia*. Using the tools of molecular genetics, monoclonal antibodies, and analytical and synthetic chemistry, we obtained insights into the molecular organization of the genus-specific epitope of chlamydial LPS.

MATERIALS AND METHODS

Bacteria and Bacterial LPS. *Salmonella minnesota* rough mutant chemotype Re (strain R595) was transformed with plasmid pFEN207 [a pUC8 plasmid containing a 6.5-kilobase insert of *Sau*3A-digested DNA from *C. trachomatis* (19)]. Bacteria were grown in a fermenter (14 liters) in the presence of ampicillin (60 μg/ml). Bacteria containing the pUC8 plasmid without the chlamydial insert were grown in parallel. LPS was extracted from the recombinant and the control strain as described (13). The extracted LPS from the recombinant strain will be referred to as R595-207; that of the control, as R595. The LPS of *Chlamydia psittaci* was prepared from purified yolk sac-grown elementary bodies as reported (14).

Bacterial and Synthetic Antigens. For serological purposes LPS were de-*O*-acylated (LPS-OH) with sodium methylate (0.5 M; 37°C for 16 hr), precipitated and washed with ethanol, and dissolved in water. The synthetic antigens of 3-deoxy-D-*manno*-octulosonic acid (KDO) are copolymerization products of allylglycosides of α-KDO*p* (poly-α-KDO), β-KDO*p* (poly-β-KDO), and the disaccharide α-KDO*p*-(2→4)-α-KDO*p* [poly-(α-KDO)$_2$] with acrylamide (P. Kosma, J. Gass, G. Schulz, R. Christian, and F. M. Unger, personal communication).

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Abbreviations: KDO, 3-deoxy-D-*manno*-octulosonic acid; SRBC, sheep erythrocytes; LPS, lipopolysaccharide(s); LPS-OH, de-*O*-acylated LPS; CI-MS, chemical ionization MS; EI-MS, electron impact MS.

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Monoclonal Antibodies. Monoclonal antibodies against KDO were obtained by conventional methods after immunization of mice with Re mutant bacteria of *S. minnesota* R595 (20). Clones 20 (IgM) and 25 (IgG3) recognize a terminal α-linked pyranosidic KDO residue and the disaccharide α-KDO*p*-(2→4)-KDO*p*, respectively (21). Clone L2I-6 (IgG3) was prepared by immunizing mice with purified elementary bodies of *C. trachomatis* (17); it reacts with a genus-specific epitope of chlamydial LPS. All antibodies were used as crude ascites.

Serology. The passive hemolysis and passive hemolysis inhibition tests were used to quantify antibodies and antigens, respectively. Sheep erythrocytes (SRBC) were sensitized with LPS-OH at a concentration of 40 μg/200 μl of packed cells. Serial dilutions of antibody (500 μl) in barbital-buffered saline were incubated with antigen-coated SRBC (0.5% suspension; 500 μl) and guinea pig complement (diluted 1:20; 250 μl) at 37°C for 1 hr. The supernatants were measured spectrophotometrically at 546 nm and 50% endpoint titers were determined. In inhibition experiments, constant amounts of antibody (250 μl) were preincubated (37°C for 15 min) with varying amounts of antigen (250 μl), followed by the addition of complement and incubation as above. Inhibition values are expressed as ng amounts causing 50% inhibition of lysis. $NaDodSO_4$/polyacrylamide gel electrophoresis (22) and electrophoretic transfer blot analysis (23) were performed as described.

Preparation of Oligosaccharides from LPS. LPS (100 mg each) from the recombinant (R595-207) and the control strain (R595) were hydrolyzed (70°C, 1 hr) in acetate buffer (5 ml; 20 mM, pH 4.4). The sample was dialyzed three times against water (20 ml each) and the dialysates were combined. To the retentate, acetate buffer (1 M) was added to a final concentration of 20 mM and hydrolysis was continued for another 2 hr at 70°C. The sample was dialyzed as before; the combined dialysates were neutralized with sodium hydroxide and lyophilized.

Gas/Liquid Chromatography (GLC) and Combined GLC/Mass Spectrometry (GLC/MS). The dry samples were carbonyl-reduced with sodium borodeuteride and methylated as described (24). The methylated derivatives were separated by GLC using a fused-silica capillary column (25 m × 0.32 mm, inner diameter) with a chemically bonded separating phase (SE-54, 0.2-μm film thickness), a temperature program of 150°C for 5 min followed by an increase of 5°C/min to a final temperature of 300°C, and H_2 (1.0 bar; 1 bar = 100 kPa) as carrier gas. Since the carbonyl reduction was not stereospecific, all compounds appeared as a mixture of the D-*glycero*-D-*galacto* and D-*glycero*-D-*talo* isomers.

RESULTS

LPS of the control (R595) and the recombinant strain (R595-207) were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis followed by electrophoretic transfer blot analysis and reaction with monoclonal antibody L2I-6, which recognizes a genus-specific epitope of chlamydial LPS (17). Positive reaction was obtained with LPS R595-207 but not with LPS R595 (data not shown), indicating the expression of the chlamydial gene in the recombinant. The results were in accordance with those reported earlier (19).

CHEMICAL ANALYSIS

Chemical analysis revealed the presence of the same components in both LPS consisting of D-glucosamine, KDO, 4-deoxy-4-amino-arabinose, phosphorus, and long-chain fatty acids (dodecanoic, tetradecanoic, hexadecanoic, and 3-hydroxytetradecanoic acid). Analyses performed to detect neutral or amino sugars, sugar acids, and sugar phosphates did not indicate the presence of an additional carbohydrate constituent in the recombinant LPS. Therefore, it was concluded that the nature of the *Chlamydia*-specific epitope is lying in structural rather than compositional variations in the KDO region.

Structure of the KDO Region in LPS of the Recombinant and Parental Strain. The LPS were hydrolyzed under conditions known to release, in addition to KDO monomer, oligosaccharides of KDO (25), which were investigated by GLC and GLC/MS as their carbonyl-reduced and methylated derivatives. Upon GLC, three peaks were observed with both LPS (peaks 1–3, Table 1). The first two peaks were identified as the D-*glycero*-D-*galacto* and D-*glycero*-D-*talo* isomers of methyl 3-deoxy-2,4,5,6,7,8-hexa-*O*-methyloctonate-2-[^{2}H] by chemical ionization MS (CI-MS) with ammonia and electron impact ionization MS (EI-MS) in comparison to similarly derivatized authentic KDO (26). The third peak was identified, also by GLC/MS, as the carbonyl-reduced and methylated derivative of α-KDO*p*-(2→4)-KDO in comparison to appropriate standards (25, 27).

In the recombinant LPS R595-207, two additional peaks were observed (peaks 4 and 5, Table 1) that were not detected in the control (R595). Peak 4 was identified as dimethyl 8-*O*-(3-deoxy-4,5,7,8-tetra-*O*-methyl-D-*manno*-octulopyranosyl)onate-3-deoxy-2,4,5,6,7-penta-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octonate-2-[^{2}H], the derivatization product of KDO*p*-(2→8)-KDO disaccharide after carbonyl reduction and methylation. This was shown by CI-MS whereby M_r = 615 was determined (spectrum not shown), indicated by the

Table 1. GLC analysis of KDO derivatives obtained from LPS R595-207 and R595

Peak	Retention time,* min	Systematic name of detected compound†	Amount, area % present in LPS‡ R595-207	R595
1, 2§	5.43/5.64	3-Deoxy-2,4,5,6,7,8-hexa-*O*-methyloctonate-2-[^{2}H]	70.1	94.7
3	24.64	Dimethyl 4-*O*-(3-deoxy-4,5,7,8-tetra-*O*-methyl-D-*manno*-octulopyranosyl)onate-3-deoxy-2,5,6,7,8-penta-*O*-methyloctonate-2-[^{2}H]	3.1	5.3
4	26.21	Dimethyl 8-*O*-(3-deoxy-4,5,7,8-tetra-*O*-methyl-D-*manno*-octulopyranosyl)onate-3-deoxy-2,4,5,6,7-penta-*O*-methyloctonate-2-[^{2}H]	26.3	ND
5	38.28	Trimethyl 4-*O*-{8-*O*-[(3-deoxy-4,5,7,8-tetra-*O*-methyl-D-*manno*-octulopyranosyl)onate]-(3-deoxy-4,5,7-tri-*O*-methyl-D-*manno*-octulopyranosyl)onate}-3-deoxy-2,5,6,7,8-penta-*O*-methyloctonate-2-[^{2}H]	0.5	ND

The LPS of the recombinant (R595-207) and the control strain (R595) were hydrolyzed under conditions known to release oligosaccharides of KDO (25) that were isolated, carbonyl-reduced (NaB^2H_4), and methylated (24). ND, not detectable.

*Determined by GLC using a fused-silica capillary column (25 m × 0.32 mm, inner diameter) with chemically bonded SE-54, a temperature program of 150°C for 5 min, and then 5°C/min to a final temperature of 300°C.

†Since carbonyl reduction of the keto group was not stereospecific, all compounds appeared as a mixture of the D-*glycero*-D-*galacto* and D-*glycero*-D-*talo* isomers.

‡The relative areas of the integrated signals from the flame ionization detector of the GLC apparatus are listed.

§In this case, the two isomers formed after reduction were resolved into two peaks.

appearance of a pseudomolecular ion peak at m/z = 633 $[M_r+18(\text{ammonia})]^+$. Upon EI-MS, the spectrum shown in Fig. 1 was obtained. Fragment ions at m/z = 292, 259, 227, and 195 are accounted to the nonreducing KDO moiety, whereas ion peaks at m/z = 308, 276, 244, and 212 are derived from the alditol chain and subsequent loss of methanol. Fragment pairs at m/z = 162 and 130 (162 − 32), 206 and 174 (206 − 32), and 250 and 218 (250 − 32) represent the primary fragments of the C-1/4, C-1/5, and C-1/6 moieties of the reducing end and their corresponding subfragments after loss of methanol therefrom. These data indicated that the compound under investigation was a KDO disaccharide that was either 2.7- or 2.8-interlinked. To determine the substitution of the reducing terminus and the ring form of the nonreducing end, the carbonyl-reduced and methylated disaccharide derivative was subjected to methylation analysis (24) whereby 2,6-di-*O*-acetyl-3-deoxy-1,4,5,7,8-penta-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octitol and 8-*O*-acetyl-3-deoxy-1,2,4,5,6,7-hexa-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octitol-2-[^{2}H] were obtained in the molar ratio of 1:1, whereas the same reaction sequence, but omitting the methylation step after carboxyl reduction, led to the formation of 1,2,6-tri-*O*-acetyl-3-deoxy-4,5,7,8-tetra-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octitol and 1,8-di-*O*-acetyl-3-deoxy-2,4,5,6,7-penta-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octitol-2-[^{2}H] in equimolar amounts. The mass spectra of these partially methylated and acetylated derivatives of 3-deoxyoctitol were identical to those described (ref. 24; H.B., A. Tacken, and R. Christian, unpublished work). The results show that the reducing KDO moiety is substituted at *O*-8 by a pyranosidic KDO residue of presently unknown anomeric configuration.

Peak 5 was identified as a KDO trisaccharide. M_r = 891 was determined by CI(ammonia)-MS [pseudomolecular ion peak at m/z = 909 $(M_r+18)^+$; spectrum not shown]. The spectrum obtained after EI-MS is shown in Fig. 2*B*. It is characterized by fragment ions of high intensity at m/z = 308, 276, 244, and 212, which are accounted to the primary fragment of a mono-*O*-substituted 3-deoxyoctonate chain after cleavage of the glycosidic bond and the corresponding daughter ions after substraction of methanol indicating that the trisaccharide is linear. Ion peaks at m/z = 177 and 145 (177−32) represent the C-5/8 moiety of the reducing terminus, which is typical for a 4-*O*-substituted KDO residue (27). The fragment ions at m/z = 291, 259, 227, and 195 are derived from a terminal nonsubstituted KDO residue. Together with the data obtained for the α-2.4- and the 2.8-linked KDO disaccharides, the structure of the KDO trisaccharide is assumed to be the one shown in Fig. 3*C*.

With the knowledge on the hydrolysis and derivatization conditions to obtain the KDO oligosaccharides in an optimal yield, their retention times in GLC, and their fragmentation pattern after EI-MS, quantities as low as 500 μg of LPS were sufficient for the detection of KDO oligosaccharides. However, the corresponding peaks were then only observed by selected ion monitoring (this technique is a computer-assisted detection mode in MS; from the mass spectrum of a reference compound characteristic fragment ions are selected to be recorded). By this sensitive detection mode, KDO monosaccharide, α-2.4- and 2.8-linked KDO disaccharides, and the KDO trisaccharide KDO-(2→8)-KDO-(2→4)-KDO were also detected in authentic LPS of *C. psittaci*. The facts that no other carbohydrates were detected and that quantitative analysis indicated that the LPS of *C. psittaci* contained 28% of KDO (14) show the significance of these structures.

SEROLOGICAL ANALYSIS

Epitope Specificities of LPS from R595, R595-207, and *C. psittaci*. To determine the epitopes present in LPS of R595-207 and R595 and in chlamydial LPS, SRBC were coated with the respective LPS and allowed to react with three different monoclonal antibodies. Two of them have been defined with regard to their epitope specificity using synthetic KDO antigens [copolymerization products of allylglycosides of α- and β-KDO*p* and of α-KDO*p*-(2→4)-α-KDO*p* disaccharide]; clone 20 reacts with a single terminal KDO residue in α-linkage, whereas clone 25 recognizes an α-KDO*p*-(2→4)-KDO disaccharide (21). The third antibody (clone L2I-6) recognizes a genus-specific epitope of chlamydial LPS (17), the chemical structure of which was hitherto unknown.

The results obtained in a passive hemolysis assay are shown in Table 2. All three antibodies exhibited a similar reactivity with chlamydial and recombinant LPS, respectively, indicating that the corresponding epitopes were equally expressed in both LPS. SRBC coated with R595 LPS reacted with clones 20 and 25 but not with clone L2I-6, indicating the absence of the *Chlamydia*-specific epitope in the parent LPS.

The same antibodies were used in inhibition experiments using LPS and synthetic KDO antigens as inhibitors. The results are shown in Table 3. The LPS of *C. psittaci* and R595-207 yielded a similar inhibition pattern. Both preparations inhibited the KDO monosaccharide-specific antibody (clone 20) with 1 and 2 ng, respectively, and the disaccharide-specific antibody (clone 25) with 16 and 8 ng, respectively. The *Chlamydia*-specific antibody L2I-6 was inhibited with 16 ng (*C. psittaci* LPS) and 63 ng (R595-207 LPS). The parent

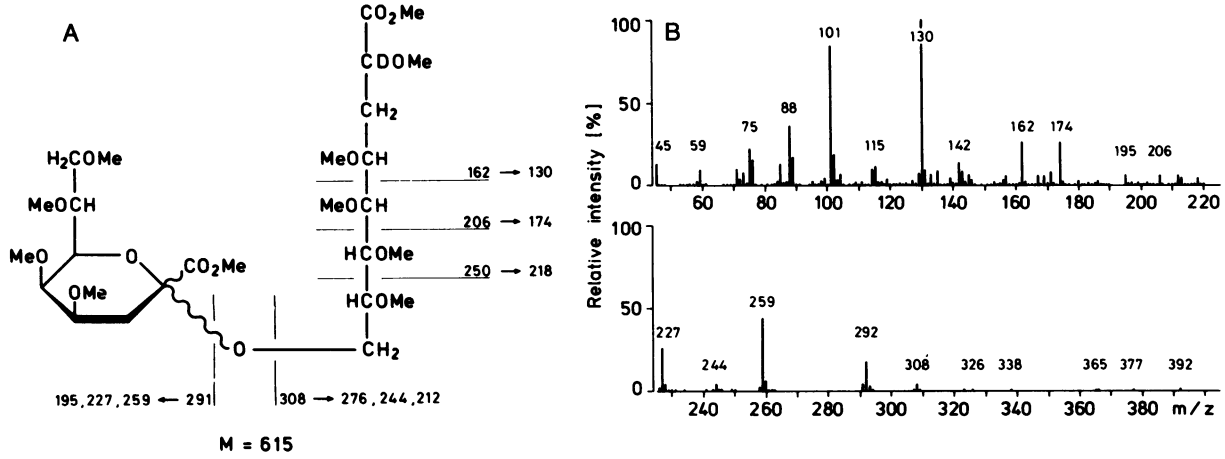

FIG. 1. Fragmentation pattern (*A*) and mass spectrum (*B*) of the carbonyl-reduced (NaB^2H_4) and methylated derivative of 2.8-linked KDO disaccharide (peak 4, Table 1). The spectrum was recorded on a GLC-coupled mass spectrometer in the electron impact mode (70 eV).

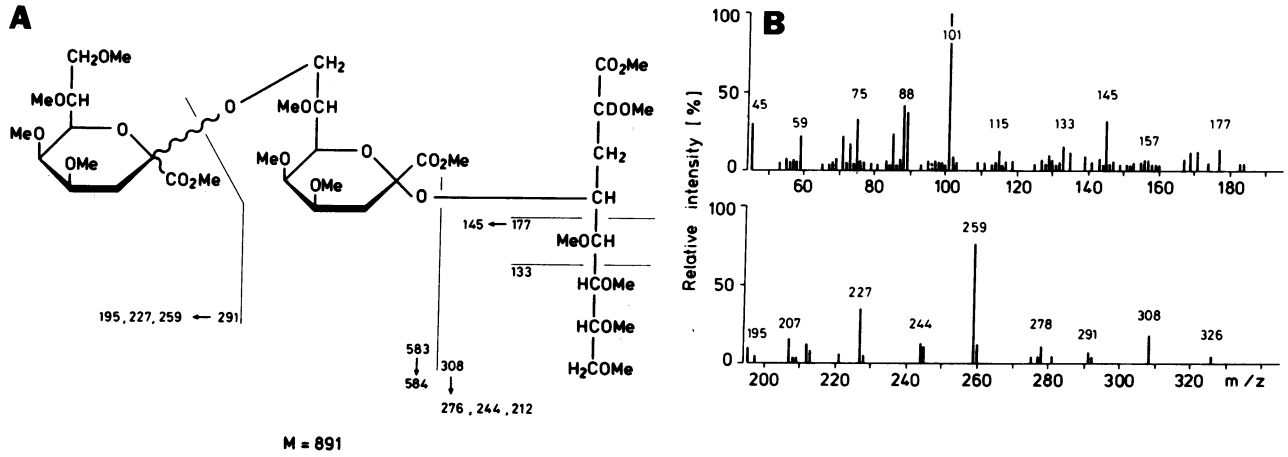

FIG. 2. Fragmentation pattern (*A*) and mass spectrum (*B*) of the carbonyl-reduced (NaB^2H_4) and methylated derivative of the trisaccharide KDO-(2→8)-KDO-(2→4)-KDO (peak 5, Table 1) after electron impact ionization.

LPS R595 inhibited clones 20 and 25 with 1 and 16 ng, respectively, but failed to inhibit clone L2I-6.

By using synthetic KDO antigens, the following results were obtained. The monosaccharide derivative poly-α-KDO was only an inhibitor for clone 20. The disaccharide derivative poly-α-KDO-(2→4)-KDO inhibited clone 20 and clone 25 with 0.5 and 4 ng, respectively, but not the *Chlamydia*-specific antibody L2I-6. The β-linked KDO was not active in either system.

DISCUSSION

The concept of this work was based on the assumption that the expression of the *Chlamydia*-specific epitope is paralleled by significant alterations of the chemical composition or/and the chemical structure of the LPS from a recombinant strain in comparison with that of the parent strain. Since we knew from our earlier work (14, 15) that the epitope was most likely located in the saccharide portion of the LPS molecule (it could be destroyed by mild acid hydrolysis and by periodate oxidation), we chose a bacterium with a short, well-characterized oligosaccharide in its LPS so as to allow chemical analysis and chemical synthesis.

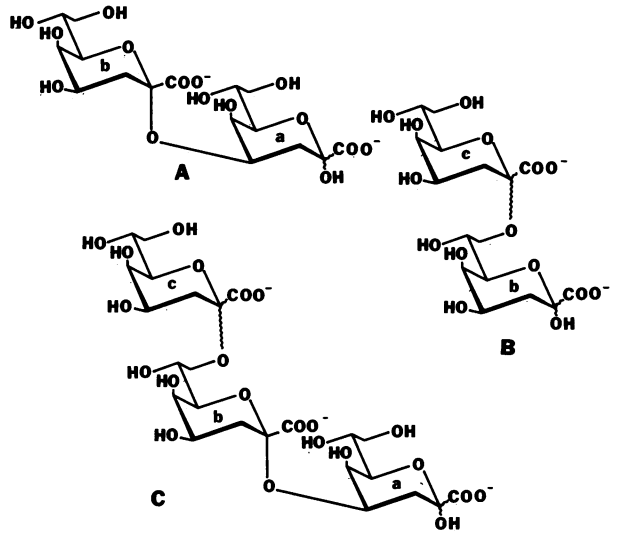

FIG. 3. Oligosaccharides of KDO released from LPS of *C. psittaci* and R595-207 after mild acid hydrolysis. The structure of the α-2.4- (*A*) and the 2.8-linked (*B*) KDO disaccharides and of the trisaccharide (*C*) was determined by GLC/MS of the carbonyl-reduced and methylated derivatives and by methylation analysis in comparison to standards. Waved lines indicate uncertain anomeric configurations. The assignment of the anomeric configuration of KDO residue b in the trisaccharide is based on the assumption that the disaccharides (*A* and *B*) are hydrolysis products of the trisaccharide (*C*).

The Re mutant of *S. minnesota* R595 (18), which contains only an α-2.4-linked KDO disaccharide in its saccharide portion (25), fulfills these requirements and, as shown recently (28), also expresses the *Chlamydia*-specific epitope after transformation with plasmid pFEN207 (19). Transformation of this strain with pFEN207 resulted in the synthesis of a LPS that differed chemically and serologically from that of the parent strain. The LPS of the recombinant strain contained two structural elements that were absent in the parent LPS: a 2.8-linked disaccharide of KDO and a trisaccharide of the sequence KDO-(2→8)-KDO-(2→4)-KDO. The disaccharide could be a hydrolysis product of the trisaccharide; however, it could be also possible that both structures are simultaneously present in the LPS. Nevertheless, the appearance of an 8-*O*-substituted KDO residue must be regarded as the result of the action of an enzyme encoded by the chlamydial DNA insert. Since this insert encodes for one or, at most, two gene products (F.E.N., unpublished data), the most likely explanation is that the enzyme is a glycosyltransferase that attaches KDO specifically to the hydroxyl group at C-8 of the terminal KDO residue of the parent LPS. Whereas other enzymes—e.g., KDO-8-phosphate synthetase (29), KDO-CMP synthetase (30), and the transferase attaching α-2.4-linked KDO disaccharide to lipid A (31)—are present in the parent strain and also in many other bacteria, this very KDO transferase seems to be a characteristic feature of the genus *Chlamydia*.

The appearance of the 8-*O*-substituted KDO in the recombinant LPS is paralleled by the expression of a genus-specific epitope of chlamydial LPS being recognized by monoclonal

Table 2. Antibody titers of monoclonal antibodies recognizing KDO epitopes in LPS of R595, R595-207, and *C. psittaci*

Monoclonal antibody clone	Hemolytic titer against SRBC coated with LPS-OH		
	R595	R595-207	*C. psittaci*
20	102,400	51,200	102,400
25	5,120	1,280	5,120
L2I-6	<10	160	320

Clones 20 and 25 are specific for a terminal α-linked KDO residue and an α-2.4-linked KDO disaccharide, respectively, whereas clone L2I-6 recognizes the genus-specific LPS epitope of *Chlamydia*.

Table 3. Inhibition of monoclonal antibodies recognizing different KDO epitopes in chlamydial LPS

Inhibitor	Inhibition value in the hemolytic antigen/antibody system, ng		
	Clone 20/R595 LPS-OH	Clone 25/R595 LPS-OH	L2I-6/*C. psittaci* LPS-OH
LPS			
R595	1	16	>1,000
R595-207	2	8	63
C. psittaci	1	16	16
Synthetic antigen*			
Poly-α-KDO	2	>1,000	>1,000
Poly-β-KDO	>1,000	>1,000	>1,000
Poly-(α-KDO)$_2$	0.5	4	>1,000

*Copolymerization products of allylglycosides of α-KDO*p* (poly-α-KDO), β-KDO*p* (poly-β-KDO), and α-KDO*p*-(2→4)-α-KDO*p* disaccharide [poly-(α-KDO)$_2$] with acrylamide.

antibody L2I-6. Since this antibody reacts with chlamydial and recombinant LPS and not with Re LPS or lipid A and, in addition, is neither inhibited by synthetic antigens containing KDO monosaccharide in α- or β-linkage nor by those containing α-2.4-linked KDO disaccharide, it appears that the epitope specificity of monoclonal antibody L2I-6 requires an 8-*O*-substituted KDO residue and that the terminal KDO residue does not seem to contribute significantly to the antigenic determinant. A similar observation was made with monoclonal antibody clone 25, which reacted with an α-2.4-linked KDO disaccharide and could not be inhibited with α-KDO monosaccharide (21).

Chlamydial LPS was investigated in parallel; α-2.4- and 2.8-linked KDO disaccharide and the trisaccharide KDO-(2→8)-KDO-(2→4)-KDO were also detected and the LPS could not be distinguished from that of the recombinant strain by serological methods. The monoclonal antibodies clones 20, 25, and L2I-6 reacted in a passive hemolysis assay as well as in inhibition experiments also with chlamydial LPS, indicating the presence of the corresponding epitopes.

Therefore, it is concluded that chlamydial LPS contains a linear KDO trisaccharide of the sequence KDO-(2→8)-KDO-(2→4)-KDO, as shown in Fig. 3*C*, and three different epitopes related to a different substitution pattern of KDO. Since natural LPS is heterogeneous, it cannot be determined whether these epitopes are expressed by the same structure—e.g., the KDO trisaccharide—or by partial structures, as shown in Fig. 3 *A* and *B*; we also do not know the expansion of the *Chlamydia*-specific epitope. Nevertheless, the 8-*O*-substituted KDO residue must be regarded as determining the specificity of the genus-specific epitope. This epitope is generated through the action of a *Chlamydia*-specific gene product encoded by the insert of pFEN207.

The results of this study allow further investigations on the biological relevance of this important surface component of Chlamydiae that will help us to gain a better understanding of the biology of Chlamydiae and may be useful in the prevention or cure of chlamydial infections.

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1. Storz, J. (1971) *Chlamydia and Chlamydia-Induced Diseases* (Thomas, Springfield, IL).
2. Hobson, D. & Holmes, K. K. (1977) *Nongonococcal Urethritis and Related Disorders* (Am. Soc. Microbiol., Washington, DC).
3. Schachter, J. & Caldwell, H. D. (1980) *Annu. Rev. Microbiol.* **34**, 285–309.
4. Moulder, J. W., Levy, N. J. & Schulman, L. P. (1980) *Infect. Immun.* **30**, 874–883.
5. Byrne, G. I. & Moulder, J. W. (1978) *Infect. Immun.* **19**, 598–606.
6. Hackstadt, T., Todd, W. J. & Caldwell, H. D. (1985) *J. Bacteriol.* **161**, 25–31.
7. Friis, R. R. (1972) *J. Bacteriol.* **110**, 706–721.
8. Caldwell, H. D., Kuo, C. C. & Kenny, G. E. (1975) *J. Immunol.* **115**, 963–968.
9. Caldwell, H. D. & Schachter, J. (1982) *Infect. Immun.* **35**, 1024–1031.
10. Dhir, S. P., Hakomori, S., Kenny, G. E. & Grayston, J. T. (1972) *J. Immunol.* **109**, 116–122.
11. Stephens, R. S., Tam, M. R., Kuo, C. C. & Novinski, R. C. (1982) *J. Immunol.* **128**, 1083–1089.
12. Nurminen, M., Leinonen, M., Saikku, P. & Mäkelä, P. H. (1983) *Science* **220**, 1279–1281.
13. Nurminen, M., Rietschel, E. T. & Brade, H. (1985) *Infect. Immun.* **48**, 573–575.
14. Brade, L., Schade, U., Schramek, S. & Brade, H. (1986) *Infect. Immun.* **54**, 568–574.
15. Brade, L., Nurminen, M., Mäkelä, P. H. & Brade, H. (1985) *Infect. Immun.* **48**, 569–572.
16. Nurminen, M., Wahlström, E., Kleemola, M., Leinonen, M., Saikku, P. & Mäkelä, P. H. (1984) *Infect. Immun.* **44**, 609–613.
17. Caldwell, H. D. & Hitchcock, P. J. (1984) *Infect. Immun.* **44**, 306–314.
18. Lüderitz, O., Galanos, C., Risse, H. J., Ruschmann, E., Schlecht, S., Schmidt, G., Schulte-Holthausen, H., Wheat, R., Westphal, O. & Schlosshardt, J. (1966) *Ann. N.Y. Acad. Sci.* **133**, 349–374.
19. Nano, F. E. & Caldwell, H. D. (1985) *Science* **228**, 742–744.
20. Appelmelk, B. J., Verweij-van Vught, A. M. J. J., Maaskant, J. J., Schouten, W. F., Thijs, L. G. & MacLaren, D. M. (1987) *FEMS Microbiol. Lett.* **40**, 71–74.
21. Brade, L., Kosma, P., Appelmelk, B. J., Paulsen, H. & Brade, H. (1987) *Infect. Immun.* **55**, in press.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Bittner, M., Kupferer, P. & Morris, C. F. (1980) *Anal. Biochem.* **102**, 459–471.
24. Tacken, A., Brade, H., Unger, F. M. & Charon, D. (1986) *Carbohydr. Res.* **149**, 263–277.
25. Brade, H. & Rietschel, E. T. (1984) *Eur. J. Biochem.* **145**, 231–236.
26. Brade, H. (1985) *J. Bacteriol.* **161**, 795–798.
27. Brade, H., Zähringer, U., Rietschel, E. T., Christian, R., Schulz, G. & Unger, F. M. (1984) *Carbohydr. Res.* **134**, 157–166.
28. Brade, L., Nano, F. E., Schlecht, S., Schramek, S. & Brade, H. (1987) *Infect. Immun.* **55**, in press.
29. Levin, D. H. & Racker, E. (1959) *J. Biol. Chem.* **234**, 2532–2539.
30. Ghalambor, M. A. & Heath, E. C. (1966) *J. Biol. Chem.* **241**, 3216–3221.
31. Munson, R. S., Jr., Rasmussen, N. S. & Osborn, M. J. (1978) *J. Biol. Chem.* **253**, 1503–1511.