Structure of Cell Wall Lipopolysaccharide from *Salmonella typhimurium*

IV. Anomeric Configuration of L-Rhamnose Residues and Its Taxonomic Implications

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A major portion of cell wall lipopolysaccharide from group A, group B, or group D, *Salmonella* corresponds to a linear polysaccharide chain, which consists of α-D-galactosyl-(1 → 2)-α-D-mannosyl-(1 → 4)-L-rhamnosyl-(1 → 3)-repeating units, and has short branches of single 3,6-dideoxyhexose residues. The groups differ in the configuration of the 3,6-dideoxyhexose present. Furthermore, it has been claimed that the anomeric configuration of the rhamnosyl residues is β-L in group B, in contrast to the α-L configuration found in groups A and D. In this study, oligosaccharides containing more than one repeating unit were isolated from a lipopolysaccharide of a group B *Salmonella*, and the anomeric configuration of the rhamnosyl residues was determined by the comparison of optical rotatory powers of these oligosaccharides with that of the repeating unit trisaccharide. The results established the configuration of rhamnose as α-L, in contrast to the β-L configuration suggested in the literature. Since rhamnosyl linkages in lipopolysaccharide of a group D, *Salmonella* are hydrolyzed in acid at exactly the same rate as are those in group B *Salmonella*, the configuration of rhamnose residues in groups D, lipopolysaccharide is also likely to be α-L. These results indicate that lipopolysaccharides of *Salmonella* groups A, B, and D share an identical main chain polysaccharide and differ essentially only in the configuration of 3,6-dideoxyhexose branches; they thus suggest close evolutionary relationship between these three serogroups of *Salmonella*.

The peripheral "O chain" portion of cell wall lipopolysaccharide (LPS) of *Salmonella* of group A, B, and D, is known to consist of a main chain to which short branches of single 3,6-dideoxyhexose residues are attached (14). The structure of O chains in these groups is supposed to differ mainly in two respects (Fig. 1). (i) The stereochemical configuration of the 3,6-dideoxyhexose is different in that group A has paratose (3,6-dideoxy-D-glucose), group B abequose (3,6-dideoxy-D-galactose), and group D, tyvelose (3,6-dideoxy-D-mannose). (ii) Although in all groups the main chain consists of the trisaccharide repeating unit α-D-galactosyl-(1 → 2)-α-D-mannosyl-(1 → 4)-L-rhamnosyl-(1 → 3)- (in strains containing a converting prophage φ27, the galactose residue is linked to the 6-position of the mannose residue [1, 7]), the rhamnosyl residues are reported to have the β-L configuration in group B (9, 10) and the α-L configuration in groups A and D, (8, 11). We thought that the second difference was important, because it suggested that, at least in terms of LPS structure, groups A and D, were more closely related to each other than either of them was to group B. The assignment of the anomeric configuration, however, was primarily based on a rather insensitive and ambiguous method of following the changes in rotation during the hydrolysis of the entire LPS (8–11). Therefore, we felt it was desirable to reexamine the situation by the use of a more direct method. In this paper we describe the isolation, from the LPS of a group B *Salmonella*, of oligosaccharide fragments containing rhamnosyl linkages, and offer evidence that, contrary to the statements in the literature, both group B and group D,
Salmonella contain α-L anomers of rhamnose.

MATERIALS AND METHODS

Bacterial strains. Salmonella typhimurium LT2 and Salmonella enteritidis SH1262 were used as representatives of serogroup B and D, Salmonella, respectively. In the latter strain, which was a kind gift from P. H. Mäkelä, Central Public Health Laboratory, Helsinki, Finland, the chromosomal region including the OaR locus (22) has been replaced by a corresponding region from a serogroup C donor, so that the α-d-glucose branches corresponding to O-12 antigen are not added to the O chain of the LPS.

Cultivation of bacteria and isolation of LPS. LPS was isolated from cells in exponential phase of growth in L broth, as described previously (24). The preparation of “uniformly” 14C-labeled LPS has been described (17); in the LPS prepared in this manner, the specific radioactivities of rhamnose and galactose were equal, but that of mannose was about 20% lower. In the calculation of molar ratios of sugars from radioactivity, correction was made for this difference in specific activity.

Paper chromatography. Solvents used were: A, butan-1-ol, pyridine, water (6:4:3, v/v); B, phenol, water (4:1, v/v); and C, butan-1-ol, acetic acid, water (upper phase of 4:1:5, v/v, mixture). Pre-washed Whatman 3MM paper (17) was always used, and the chromatography was carried out in the descending manner at room temperature.

The paper strips were scanned for radioactivity with a Nuclear-Chicago Corp. scanner. In quantitative experiments, 5-mm-wide segments were cut out from the strip, and the radioactivity in each segment was determined by the use of a Nuclear-Chicago Corp. liquid scintillation spectrometer.

Partial and complete acid hydrolysis of LPS and oligosaccharides. For partial hydrolysis, samples were treated in N H2SO4 at 100 C for 5 to 10 min, and the acid was removed by adding excess Dowex-1 (HCO3 -). Complete acid hydrolysis was done by heating samples in N HCl in sealed tubes at 100 C for 3 or 4 hr; the acid was removed by drying the hydrolysates in an evacuated desicator containing KOH pellets.

Reduction of oligosaccharides with NaBH4. This was carried out as described earlier (17).

Optical rotation of oligosaccharides. Rotation was measured at 22 C by using a Bendix series 1100 automatic polarimeter that had a full scale range of ±0.1°. The optical length of the tube was 5 cm, and the concentration of the oligosaccharides was in the range of 2 to 5 mg/ml. Since this instrument measured optical rotation at 546 nm, the obtained [α]D values were multiplied with 0.85 in order to obtain the approximate [α]D values.

The oligosaccharides were quantitated by the cysteine-H2SO4 reaction for 6-deoxyhexoses (4). To quantitate the reduced trisaccharide, it was first mixed with 14C-labeled trisaccharide before reduction, and the specific radioactivity of the mixture was determined; this allowed us to quantitate the reduced compound on the basis of its radioactivity.
Theoretically expected specific rotations of oligosaccharides were calculated by the use of Hudson’s isorotation rules (20). Thus the molecular rotation of the oligosaccharides was assumed to be close to the algebraic sum of the rotation due to the anomeric carbon (α-A for α-D- and β-L-α-momers, β-A for β-D- and α-L-α-momers) and the rotation due to the remaining portion (B) of the component sugars. The values of A and B were calculated (20) from the published values (3) of molecular rotations for α- and β-methyl-glycosides. Since aqueous solutions of free L-rhamnose at equilibrium are mixtures of about 56% α-form and 44% β-form as judged by the optical rotation, the contribution of the anomeric carbon of reducing end rhamnose was neglected in the calculation for the “theoretical” rotation of oligosaccharides.

**Rate of acid hydrolysis of rhamnosyl linkages.** This rate can be measured easily due to the availability of the cysteine-H2SO4 reaction (4) which can determine rhamnose in polysaccharides without prior hydrolysis. Lipid A-free, polysaccharide preparations were obtained by treating LPS in n acetic acid at 100 C for 90 min and then dialyzing the clear supernatant liquid extensively against water. The polysaccharide, containing 1.5 μmoles of rhamnose, was mixed with H2SO4 (0.2 m mole), and the mixture (1.0 ml) in a small tube with a Teflon-lined screw cap was heated in a vigorously boiling water bath. At 10-min intervals, 0.05-ml portions were rapidly taken out and transferred to test tubes kept in an ice bath. After all the samples were taken, 0.45 ml of an ice-cold aqueous solution of NaBH4 (10 mg/ml) was added to each tube, and the tubes were kept for 2 hr at room temperature. This procedure was followed by the determination of 6-deoxyhexose by the cysteine-H2SO4 procedure (4). The hydrolysis of rhamnosyl linkages releases oligo- and polysaccharides with rhamnol residue as reducing ends; these residues are reduced with NaBH4 and produce no color in the subsequent cysteine-H2SO4 reaction. Thus the procedure measures only the rhamnosyl residues that are a part of unhydrolyzed, rhamnosyl linkages.

**RESULTS**

Isolation of oligosaccharides I and II. Many oligosaccharide bands were seen when partial acid hydrolysates (see Materials and Methods) of 14C-labeled LPS from S. typhimurium LT2 were chromatographed on paper with solvent A (Fig. 2). The two major oligosaccharide bands with lower $R_F$ values than the trisaccharide were designated I and II in the order of increasing $R_F$ (see Fig. 2). 14C-labeled I and II were purified as follows. 14C-LPS from strain LT2 (106 counts per min, 10 mg) was hydrolyzed in 10 ml of n H2SO4 for 5 min at 100 C, and, after the removal of the acid with Dowex-1 (HCO3-), the hydrolysate was chromatographed on paper with solvent A. 14C-oligosaccharides were eluted from the areas corresponding to I and II (see Fig. 2), and were purified by successive paper chromatography in solvents B and C. The oligosaccharides obtained contained 86,500 counts per min (I) and 28,400 counts per min (II). The $R_{galactose}$ values of these oligosaccharides are shown in Table 1.

A typical large-scale preparation was done as follows. LPS from LT2 (0.76 g) was hydrolyzed in 40 ml of n H2SO4, as described above. The acid was removed, and the oligosaccharides were isolated by successive paper chromatography as above, except that the procedure was scaled up. For example, in one experiment the first paper chromatography was performed on six sheets of paper, each 25 cm in width; in another experiment column chromatography (Whatman CF-II cellulose powder, 50 g) with

**Table 1. Paper chromatographic mobilities of oligosaccharides**

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>$R_{galactose}$ in solvent:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>I</td>
<td>0.22</td>
</tr>
<tr>
<td>II</td>
<td>0.40</td>
</tr>
<tr>
<td>Trisaccharide*</td>
<td>0.72</td>
</tr>
</tbody>
</table>

* α-D-galactosyl-(1 → 2)-α-D-mannosyl-(1 → 4)-L-rhamnose (25).

![Fig. 2. Paper chromatography of a partial acid hydrolysate of 14C-labeled S. typhimurium LPS. Solvent A was used. Tri and Di refer to the trisaccharide galactosyl-mannosyl-rhamnose and the disaccharide mannosyl-rhamnose, respectively. For other abbreviations see Fig. 1.](http://jb.asm.org/Downloaded from http://jb.asm.org/ on October 26, 2017 by guest)
solvent A was used instead of the paper chromatography. The yields of I and II in one experiment were 30 mg and 5.7 mg, respectively.

Structure of oligosaccharides I and II. The oligosaccharides labeled uniformly with $^{14}$C were chromatographed on paper (solvent A) after complete acid hydrolysis. Three radioactive bands, corresponding to galactose, mannose, and rhamnose, were found in each case. But their molar ratios, determined as described in Materials and Methods, were different: for I, it was 2.0:2.0:2.0, whereas for II, it was 1.0:1.9:2.0.

When the uniformly labeled I and II were first reduced with NaBH$_4$ (Materials and Methods) and were then hydrolyzed, in each case about half of the rhamnose was found to have been converted to rhamnitol on the basis of paper chromatography in solvent B. The ratios of $^{14}$C-rhamnitol to $^{14}$C-rhamnose, determined as described in Materials and Methods, were in two experiments 0.7 and 1.0 for the hydrolysates of the reduced I, and 0.8 and 0.8 for those of reduced II.

The size of the labeled oligosaccharides I and II was determined by applying them on a gel filtration column (1.27 by 51 cm) (Bio-Gel P-4, 200 to 440 mesh, Bio-Rad Laboratories, Richmond, Calif.) that had been calibrated with blue dextran and a tetrasaccharide, glucosyl-galactosyl-mannosyl-rhamnose (23). Both I and II were eluted at a position expected for pentasaccharides, i.e., about 5 ml ahead of the tetrasaccharide marker. If they each contained three repeating units, then they would have been eluted at the position expected for a nonasaccharide, i.e., with 8 ml less eluent than was actually needed. The gel filtration thus showed that both I and II contained between five and seven sugar residues.

The results so far described, if taken together with our knowledge of the structure of S. typhimurium O side chain (Fig. 1), suggest that the oligosaccharide I is a hexasaccharide, galactosyl-mannosyl-rhamnosyl-galactosyl-mannosyl-rhamnose, and that II is a pentasaccharide, mannosyl-rhamnosyl-galactosyl-mannosyl-rhamnose. This conclusion was further strengthened by the results of partial acid hydrolysis of the oligosaccharides shown in Fig. 3. Clearly a brief hydrolysis of I produces almost quantitatively a compound with an $R_f$ of the trisaccharide, $\alpha$-d-galactosyl-(1 $\rightarrow$ 2)-$\alpha$-d-mannosyl-(1 $\rightarrow$ 4)-l-rhamnose, that has been characterized earlier (8, 25). This product was shown to have an $R_{galactose}$ value identical to that of the authentic trisaccharide also in solvent C. Furthermore, complete acid hydrolysis of this compound produced equimolar amounts of $^{14}$C-galactose, $^{14}$C-mannose, and $^{14}$C-rhamnose as expected.

Partial acid hydrolysis of II, on the other hand, produced two major products (Fig. 3b). One was identified as the galactosyl-mannosyl-rhamnosyl trisaccharide by the procedures described above. The other, which had the $R_f$ identical to that of mannose in solvent A (Fig. 3b), co-chromatographed with the already characterized disaccharide, $\alpha$-d-mannosyl-(1 $\rightarrow$ 4)-l-rhamnose (25), in solvents B and C. Furthermore, the complete acid hydrolysis of this compound produced $^{14}$C-mannose and $^{14}$C-rhamnose in equimolar quantities. These results thus established that II was mannosyl-rhamnosyl-galactosyl-mannosyl-rhamnose and I was a hexasaccharide with an additional galactose residue added onto the nonreducing end of II.

Nature of the rhamnosyl-galactose linkages. Positions and anomeric configurations of d-mannosyl and d-galactosyl linkages in I and II are clear from the fact that the partial acid hydrolysis of these oligosaccharides produce $\alpha$-d-galactosyl-(1 $\rightarrow$ 2)-$\alpha$-d-mannosyl-(1 $\rightarrow$ 4)-l-rhamnose and $\alpha$-d-mannosyl-(1 $\rightarrow$ 4)-l-rhamnose. However, these results do not tell us the nature of the rhamnosyl-galactose linkages in I and II.

Hellerqvist and co-workers (7-11) carried out methylation analysis on the LPS of groups A, B, and D, Salmonella, and concluded that the rhamnosyl residues are attached to C-3 of the galactose residue in all cases. This point was confirmed in our periodate oxidation studies (unpublished data) on $^{14}$C-labeled oligosaccharide I. Thus, of the two galactose residues present, one was resistant to periodate presumably because it was substituted at C-3.

Specific optical rotation of oligosaccharides was measured as described in Materials and Methods, by using the oligosaccharides that were isolated in the large-scale experiments described already. They were applied to columns of Bio-Gel P-2 (1.27 by 50 cm), and the oligosaccharide peaks were analyzed for 6-deoxyhexose and for optical rotation, as described in Materials and Methods. As seen in the upper half of Table 2, the values of specific rotation were more or less constant throughout each peak, except in a single, very dilute fraction at the end of the oligosaccharide II; this suggests that the oligosaccharides are sufficiently pure and that the rotation values obtained are reliable.

However, in view of the presence of the single fraction showing an unusually high rotation,
Fig. 3. Paper chromatography of partial acid hydrolysate of oligosaccharides I (a) and II (b). Uniformly $^{14}$C-labeled oligosaccharides were treated for 5 min at 100°C with $\text{H}_2\text{SO}_4$, and the hydrolysates were chromatographed in solvent A after the removal of the acid (Materials and Methods). Di, Tri, I, and II indicate the positions of the disaccharide mannosyl-rhamnose, the trisaccharide galactosyl-mannosyl-rhamnose, oligosaccharide I, and oligosaccharide II on control strips developed in parallel.

### Table 2. Specific rotation of oligosaccharides

<table>
<thead>
<tr>
<th>Substance</th>
<th>$[\alpha]_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>values*</td>
</tr>
<tr>
<td>I</td>
<td>78, 91, 80</td>
</tr>
<tr>
<td>II</td>
<td>71, 79, (93)*</td>
</tr>
<tr>
<td>Triaccharide</td>
<td>105, 109, 103</td>
</tr>
<tr>
<td>Reduced oligosaccharides</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>95, 86, 103</td>
</tr>
<tr>
<td>II</td>
<td>72, 70, 73</td>
</tr>
<tr>
<td>Triaccharide</td>
<td>110, 106, 105</td>
</tr>
</tbody>
</table>

*Specific rotation was measured on fractions eluted from Bio-Gel P-2 columns. Three fractions with highest concentrations of the oligosaccharide were used.

*This fraction contained much less oligosaccharide than the other two, and the accuracy of the $[\alpha]_D$ value obtained was doubtful.

The experiment was repeated with a different batch of oligosaccharides. Furthermore, the oligosaccharides were reduced with NaBH$_4$ to eliminate the complication that could arise from the muta-rotation of the rhamnose at the reducing end, and then the reduced oligosaccharides were purified by gel filtration through columns of Bio-Gel P-2. The lower half of Table 2 shows that the results were very similar to those obtained with the reducing oligosaccharides.

The trisaccharide had a specific rotation, $[\alpha]_D$ of +106°. This is in good agreement with an expected value (see Materials and Methods) for $\alpha$-$\text{D}$-galactosyl-$\alpha$-$\text{D}$-mannosyl-$\text{L}$-rhamnitol, $[\alpha]_D = +114°$. It also agrees with the reported $[\alpha]_D(+115°)$ of the trisaccharide isolated from a group A Salmonella LPS (8). The presence of a single $\beta$-linked sugar would reduce the $[\alpha]_D$ to about one-half of these values, and we can conclude safely that mannos and galactose are both $\alpha$-$\text{D}$-anomers.

The expected specific rotation $[\alpha]_D$ for the hexasaccharide (i.e., I) is +93° or +121°, depending on whether the rhamnosyl linkage is $\alpha$-$\text{L}$- or $\beta$-$\text{L}$-, respectively. The observed rotation (+83° and +95° for I and reduced I) is clearly indicative of the presence of an $\alpha$-$\text{L}$-linkage. Similarly, the expected $[\alpha]_D$ for the pentasaccharide (II) is +65° and +99° for the isomers containing an $\alpha$-$\text{L}$- and $\beta$-$\text{L}$-linked rhamnose. The observed values (+75° and +72°) again indicate the presence of an $\alpha$-$\text{L}$-rhamnose residue.

For the final confirmation of the anomeric configuration of rhamnose residues, oligosaccharides were treated briefly with acid so that mostly rhamnosyl linkages are hydrolyzed, and
changes in optical rotation accompanying this process were measured. Oligosaccharides I and II (both labeled with $^{14}$C) were dissolved in $\text{H}_2\text{SO}_4$, and one-half of the solution was heated at 100°C for 10 min. The optical rotation of these partial hydrolysates was measured together with that of the remaining, unhydrolyzed samples. The interpretation is complicated, because some of the $\alpha$-d-mannosyl and $\alpha$-d-galactosyl linkages are also hydrolyzed under these conditions. Therefore, after the measurement of rotation, the partial hydrolysates were chromatographed on paper with solvent A, and the extents of hydrolysis of galactosyl and mannosyl linkages were calculated, as a first approximation, from the amounts of free $^{14}$C-galactose and $^{14}$C-rhamnose found. It was found that hydrolysis occurred in about 80% of rhamnosyl linkages as well as in at least 20 and 15% of galactosyl and mannosyl linkages. Calculations according to Hudson's rules (Materials and Methods) indicate that the partial hydrolysis should produce little change (less than 5%) in rotation if the rhamnosyl residue in I and II is an $\alpha$-L-anomer, but a drastic decrease (27% and 40% for I and II) in rotation should occur if the rhamnose is a $\beta$-L-anomer. The observed changes (2% decrease for I and 1% increase for II) after partial hydrolysis showed clearly that the rhamnose was an $\alpha$-L-anomer.

Although our data alone do not exclude the possibility that the rhamnose residues are $\beta$-anomers, this is most unlikely in view of the work of Krüger and co-workers (13), who showed convincingly that rhamnose in LPS of groups A, B, and D1 Salmonella belong to the $\beta$-series.

Comparison of rhamnosyl linkages in groups B and D1. The results described in the preceding section established that the rhamnosyl residues in the LPS of a group B Salmonella are $\alpha$-L-anomers, contrary to the reports in the literature. We then wanted to examine whether the report (11) that LPS of group D1 Salmonella contains $\alpha$-L-rhamnosyl residues can be confirmed. For this we compared the rates of acid hydrolysis of rhamnosyl linkages in LPS of group B and D1 Salmonella. The rates of acid hydrolysis of glycosidic bonds between two given sugars are dependent on the position of attachment and the anomic configuration of the glycosidic residue (2). In LPS from group B and D1, the rhamnosyl residues are attached to C-3 of the galactose residues; thus only the anomic configuration of the rhamnose residues will affect the rate of hydrolysis. (In the group B Salmonella used, i.e., S. typhimurium LT2, most galactose residues do not carry branches of glucose [17].) In view of the possible effect of such branches on the rate of cleavage of rhamnosyl linkages, a group D1, S. enteritidis strain that similarly lacked glucosyl branches, SH1262, was used for the preparation of group D1 LPS.) The time course of acid hydrolysis, measured as described in Materials and Methods, is shown in Fig. 4. After a short initial lag, which can probably be attributed to the known difficulty in the cleavage of internal linkages in a long, linear polysaccharide (2), the hydrolysis proceeds with typical first-order kinetics and the rate constants are equal for the polysaccharides of groups B and D1. When the glycosidic linkage is $(1 \rightarrow 2)$, $(1 \rightarrow 3)$, or $(1 \rightarrow 4)$ between two hexoses, there is usually a two- to threefold difference in the rates of acid hydrolysis between $\alpha$- and $\beta$-linkages (2). Thus the results very strongly suggest that the anomic configuration of the rhamnosyl residues is identical in group B and D1 Salmonella, i.e., it is $\alpha$-L.

**DISCUSSION**

The results unequivocally establish the anomic configuration of rhamnose residues in the LPS of S. typhimurium LT2 as $\alpha$-L. This is contrary to the conclusions in the literature (9, 10), which were based on two lines of evidence. (i) Optical rotation of the whole LPS continued to decrease during its acid

![Fig. 4. Rates of acid hydrolysis of rhamnosyl linkages in the O side chain. The course of hydrolysis in 0.4 N H$_2$SO$_4$ at 100°C was followed as described in Materials and Methods. LPS preparations were obtained from S. typhimurium LT2 (group B) and S. enteritidis SH1262 (group D1).](http://jb.asm.org/)
hydrolysis. (ii) The untreated LPS was rather strongly dextrorotatory; this suggested the presence of more dextrorotatory, \( \beta\)-L-rhamnosyl residues, especially in view of the presumed presence (i) of levorotatory, \( \beta\)-D-mannosyl residues. However, the first evidence (i) is not convincing, because many different linkages are hydrolyzed at the same time and all of these contribute to the overall change of rotation. The latter line of evidence (ii) can also be disregarded, since the mannose residues are now definitely known to be the dextrorotatory, \( \alpha\)-D-anomers in the LPS of group A, B, and D. Salmonella, as shown by the results of treatment with mannosidases (6) and by the \([\alpha]_D\) of the repeating unit trisaccharide (Results; also see reference 8). Thus the strong dextrorotatory power of LPS from group B Salmonella can now be explained by the presence of three \( \alpha\)-D-linked sugars, i.e., mannose, galactose, and abequose, and there is no need to assume in addition the presence of the strongly dextrorotatory \( \beta\)-L-rhamnose.

Very recently a new technique was used for the determination of anomeric nature of L-rhamnose in *S. typhimurium* LPS (12). Here acetylated polysaccharide is treated with chromium trioxide, which apparently oxidizes only those sugar residues carrying the aglycons at the equatorial position of C-1. The authors concluded that the L-rhamnose residues in this LPS are oxidized and therefore must be \( \alpha\)-L-anomers (12). However, only about 20% of rhamnose residues were oxidized after 1 hr of treatment, which destroys 100% of the reference oligosaccharides that carry aglycons at equatorial positions. We therefore conclude that the reported results (12) actually suggest that the rhamnosyl residues are essentially resistant to chromium oxide, and have the \( \alpha\)-L-configuration.

This study has also confirmed the \( \alpha\)-L-configuration of rhamnose residues in the LPS of a group D, *Salmonella*. The report of the presence of \( \alpha\)-L-rhamnose in LPS of a group A *Salmonella* (8) is also likely to be correct, although we have not examined group A strains. These results, taken together with the extensive results of Herrqvist and co-workers (7-11), indicate that the O side chains of *Salmonella* of groups A, B, and D, all share a common main chain composed of a trisaccharide repeating unit which can be written as the "chemical repeat unit," \( \alpha\)-D-galactosyl-\( \alpha\)-D-mannosyl-\( \alpha\)-D-mannosyl-(1 \( \rightarrow \) 4)-\( \alpha\)-L-rhamnoseyl-(1 \( \rightarrow \) 3); or as the "biological repeat unit," \( \alpha\)-D-mannosyl-(1 \( \rightarrow \) 4)-\( \alpha\)-L-rhamnosyl-(1 \( \rightarrow \) 3)-\( \alpha\)-D-galactosyl. The linkage between the biological repeating units is normally (1 \( \rightarrow \) 2), which is changed into (1 \( \rightarrow \) 6) upon lysogenization by a converting bacteriophage \( \phi_27\) (1). Thus, the major difference between the O chains of these three groups seems to be the stereochemical configuration of the 3,6-dideoxyhexose branches. It should be noted that these 3,6-dideoxyhexoses are synthesized by a pathway (5, 16, 19), the major portion of which is shared by all three groups.

\[
\begin{align*}
\text{glucose 1-phosphate} & \rightarrow \text{CDP-glucose} \rightarrow \text{CDP-4-keto-3,6-dideoxyhexose} \rightarrow \text{CDP-4-keto-3,6-dideoxyhexose} \\
& \rightarrow \text{CDP-tyvelose} \rightarrow \text{CDP-tyvelose (group A)} \\
& \rightarrow \text{CDP-tyvelose (group D)}
\end{align*}
\]

(The abbreviations are as follows: CTP, cytidine triphosphate; CDP, cytidine diphosphate, PP\(_i\), inorganic pyrophosphate.)

Although the detailed mechanism of the conversion of CDP-4-keto-3,6-dideoxyhexose into CDP-paratose and CDP-abequose is not known, it probably involves a simple stereospecific reduction of C-4 by a single enzyme. If so, the difference between groups A and B could essentially be the different stereospecificity of a single enzyme.

The interconversion of CDP-paratose and CDP-tyvelose is catalyzed by a single epimerase (15). As was pointed out by Matsushashi and Strominger (15), it seems possible that group A strains are mutants of group D, strains that have lost this enzyme. Indeed the known group A serotypes seem to be related to some group D, serotypes in various properties, e.g., H-antigen specificity, fermentation pattern, and clinical characteristics (15).

The value of LPS structure as a taxonomic marker has been stressed (18). The consideration described above shows that groups A, B, and D, of *Salmonella* are more closely related to each other, at least in terms of LPS structure, than was previously assumed. This notion fits with many observations. (i) These three groups contain the most potent human pathogens capable of producing enteric fever*-type infection (21). (ii) They share O-antigen \( 12_i, 12_s \), and (in \( 12_s \)-positive forms) \( 12_{14} \) (14). (iii) They are infected by bacteriophage P22, and produce antigen \( 12 \) when lysogenized (14). (iv) Group B "polymerase" can polymerize the O repeating units of group D, *Salmonella*; conversely, group D, "polymerase" can act on...
group B-type repeating unit tetrasaccharide (22).

The argument described above, however, does not necessarily mean that there is no difference between groups A, B, and D, Salmonella except for the enzymes in the last steps of CDP-dideoxyhexose synthesis. Individual strains are indeed known frequently to have characteristic additional structure on the O side chain. For example, O-acetyl groups have been found on rhamnose (group A), abequose (group B), and glucose (group D), residues in certain strains; O side chains in many strains also carry short branches of α-D-glucose (8-11). Furthermore, there are sometimes clear differences in fermentation pattern, nutritional requirement, virulence, and host specificity even among the strains that produce LPS of very similar structure (19). These observations indicate that more extensive studies are needed for the better understanding of the evolutionary relationships between these serogroups of Salmonella.

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