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On the Structure of the Lipopolysaccharide Core in the cell Wall of *Escherichia coli* K12 W2252-11U⁻ and its Ter-Mutant Cells.

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SUMMARY : From *Escherichia coli* K12 W2252-11U⁻ (wild type I and II) and its Ter-mutants, lipopolysaccharides were isolated and the primary structure of its core region was elucidated. the configuration of the glucose III(1⟶2)-linked to the glucose II residue in the core of parents (wild type I and II) changes into the configuration of the glucose (1⟶3)-linkage by the Ter-mutation. Also, the structure of the core in the Ter-15 mutant differs from that in the parent (wild type I) and is similar to that in *Escherichia coli* C used as an indicator for φx174 phage. D-ribose residue in the core of the Ter-21 mutant links to the glucose I residue instead of the galactose residue in the core of the parent (wild type II).

The cell wall lipopolysaccharides of *Escherichia coli* K12 strain consist of the inner lipid A portion and of the core oligosaccharides (1). *Escherichia coli* K12 W2252-11U⁻ (wild type I and II) used here, previously reported in Biochem. Biophys. Res. Comm. (2), do not possess a receptor site in the core oligosaccharides for the adsorption of φx174 phage (wild type), so the cells are resistant to this phage. Also, the core oligosaccharides in lipopolysaccharides (LPS) of the cell wall in the Ter-mutant cells (3) exhibit variability in their sugar components. The Ter-15 mutant isolated from *Escherichia coli* K12 W2252-11U⁻ (wild type I) becomes sensitive to this phage and possesses a receptor site in the core for this phage, but the Ter-21 mutant from *Escherichia coli* K12 W2252-11U⁻ (wild type II) is insensitive to this phage, as a receptor site is not produced in the core. Thus, the structure of the core oligosaccharides in LPS from these mutant cells differs from its structure in LPS from those parent cells (wild type I and II). In this paper, we therefore undertook the elucidation of the structure in the core of the Ter-mutant and parent cells.

Abbreviations used : LPS, lipopolysaccharide ; KDO, 2-keto-3-deoxymannulosoctonic acid ; G.L.C., Gas-liquid chromatography ; GlcNAc, N-acetylglucosamine.

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Materials and Methods

Bacteria : *Escherichia coli* K12 W2252-11U$^-$ (thy$^-$,ura$^-$,met$^-$)RC^{str} (wild type I) and its Ter-15 mutant, and *Escherichia coli* K12 W2252-11U$^-$ (thy$^-$,ura$^-$,met$^-$)RC^{rel} (wild type II) and its Ter-21 mutant are as described in a previous report (2). *Escherichia coli* C strain is used as the sensitive cell type for φx174 phage infection.

Chemicals : Hexose, pentose and other chemicals were purchased from Wako Pure Chemical Company, LTD, Japan.

Isolation and analysis of LPS core oligosaccharides :

The LPS in the outer membrane of *E. coli* K12, its Ter-mutant and *E. coli* C (as a control) strains were extracted by the methods of Galanos et al (4) and of Westphal et al (5). The core oligosaccharides in LPS of the cell wall were purified as in the previous report (2).

The aldose analysis of the core was carried out as described in the previous report (2). The core materials were also methylated with methylsulfonyl carbanion in dimethyl sulfoxide (6). The methylated products, after drying the chloroform extracts, were hydrolysed with formic/sulfuric acid (7), and neutralyzed with barium hydroxide. The methylated monomers thus obtained were reduced with sodium borohydride (8) and neutralized with Dawex 50 X8 (H-form). These products were acetylated with pyridine and acetic anhydride (9), and extracted with chloroform. The chloroform extracts were purified by passage over Wakogel C-200 (Silica Gel) in a column (1.0 X 0.5 cm). After drying, these alditol acetates (10) were identified by gas-liquid chromatography (G.L.C.)(Shimadsu Gas Chromatograph, model GC 6AM, column 2m, 2.6 mm ID, ECNSS-M, 3%, chromosorb-P, AW-DMCS, mesh 60 - 80, carrier gas N_2 , 60 ml/min, 2Kg/cm^2, Temperature 180°C).

Results and Discussion

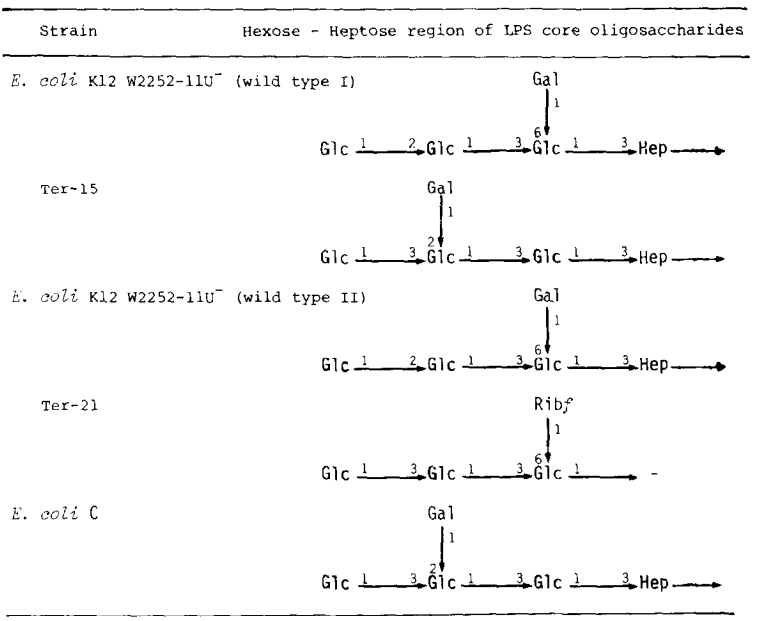
By analysis of the core oligosaccharides in LPS from *E. coli* K12 W2252-11U$^-$ (wild types) and its Ter-mutant cells (2), galactose, glucose, heptose and KDO were found present in the core from *E. coli* K12 (wild type I and II) and the Ter-15 mutant cells, but in the Ter-21 mutant cells glucose, ribose and KDO were present. Neither galactose nor heptose could be found in the core of the Ter-21 mutant. *Escherichia coli* K12 strains are not sensitive to φx174 phage infection, but the Ter-15 mutant becomes sensitive to this phage. Also, the Ter-21 mutant is insensitive to this phage. Regarding these data, the structure (in particular, the linkage between galactose and glucose) of the core in LPS of the mutant cells seems to differ from that of the core in LPS of the parent cells (wild types).

In Table 1 and Figure 1, it can be found that the retention time of the 2,4,6-Glc differs at 0.03 from that of the 3,4,6-Glc on ECNSS-M column as the alditol acetates, although Feige & Stirm (11) reported that 2,4,6-Glc

Table 1. Methylation and Gas-liquid Chromatography of *Escherichia coli* K12 W2252-11U$^-$ and its Ter-mutant Cell Wall LPS Core Oligosaccharides.

Alditol Derivative[a]	T[b] Lit.[c]	T[b] Found	Approximate Molar Ratio from LPS core in *E. coli*[d]: K12(wild type I)	Ter-15	K12(wild type II)	Ter-21	C
2.3.4.6-Glc	1.00[e]	1.00[e]	0.70	1.10	0.9	1.00	1.00
2.3.4.6-Gal	1.25	1.24	1.00	1.00	1.00	-	1.00
2.4.6-Glc	1.95	1.93	-	1.00	-	0.95	-
3.4.6-Glc	1.98	1.96	1.00	-	0.90	-	1.00
2.3.5-Rib	0.40	0.39	-	-	-	1.00	-
2.3.4.6.7-Hep	2.13	2.14	0.90	0.90	0.98	-	0.8
4.6-Glc	4.02	3.83	-	0.80	-	-	0.93
2.4-Glc	5.10	4.90	1.00	-	0.89	0.97	-
2.4.6.7-Hep	4.40	4.31	0.70	0.88	0.80	-	0.90

a. 2.3.4.6-Glc = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol etc. b. Retention time relative to 2.3.4.6-Glc (T = 1.00) and 2.3-Glc (T = 5.39) on ECNSS-M (10,11,16). c. (10, 16).
d. Peak Ratio relative to 2.4.6-Glc = 1.0. e. Standard.



Sugars except D-ribose occur as pyranoses, and it is not known if the branch heptose is located on the distal, or on the proximal chain heptose.

Figure 1. Cell Wall Lipopolysaccharide Core Structures in *Escherichia coli* K12 W2252-11U⁻ and its Mutants.

and 3,4,6-Glc did not separate on ECNSS-M as the alditol acetates. Therefore, the configuration of the (1⟶2)-linked glucose in *E. coli* K12 W2252-11U⁻ (wild type I and II) changes into the configuration of the (1⟶3)-linked glucose in the Ter-mutant cells. Also, 2,4-Glc is found in the core of *E. coli* K12 W2252-11U⁻ (wild type I and II) and the Ter-21 mutant cells, but 4,6-Glc is found in the core of the Ter-15 mutant cells. According to many reports about the configuration of the linked galactose in *E. coli* K12 and C strains (12 - 14), the data obtained here show that the configuration of the (1⟶6)-linked galactose in the core of *E. coli* K12 W2252-11U⁻ (wild types) changes into the configuration of the (1⟶2)-linked galactose in the core of the Ter-15 mutant cells, and is similar to that of the (1⟶2)-linked galactose in the core of *E. coli* C cells which are sensitive to ϕx174 phage.

From reports of Lindberg et al (15) in *S. typhimurium*, and Feige & Stirm (11) in *E. coli* C and its phage resistant mutants, ϕx174 phage is

specific for α(1⟶2)-linked terminal hexoses and an α-linkage is proposed for the terminal galactose. As thus proposed, loss of the galactose II residue in an *E. coli* C mutant, strain C/15, resulted in loss of φx174 adsorption and susceptibility (14). The presence of the terminal glucose III residue in the core oligosaccharides was apparently not required, since φx174 was adsorbed by and lysed a mutant strain lacking that constituent. But for the *E. coli* C23 mutant, loss of α(1⟶2)-linked galactose II exhibits a residual adsorption of this phage (11).

In the core of LPS in *E. coli* K12, one molecule of galactose in its molar ratio is detected and shown as the configuration of the branched galactose (1⟶6)-linked to the glucose I residue, and so the core exhibits the following configuration :

In the core of *E. coli* K12 W2252-11U⁻ and its Ter-mutant cells used here, one molecule of galactose and three molecules of glucose in its molar ratio were detected by methylation analysis with G.L.C., and were determined as the configuration shown in Fig. 1. Thus, the galactose residue does not increase in the core of the Ter-15 mutant cells, and the configuration of the linkage between galactose and glucose would change into a configuration similar to both the linkage of sugars in the core of *E. coli* C cells used here and those of the *E. coli* C 23 mutant isolated by Feige & Stirm (11).

Because the 3,4,6-tri-O-methyl-D-galactitol derivative cannot be detected in the core of *E. coli* C cells used here, the terminal (1⟶2)-linked galactose II residue seems to be lacking in this core.

In accordance with the results presented here, it is considered that φx174 phage adsorption is not specific for the core terminal sugar itself which differs from other reports, but possibly for the configuration around the linkage of glucose II residue.

The core of the Ter-21 mutant contains one molecule of D-ribose in its molar ratio, and the structure shows the ribose (1⟶6)-linked to the glucose I residue instead of the (1⟶6)linked galactose in the core of *E. coli* K12 W2252-11U⁻ (wild type II) by examination of the data in Table 1. Also, the terminal glucose residue changes into the configuration of (1⟶3)-linked form in the glucose II residue by Ter-mutation.

From these facts it is proposed that Ter-mutation induces a different configuration of sugar linkages in its core compared to those in the core of its parent cells (wild type I and II). But we cannot explain why D-ribose residue is contained in the core of the Ter-21 mutant cells.

By the reports of Prehm et al (12,13), the *E. coli* K12 strain contains as a basal structure N-acetylglucosamine (GlcNAc)(1⟶6)-linked to the terminal glucose residue, but we could not detect the GlcNAc residue in *E. coli* K12 W2252-11U⁻ and its Ter-mutant cells used here. Therefore, the (1⟶2)-linked N-acetylglucosamine residue is lacking in these *E. coli* K12 cells.

Finally, in the previous report (B.B.R.C. (2)), we calculated the sugar components in its molar ratio by trimethylsilylation analysis with gas-liquid chromatography (G.L.C.) after methanolysis of the core oligosaccharides and obtained results showing one molecule of galactose and two molecules of glucose in the parent and in the Ter-15 mutant cells, or one molecule of ribose and two molecules of glucose in the Ter-21 mutant cells. But, from the study of the structure of the core oligosaccharides by the methylation analysis, three molecules of glucose versus one molecule of galactose or ribose were detected in the core of *E. coli* K12 W2252-11U⁻ and its Ter mutant cells. As the former analytical data shows insufficient amounts of glucose in the core, even though the methanolysis was carried out completely for a long period, it is considered to be a doubtful factor in the calculation of the amounts of sugar from the data obtained by this method.

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